Int. J. Plant Sci. 168(6):775–799. 2007.
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1058-5893/2007/16806-0002$15.00

THE IDENTIFICATION OF A-, B-, C-, AND E-CLASS MADS-BOX GENES AND IMPLICATIONS FOR PERIANTH EVOLUTION IN THE BASAL EUDICOT *TROCHODENDRON ARALIOIDES* (TROCHODENDRACEAE)

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Trochodendron aralioides is the sole member of the family Trochodendraceae, which belongs to the basal eudicots, has vesselless wood, and lacks a distinct perianth. Our observations confirmed that there are numerous perianth-like structures and that the number of these structures differs between protandrous and protogynous flowers and among the positions within an inflorescence. The epidermal cells on many floral parts of *T. aralioides* are papillate or conical, similar to the ones of ordinary showy petals of other species. The data in this article support the hypothesis that the perianth of *Trochodendron* has been secondarily lost and suggest that some aspects of petal identity, e.g., papillate cells, have been retained that might be important for pollinator attraction. We have identified 11 homologues of floral organ identity genes—two A-class, three B-class, two C-class, and four E-class homologous genes—from *T. aralioides*. Phylogenetic analysis shows that all of the genes arose before a major duplication of MADS-box genes at the base of the core eudicots. Expression patterns for those floral organ identity gene homologues was determined by reverse transcriptase PCR, which showed variations that do not conform well to the current floral ABCDE model. In addition, all paralogous genes have distinct expression patterns, suggesting that they had undergone functional divergence.

Keywords: Trochodendron aralioides, ABCDE model, perianth evolution, MADS-box genes.

Introduction

Trochodendron aralioides Sieb. & Zucc. is a unique plant with vesselless wood and without a distinguishable perianth or showing only highly reduced tepal-like scales (Endress 1986). It is restricted to Taiwan, the Ryukyu Islands, Japan, and South Korea, although the genus *Trochodendron* was once widely distributed in the Northern Hemisphere during the Miocene, according to the fossil records (Manchester et al. 1991). Because of its "primitive" features, *Trochodendron* has been suggested to be one of the earliest angiosperms or close to the hamamelid dicots. However, recent systematic studies have placed *Trochodendron* in a rather isolated position in the lower eudicots close to Buxales, another taxon that shows perianth reduction.

Molecular phylogenetic studies based on nuclear and organelle nucleotide sequences have revealed a well-supported clade comprising two genera, *Trochodendron* and *Tetracentron*, in the basal eudicots (APG 1998; APG II 2003; Hilu et al. 2003; Qiu et al. 2005). However, the exact phylogenetic position of the *Trochodendron*/*Tetracentron* clade is disputable. In most cases, this clade and the family Buxaceae are sister groups or form a grade to the core eudicot clade, as suggested by chloroplast *rbcL* (Chase et al. 1993; Qiu et al. 1998), *matK* (Hilu et al. 2003) and combined mitochondrial/nuclear (Parkinson et al. 1999) and chloroplast/nuclear data sets (Hoot et al. 1999; Savolainen et al. 2000; Soltis et al. 2000; Kim et al. 2004*a*). Regardless, it is clear that *Trochodendron* and *Tetracentron* are sister groups, and whether to place them in the same or separate families is equivocal under APG II (2003). These two genera share many similar characters, such as their sessile connate carpels, horizontally extended and nectariferous ovaries, floral organs with oil cells, vesselless wood, follicles, and small seeds (Endress 1986).

Trochodendron is generally described as lacking a perianth. The inflorescence of *Trochodendron* is a botryoid, i.e., a raceme with a terminal flower, covered with numerous bracts when in a bud (fig. 1). The terminal flower has many scalelike bracts called metaxyphylls (Endress 1986). Each lateral flower in an inflorescence is subtended by a bract (pherophyll) and has two prophylls in the transversal position above the pedicel (fig. 1*B*). The length of the prophylls is variable; prophylls are much longer in lower lateral flowers and smaller in upper lateral flowers of an inflorescence. In specimens of *Trochodendron* from Taiwan, each flower contains numerous stamens in three to five superimposed series (whorled phyllotaxis), but it is interesting to note that the floral phyllotaxis in *Trochodendron* is indeed diverse in having both spiral and whorled flowers (Endress 1990). There are five to seven carpels, fewer in number than in other reports (Nast and Bailey 1945; Endress 1986), fused laterally to form a whorl (Li and Chaw 1996). Endress (1986) found a few residual scales, clearly visible under scanning electronic microscopy (SEM), located between prophylls and stamens in young flowers of *Trochodendron*; he suggested that those scales are residual perianth

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Manuscript received November 2006; revised manuscript received February 2007.

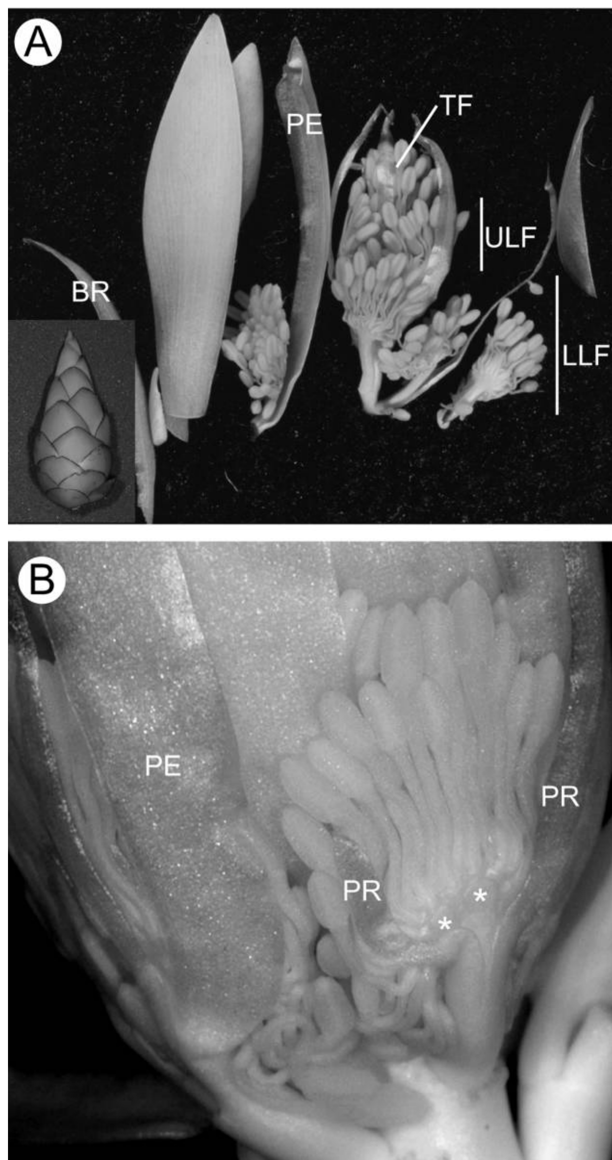

Fig. 1 Morphology of *Trochodendron* flowers. *A*, Inflorescence bud (inset) and dissections showing a series of flower-subtending pherophylls (*PE*). *BR* = bracts covering the inflorescence bud; *LLF* = lower lateral flowers; *TF* = terminal flower; *ULF* = upper lateral flowers. *B*, Two elongated prophylls (*PR*) found at the base of a lower lateral flower from a protogynous plant. Two tepal-like scales are marked (asterisks) between stamens and prophylls.

and called the scales "tepals" (visible in fig. 1*B*). These tepals are not considered to be bracts because there is a developmental time lag between the two prophylls and they are different from the scalelike bracts (metaxyphylls) of the terminal flower (Endress 1986). However, morphologically it is usually difficult to discriminate a perianth from a series of bracts, such as in *Buxus* of Buxaceae (von Balthazar and Endress 2002), and so it remains ambiguous whether the scales are indeed residual perianth or bractlike organs.

Flowers lacking a distinct perianth are known to have evolved at least six times from ancestors with a perianth, such as Chloranthaceae, Piperaceae, and Trochodendraceae (Albert et al. 1998; Soltis et al. 2005; Endress 2006). Although some of those taxa have residual perianth-like structures, sometimes it is difficult to identify whether they are reduced perianths or bracts. The question of the origins of the perianth in angiosperms is complex and has been subject of several competing hypothesis (Albert et al. 1998). The perianth may originate completely from bracts (bracteopetals), originate completely from stamens (andropetals), or have mixed origins, i.e., sepals from bracts and petals from stamens (Weberling 1989; Takhtajan 1991; Endress 1994). Within the family Magnoliaceae, all transitional stages from undifferentiated perianth to sepal/petal bipartite can be observed, indicating a similar origin of petals and sepals in this group. In comparison, the petals of Ranunculaceae are clearly staminodal in origin (Takhtajan 1991; Bowman 1997). Both scenarios can be readily explained by a "sliding-boundary" model that suggests that the perianth partition can be regulated by the different patterning of floral organ identity genes among whorls (Bowman 1997; Albert et al. 1998; Baum 1998; Baum and Whitlock 1999). However, the whorl numbers of the perianth in the lower eudicots are highly variable, ranging from zero, as in Buxaceae, and one, as in Papaveraceae, to many, as in Berberidaceae; this condition is not so easily explained by the sliding-boundary model. It is worth noting that *Tetracentron*, the sister group of *Trochodendron* (Qiu et al. 1998; APG II 2003), does have a distinct perianth, which is thought to be sepals (Nast and Bailey 1945; Endress 1986). Nonetheless, the absence of perianth in *Trochodendron* might be explained by a lack of expression of proper petal-specifying genes in the region corresponding to perianth formation.

Recent progress on the floral developmental program comes mostly from studies on two model systems, *Arabidopsis thaliana* and *Antirrhinum majus*. Genetic studies of these two systems have shown that floral organ identities are determined by a combination of five classes of genes (A, B, C, D, and E) for an expanded ABCDE model (Coen and Meyerowitz 1991; Theissen and Saedler 2001). In this model, sepals, the first whorl of a flower, are determined by the expression of A- and E-class genes. Petals, the second whorl of a flower, are determined by a combination of A-, B-, and E-class genes. Stamens, the third whorl organs, are determined by a combination of B-, C-, and E-class genes, and the identity of carpels is determined by C- and E-class genes (D-class genes are involved in ovule development). In angiosperms, the A-class genes are represented by homologues of the *A. thaliana APETALA1* (*AP1*) and *APETALA2* (*AP2*) genes, whereas the B-class genes are represented by homologues of the *A. thaliana APETALA3* (*AP3*) and *PISTILLATA* (*PI*) genes, and the C-class genes are represented by homologues of the *A. thaliana AGAMOUS* (*AG*) gene. All of the A-, B-, C-, and E-class genes are MADS-box genes except for *AP2*, which belongs to a unique family found only in plants. Studies of many other eudicots show a very high degree of conservation among these genes, indicating that the eudicots may all share a similar developmental program, although gene function has been investigated in only a few species (Bowman 1997; Kramer et al. 1998; Ma and dePamphilis 2000; Ferrario et al. 2004). Among these genes, B-function genes may have played a major role in morphological evolution of angiosperm perianth and stamens, because they are

highly conserved and share similar expression patterns in angiosperms (Bowman 1997; Albert et al. 1998; Baum 1998; Kramer and Irish 2000).

Major duplications of floral homeotic MADS-box genes have been identified and discussed for the A, B, C, and E subfamilies of MADS-box genes (Kramer et al. 1998, 2003, 2004; Irish 2003, 2006; Zahn et al. 2005*b*; Kramer and Zimmer 2006). One major duplication event occurred before the origin of the angiosperms and gave rise to two B lineages, paleo-*AP3* and *PI* (Kramer et al. 2003; Kim et al. 2004*b*); a C/D lineage, basal *AG*-like and D (Kramer et al. 2004); and two E lineages, *AGL2/3/4*-like and *AGL9* (Zahn et al. 2005*a*), or *LOFSEP* and *SEP3* (Malcomber and Kellogg 2005). A second major duplication occurred near the basal eudicots and gave rise to the following lineages in core eudicots: eu*AP1* and eu*FUL* lineages of A-class genes (Litt and Irish 2003), eu*AP3* and *TM6* lineages of B-class genes (Kramer et al. 1998; Kramer and Irish 1999, 2000), eu*AG* and *PLE* lineages of C-class genes (Kramer et al. 2004), and *AGL2/4* and *AGL3* lineages of E-class genes (Zahn et al. 2005*a*).

The phylogenetic position of *Trochodendron* is very close to that of the second major duplication of these ABCE lineages. We have recently shown that an *AP3* homologue from *Trochodendron* (*TroAP3*, here renamed as *TraAP3* for consistency with other names) is indeed the sister to the eu*AP3* and *TM6* clades; therefore, it is designated as the paleotype of *AP3* genes (Kramer et al. 2006). A detailed phylogenetic analysis for other floral organ identity genes from *Trochodendron* is thus critical for elucidating the gene evolution near the major duplication event.

Trochodendron is a very interesting plant for examining the mechanism of floral development because it has no distinct perianth. On the basis of previous phylogenetic analysis and morphological studies (APG 1998; APG II 2003; Hilu et al. 2003; Qiu et al. 2005), the absence of a distinct perianth in *Trochodendron* appears to be the result of a secondary loss in evolution, rather than being a primitive condition. In this study, we aim to examine the possible mechanism of floral development in *Trochodendron* by studying the expression pattern of floral organ identity gene homologues. The expression pattern of a *LEAFY* (Schultz and Haughn 1991) homologue in *T. aralioides*, *TraLFY*, was carried out for comparison. We also provide additional detailed description on the epidermal cells in different floral parts with SEM.

Material and Methods

Plant Material

Trochodendron aralioides material was collected from ErhKeShan, ChiuFen ShuMeiPing, and YangMingShan, located in northern Taiwan. Since protandrous and protogynous flowers are found among individuals (Chaw 1992), both types of flowers were collected. The floral buds and floral parts were collected and immersed in liquid nitrogen or FAA fixation buffer (63% ethanol, 5% acetic acid, and 2% formalin) in the field. For identifying floral organ identity gene homologues, young flowers that were still in bud were used. For reverse transcriptase (RT) PCR, floral organs were separated by forceps into six categories—leaves, inflorescence and floral bracts (pherophylls), pedicels, stamens, "carpels" (including all floral parts above pedicels except the stamens or distinguishable prophylls), and fruits—and were immersed immediately in liquid nitrogen. Pedicels, stamens, and carpels were collected from mature flowers, whereas the fruits were greenish but had a narrow opening on the top. The presumed scalelike perianth and most of the prophylls were inseparable from the bottom of the flowers and were all included in the "carpel" partition during sample preparation.

Gene Sequence Determination

Total RNA was extracted following the pine tree method (Chang et al. 1993), with minor modifications. First-strand cDNA was reverse transcribed using the SuperScript II RNase H^- reverse transcriptase kit with manufacturer's instructions (Invitrogen, Carlsbad, CA) and used as a template for amplifying the floral organ identity gene homologues. Degenerate primers for initial amplification of different genes were based on published studies of A/E-class (Litt and Irish 2003), B-class (Stellari et al. 2004), and E-class genes (Kramer et al. 2004). The PCR reagents were composed of 5 μL 10× Advantage 2 PCR buffer, 1 μL 10-mM dNTP (2.5 mM each), 1 μL of each specific primer (10 mM each), 2 μL template, and 0.5 μL Advantage 2 polymerase mix (Clontech, Palo Alto, CA) and adjusted with water to a final volume of 50 μL. The PCR was first heated at 95°C for 5 min; there followed 35 cycles of 95°C for 30 s, 50°C for 45 s, and 68°C for 1 min and an extension step of 5 min at 68°C. Platinum *Taq* DNA polymerase (Invitrogen) was used to amplify DNA fragments; the PCR reagent mix contained 5 μL 10× High Fidelity PCR buffer, 1 μL 10-mM dNTP (2.5 mM each), 1 μL of each specific primer (10 mM each), 2 μL 50-mM $MgSO_4$, 1 μL template, and 0.2 μL Platinum *Taq* Polymerase High Fidelity and was adjusted with water to a final volume of 50 μL. The PCR was heated at 94°C for 2 min; there followed 35 cycles of 94°C for 30 s, 50°–55°C for 45 s, and 68°C for 1 min and an extension step of 5 min at 68°C. The PCR products were purified with the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and cloned into the pGEM-T Easy Vector system (Promega, Madison, WI) using Advantage 2 polymerase mix to amplify the PCR products. However, when a polymerase with proofreading function, such as Platinum *Taq* DNA Polymerase High Fidelity, was used, *VioTaq* DNA polymerase (Viogene, Taipei) was added before using these products in the ligation reaction. Ligation products were transformed into *Escherichia coli* DH5α- or ECOS-competent cells (Yeastern Biotech, Taipei) following the manufacturer's instructions.

We used the 5′ RACE system kit (Invitrogen) to determine the 5′ terminus for the obtained sequences of selected floral homeotic gene homologues from 3′ RACE results described above. Primers are listed in table 1. The QIAquick PCR kit was used to purify the RT-PCR products with modifications: the column was washed with PE buffer twice and the cDNA was eluted with diethylpyrocarbonate (DEPC)-treated water.

Phylogenetic Analyses

The sequences of selected species were downloaded from the National Center for Biotechnology Information GenBank

Table 1

Primers Used in 5′ RACE Screening and Gene-Specific Primers Used for Reverse Transcriptase PCR in This Study

Primer name	Sequence (5′ to 3′)	Direction
TraFUL1-CF	GGAGCAGCAAAACCAAGTTC	Forward
TraFUL1-3UTR	TTCAAACAGGCCACTTTTGG	Reverse
TraFUL2-CF	GGAGCAGGGAAACCAAGTTC	Forward
TraFUL2-3UTR	ACCAAAACTTGCTGACCAGA	Reverse
TraFUL-KR	TGAAGCTCGGAAATGGATTC	Reverse (5′ RACE)
TraAP3-KF1	ACTGAGACTTACAGGAAAAA	Forward
TraAP3-3UTR	GATACACAAACTTCTAGTAAT	Reverse
TraPI1-KF1	AACGGCGTCACCAACATTCGTCAC	Forward
TraPI1-3UTR	AGGGGTTTAATACCACGAACA	Reverse
TraPI2-F	AGAGGAAGCCCTGGAAAATG	Forward
TraPI2-3UTR	CATGGTGATCCACAAGCATC	Reverse
TraAP3-KR1	CTTTTTCCTGTAAGTCTCAGTCTG	Reverse (5′ RACE)
TraPI-CR1	GTCCCTCATTTTCGGAGCATACCC	Reverse (5′ RACE)
TraAG1-KF2	GGAACTCAGGARCCTGGAGAC	Forward
TraAG2-KF	AACGAGTTGYTGTTTGCCGA	Forward
TraAG1-3UTR	AGCGGAAACCTTTCGTCCTT	Reverse
TraAG2-3UTR	CTTCACCTCTGGCTCTCACC	Reverse
TraMADS1-CF	GAGTGTGAGCCCACGTTACA	Forward
TraMADS1-3UTR	AACGAACCCCATGTTCAAAT	Reverse
TraMADS2-CF	TGAGCCCACATTACAAATCG	Forward
TraMADS2-3UTR	GAACATCCTGTTTGAATGATCC	Reverse
TraMADS3-CF	GAACCAGAGGGATTCTTCCA	Forward
TraMADS3-3UTR	ACAGGTTGCATCACCAGATT	Reverse
TraMADS4-CF	ACAGCCAGCTCATCCACAG	Forward
TraMADS4-3UTR	GAGCACCATTCGCTTTAACC	Reverse
TraSEP-KR	GCATTTGTTCCBTTCTTTGAA	Reverse (5′ RACE)
TraLFY-F	GAAACAGCGTCGAAAGAAGG	Forward
TraLFY-R	CAACGAGGGGTTTGTAGCAT	Reverse
TraACTIN-F3	GCACCGCCTGAGAGAAAGTA	Forward
TraACTIN-3UTR	TAAAGAGGAACGGCGAAAGA	Reverse

Note. The primers were named according to genes followed by their locations within the genes, e.g., C for C domain and K for K domain. The reverse primers ending with "3UTR" were all located at the 3′ untranslated region (UTR), except for *TraLFY*. RACE = rapid amplification of cDNA ends.

(appendix). The taxa were selected on the basis of representing angiosperm diversity at the order level. Only one taxon was selected per order, and those with more complete sequences and/or with genomic/EST data were favored. All homologues or paralogues from each selected taxon were included in the analyses. Nucleotide sequences were used for separate phylogenetic analyses of each gene class. Alignments were conducted using Clustal X 1.83 (Thompson et al. 1997) and were modified in MacClade 4.06 (Maddison and Maddison 2000) according to amino acid alignment. Phylogenetic analyses for each matrix were conducted with Bayesian inference (BI) using MRBAYES 3.0b4 (Huelsenbeck and Ronquist 2001). The BI analyses were run with four chains of Markov chain Monte Carlo simulation, sampling one tree per 500 generations for 1,500,000 generations, and the general time reversible nucleotide substitution model with gamma distribution for rate categories was incorporated; this model was evaluated as optimal by the Modeltest program (Posada and Buckley 2004). The first 1000 trees were discarded before the node probability was calculated. Neighbor-joining (NJ) and maximum-parsimony (MP) methods were conducted with PAUP* 4.0b10 (Swofford 2002). For NJ analyses, the HKY85 nucleotide substitution model was used, and support for the branches was assessed using bootstrap analyses with 1000 replicates. For MP analyses, heuristic searches were conducted with 500 random addition replicates and tree bisection-reconnection (TBR) branch swapping with steepest-descent option in effect. Support for the branches was assessed using bootstrap analyses with 1000 replicates, each with one random stepwise addition and TBR branch swapping with steepest-descent option in effect.

RNA Expression

The concentration of extracted RNA was determined by GeneQuant (Amersham Biosciences, Little Chalfont, UK). RQ1 RNase-free DNase (Promega) was added to RNA to remove DNA contamination, and the concentration was adjusted to 100 ng/μL. The locus-specific forward primers in the K or C domain and the specific reverse primers located in 3′ untranslated region (UTR; table 1) were used to amplify cDNA from different tissues. These primers were designed with the software Primer3 Input (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and selected so that the PCR products span intron(s) in genomic sequences according to gene structure of *Arabidopsis* orthologues. The PCR products from mRNA and genomic DNA could therefore be easily discriminated by their sizes, and putative genomic contamination of RT-PCR could be identified. The PCR products

made from genomic DNA template were sequenced to determine the position of intron(s) in selected genes. The *Actin* gene of *T. aralioides* was identified and used as an internal control for RT-PCR. New gene-specific primers were designed (table 1) to clone *TraLFY* (*LEAFY* homologue of *T. aralioides*) based on the published sequence (Frohlich and Parker 2000). Each first-strand cDNA sample was synthesized from 2 μg of total RNA using the Superscript III RNase H^- reverse transcriptase kit system described above. PCR reactions were carried out using 0.25 μL Advantage 2 polymerase mix, 1.25 μL 10× Advantage 2 PCR buffer, 0.25 μL 10-mM dNTP, 0.25 μL each of 10-mM specific forward and reverse primers, and 0.5 μL of cDNA in 12 μL of final reaction volume. Reaction conditions were 50°–55°C for 30 min and 94°C for 2 min, followed by 30 or 35 cycles of 94°C for 15 s, 50°–55°C for 30 s, and 68°C for 1 min and a final extension at 68°C for 3 min. All of the RT-PCRs were repeated at least twice for 30-cycle reactions and at least four times for 35-cycle reactions from independent RNA preparations.

Scanning Electron Microscopy

Floral buds and mature flowers of *T. aralioides* were fixed in FAA and then dehydrated in steps, in 70% EtOH for 10 min, 85% EtOH for 20 min, 95% EtOH for 20 min, and 100% EtOH for 20 min twice. The dehydrated samples were stored in 100% EtOH and were critical-point dried and mounted with double-sided tape on aluminum stubs. The specimens were coated with gold and viewed with an FEI Quanta 200 scanning electron microscope at 10 kV.

Results

Screening and Phylogenetic Analyses of Floral Homeotic Genes

In total, 11 floral organ identity gene homologues were obtained from 286 cDNA clones screened from *Trochodendron* flowers. Ninety-four of these clones were obtained by rapid amplification of cDNA ends (RACE) using degenerated A/E-class gene-specific primers. Forty-four of those clones had the expected insert size and were sequenced, and 25 showed homology to floral A/E-class genes via GenBank blasting. Among them, three of the clones were identical and close to *FUL*-like genes and are therefore here named *TraFUL1*. An additional *TraFUL2* sequence was obtained from two independent clones when screening from 5′ RACE products of *TraFUL1*, and a specific primer was designed to conduct 3′ RACE of *TraFUL2* in order to obtain its 3′ sequence. Four *SEP* homologues were identified: *TraMADS1*, *2*, *3*, and *4*. *TraMADS1* was identified from nine clones, *TraMADS2* was identified from 10 clones, *TraMADS3* was identified from a single clone, and *TraMADS4* was identified from two independent clones. For C-class homologues, a total of 78 clones were screened, and 46 clones with expected insert size were sequenced. Among them, two *AG* homologues were identified: *TraAG1* was identified from 15 clones with identical sequences, and *TraAG2* was identified from three clones. Four B-class homologues were identified from 114 cDNA clones screened: *TraPI1* was identified from 14 clones, *TraPI2* was identified from eight clones, *TraPI3* was identified from five clones, and *TraAP3* was identified from 10 independent clones. *TraPI3* is identical to *TraPI2* except that it is missing the putative exons 3 and 4 corresponding to the exon-intron structure of *Arabidopsis PI*.

The sequences from the 11 identified floral organ identity gene homologues from *Trochodendron* were aligned with the respective ABCE genes, and phylogenetic analyses were performed. *TraFUL1* and *TraFUL2* are sister groups and form a clade with other *FUL*-like homologues of basal eudicots, *PatFL1*/*PatFL2* of *Pachysandra terminalis* (Buxaceae) and *AktFL1* of *Akebia trifoliata* (Lardizabalaceae) with moderate bootstrap support under NJ (92%) and MP (68%) and a posterior probability of 0.76 (fig. 2). This clade clearly branches off before the separation of the two well-supported eu*FUL* and eu*AP1* lineages, and the clade consists of five *FUL*-like genes from core eudicots (fig. 2).

The *AP3* homologue of *Trochodendron*, *TraAP3*, is a sister group of two sequences, *PtAP3-1* and *PtAP3-2* of *Pachysandra terminalis*, on BI phylogeny (fig. 3) but not on NJ and MP analyses, either unsolved or mixed with sequences from basal eudicots (data not shown). *TraPI1* and *TraPI2* formed a grade or were unresolved but close to the core eudicot clade (fig. 3). The two *Houttuynia* (Saururaceae) *PI*s, *HtcPI1* and *HtcPI2*, are unexpectedly grouped with *TraPI1*, receiving a BI posterior probability of 0.75; they are within the eudicot clade with 0.99 BI posterior probability. This relationship was not found in either the NJ or the MP analyses, in which *HtcPI1* and *HtcPI2* are both grouped with basal angiosperms, though with low bootstrap support.

The two AG homologues from *Trochodendron*, *TraAG1* and *TraAG2*, are again near the base of the core eudicots (fig. 4) before the separation of the two major eu*AG* clades, *SHP*/*PLE* and *AG*/*FAR*. The two major eu*AG* lineages are both monophyletic, with posterior probabilities of 1.0. *TraAG1* and *TraAG2* are either paraphyletic branches in BI and MP analyses or form a sister group in NJ analysis (bootstrap 96% support; not shown in fig. 4).

The four *SEP* homologues from *Trochodendron* are separated into two groups, *TraMADS3* in the *LOFSEP* clade and *TraMADS1*, *TraMADS2*, and *TraMADS4* in the *SEP3* clade (fig. 5). *TraMADS3* is close to *PatSEP1* of *Pachysandra terminalis* (Buxaceae) but received low support. *TraMADS1*, *2*, and *4* form a monophyletic group with low to moderate support (fig. 5). This clade is the sister group to *GRCD1* of *Gerbera hybrida* (Asteraceae) in BI phylogeny but is not found in NJ or MP analysis (data not shown).

Expression of ABCE and LEAFY *Homologues in* Trochodendron

RT-PCR was used to examine the expression patterns of floral identity gene homologues among different organs, and the results using 30 and 35 cycles of PCR are shown in figure 6. Although signals in the 35-cycle reaction are likely saturated, as in *TraActin*, we presented these results here in order to magnify the potential RT-PCR products for the material. The results of the 30-cycle RT-PCR were used to infer the expression patterns of the identified genes and are summarized in table 2. There are a few inconsistencies in the results from repeated RT-PCRs, which are mostly in reactions for bracts,

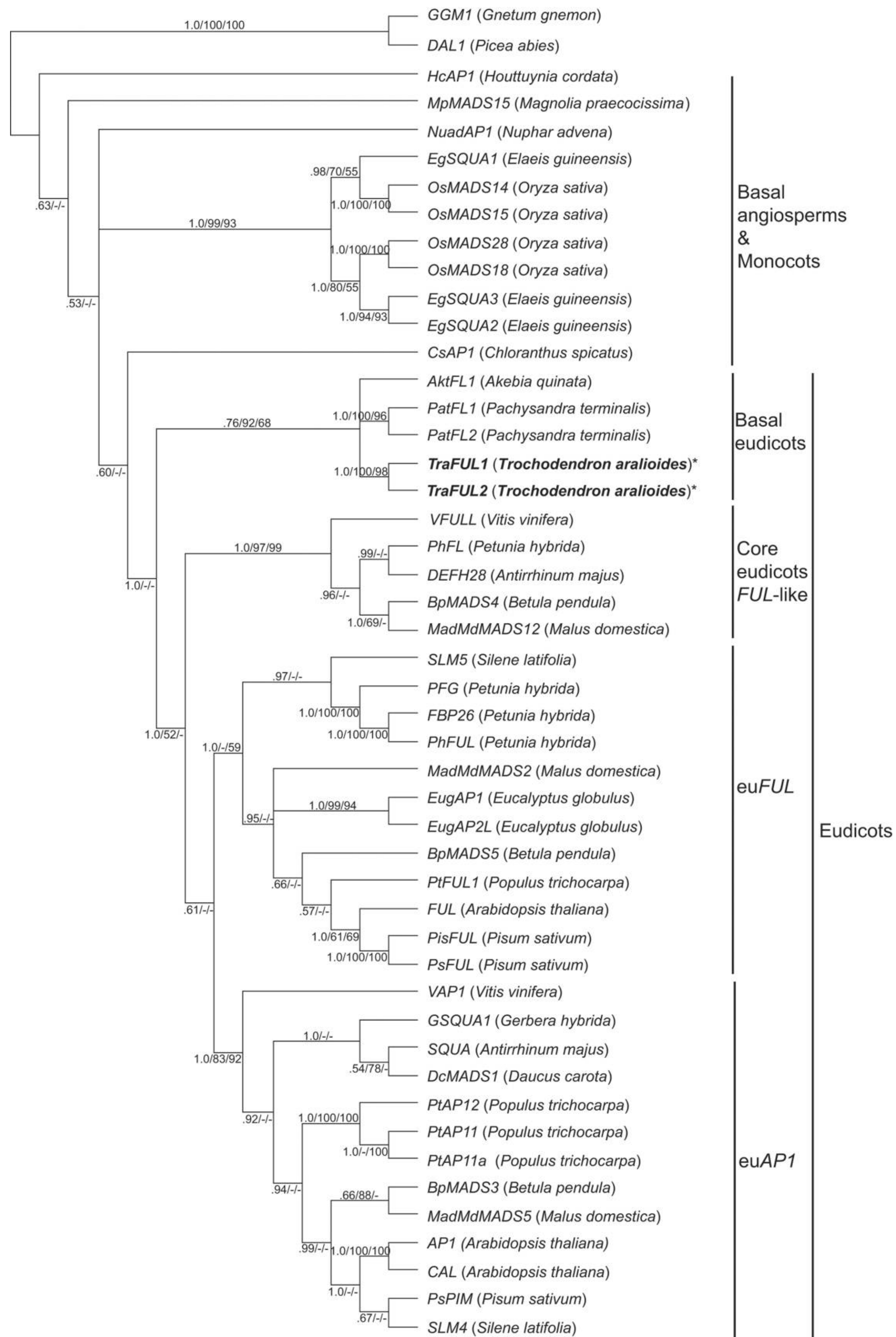

Fig. 2 Phylogenetic tree of A-class genes based on Bayesian inference (BI) analysis. *GGM1* and *DAL1* are used as outgroups. Numbers on the branches are posterior probabilities from BI, followed by bootstrap supports from neighbor-joining and maximum-parsimony analyses.

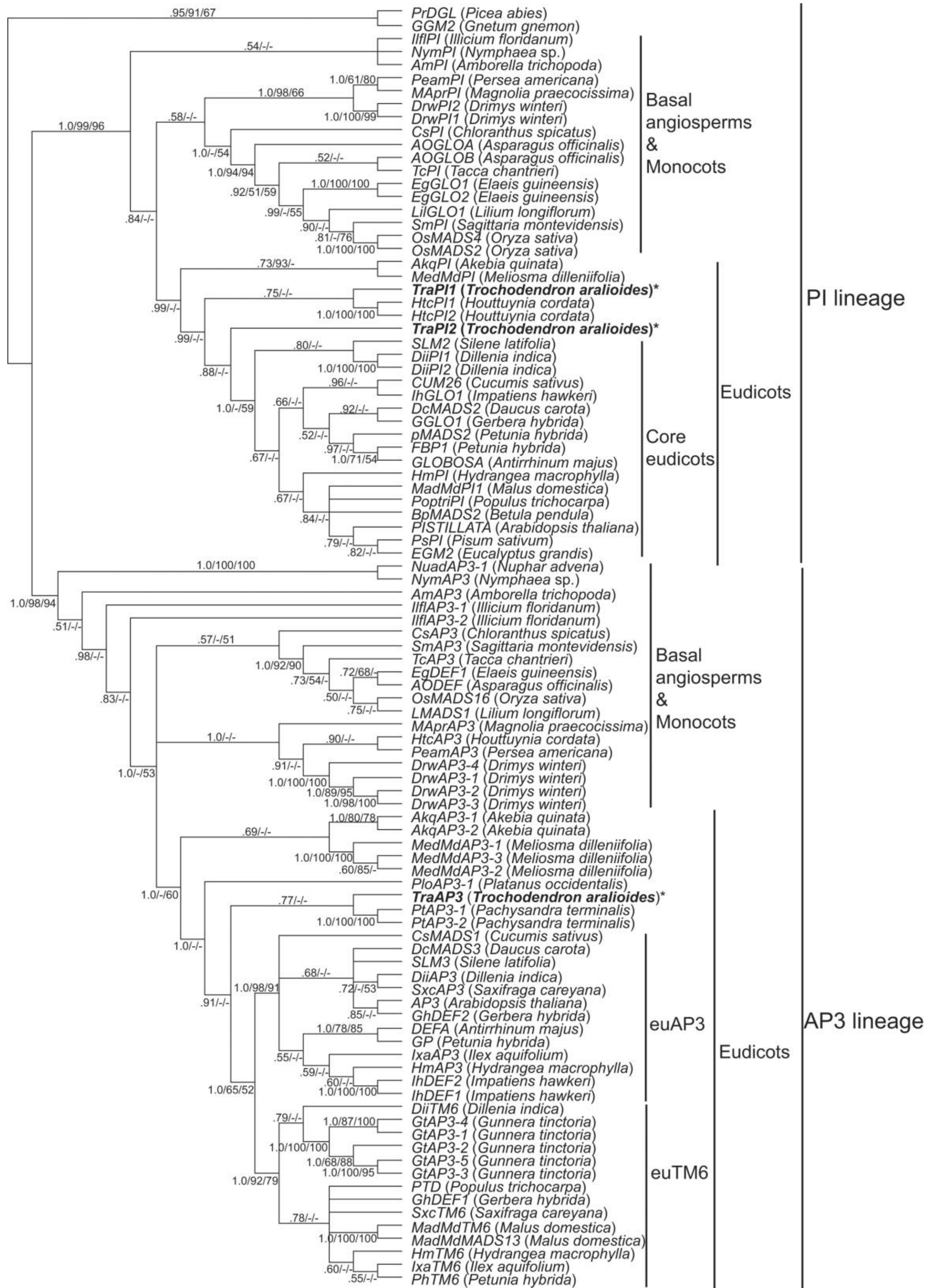

Fig. 3 Phylogenetic tree of B-class genes based on Bayesian inference (BI) analysis. *PrDGL* and *GGM2* are used as outgroups. Numbers on the branches are posterior probabilities from BI, followed by bootstrap supports from neighbor-joining and maximum-parsimony analyses.

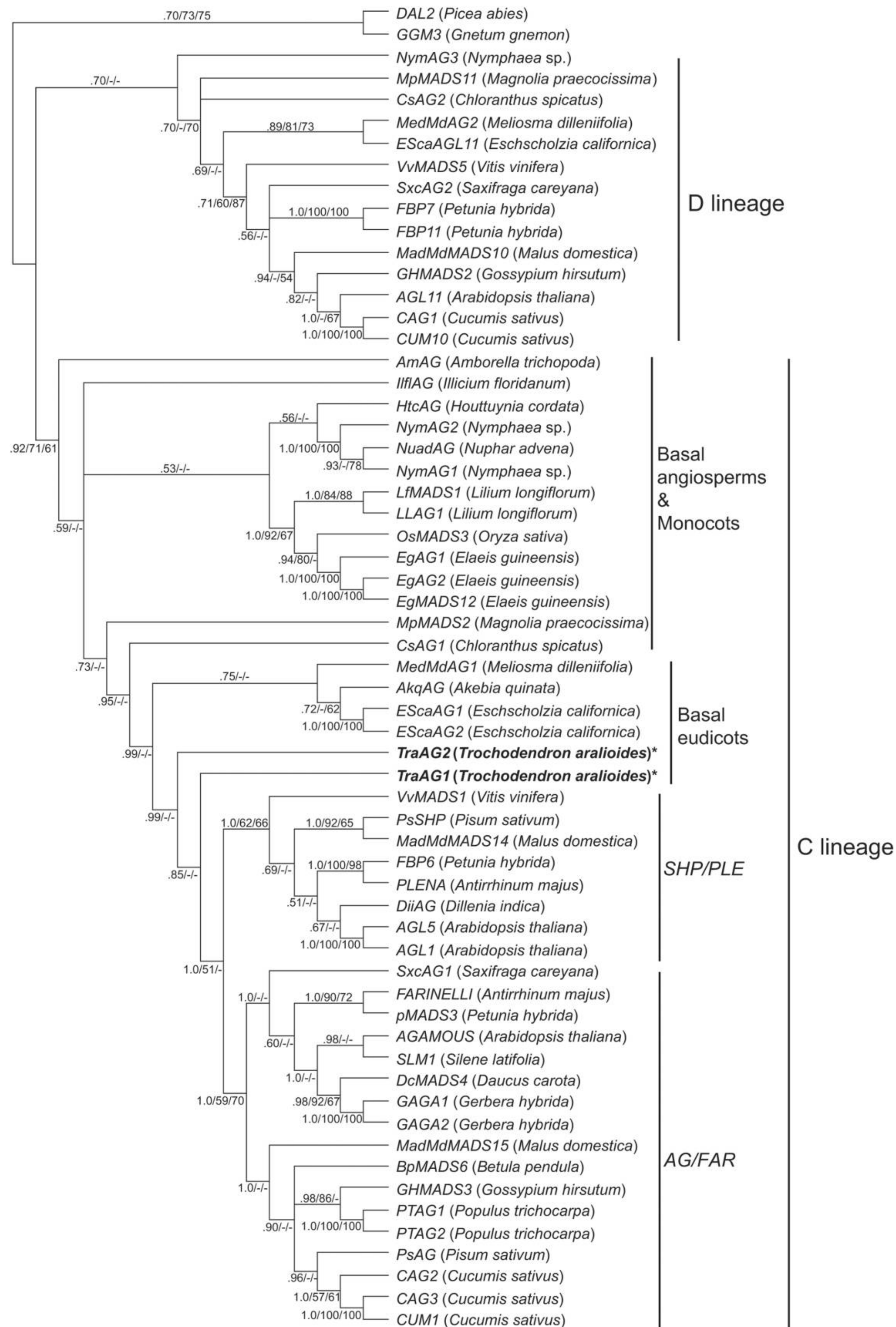

Fig. 4 Phylogenetic tree of C- and D-class genes based on Bayesian inference (BI) analysis. *DAL2* and *GGM3* are used as outgroups. Numbers on the branches are posterior probabilities from BI, followed by bootstrap supports from neighbor-joining and maximum-parsimony analyses.

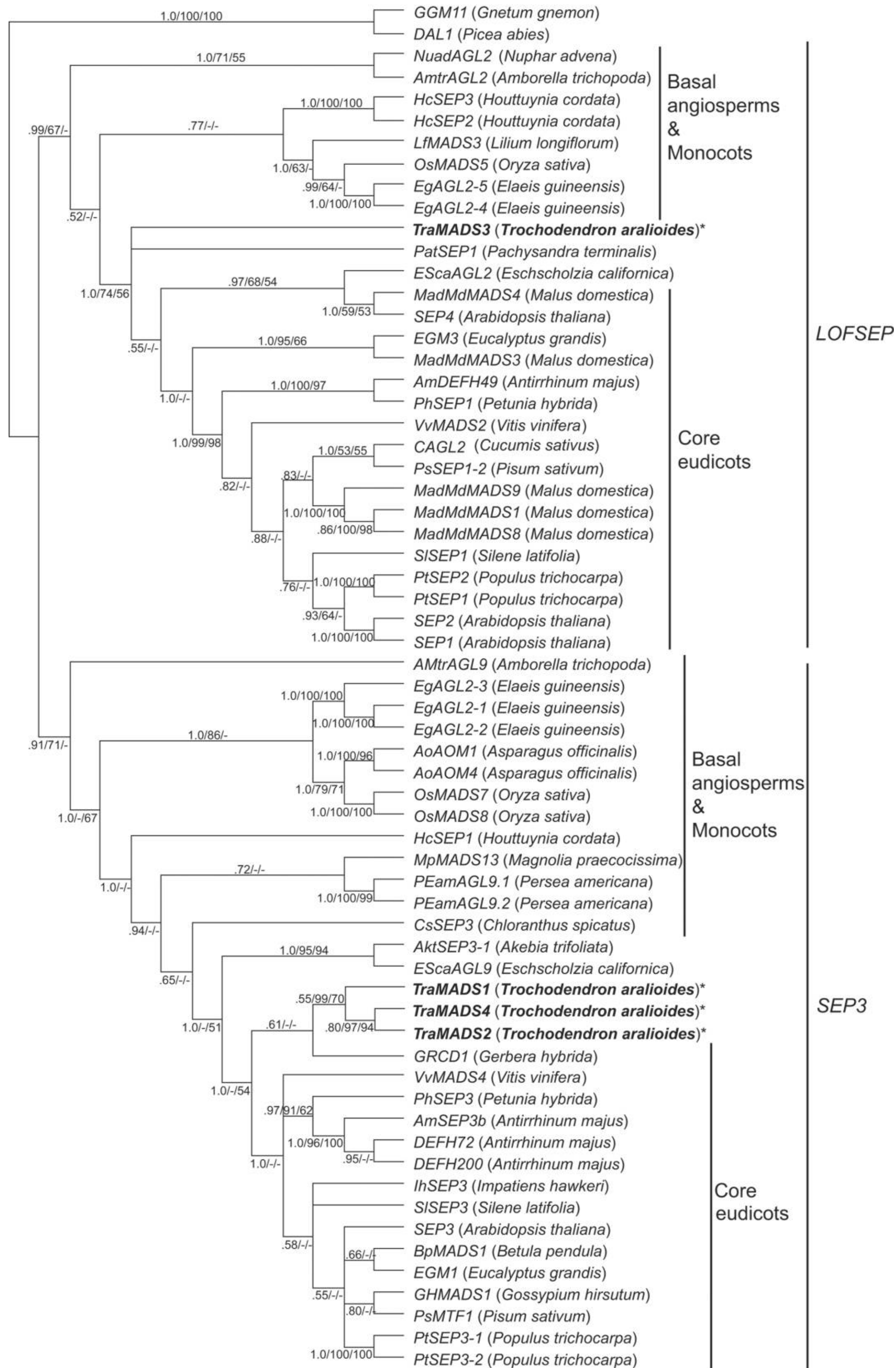

Fig. 5 Phylogenetic tree of *SEP* homologues based on Bayesian inference (BI) analysis. *DAL1* and *GGM11* are used as outgroups. Numbers on the branches are posterior probabilities from BI, followed by bootstrap supports from neighbor-joining and maximum-parsimony analyses.

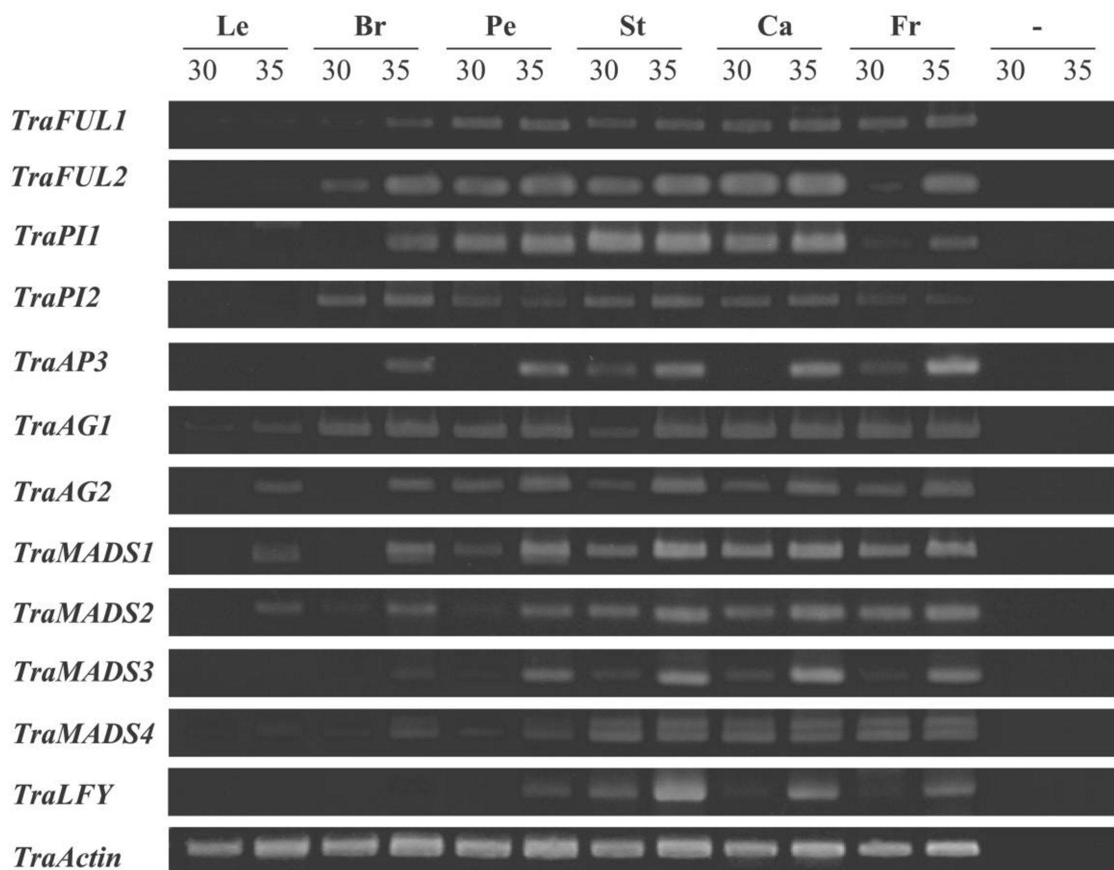

Fig. 6 Reverse transcriptase PCR of floral organ identity gene homologues and *LFY* homologue, showing expression in different organs; both 30- and 35-cycle reactions are shown. The portion marked "*Ca*" (carpels) contains floral parts without stamens and pedicels; i.e., it contains the scalelike structure, presumably as reduced perianth. The TraActin gene is used for internal control. *Le* = leaves; *Br* = bracts; *Pe* = pedicels; *St* = stamens; *Ca* = "carpels"; *Fr* = fruits; hyphen = negative control.

i.e., in *TraFUL2*, *TraPI2*, *TraAG1*, and *TraMADS4* (table 2). We did not perform a third 30-cycle reaction because of the limited plant material at hand. However, the four genes all show moderate expression in 35-cycle RT-PCR, which was repeated at least four times. Further confirmation is necessary in the future.

In addition to the internal control (*TraActin*), *TraFUL1* and *TraAG1* are the only genes expressed in all organs of flowers, bracts, fruits, and leaves, whereas expression of the other genes is mostly restricted to floral parts (table 2). *TraFUL2* and *TraPI2* have similar expression patterns, expressed in bracts, pedicels, stamens, "carpels," and, weakly, fruits but absent from leaves. *TraPI1* is expressed mostly in pedicels, stamens, and "carpels" and is absent from other parts. In comparison, *TraAP3* expression is restricted to stamens but occurs weakly in other floral parts in 35-cycle reactions. *TraAG1* is ubiquitously present, but the expression is stronger in "carpels" and fruits. *TraAG2* is expressed in pedicels, stamens (low level), "carpels," and fruits. *TraMADS1*, *TraMADS2*, and *TraMADS4* all have patterns similar to that of *TraAG2*, except that *TraMADS2* and *TraMADS4* have weaker expression in pedicels. *TraMADS3* is weakly expressed in stamens and "carpels" and is barely detectable in pedicels and fruits.

The results of RT-PCR show quite different patterns when the reaction was prolonged to 35 cycles of PCR. Almost all of the identified MADS-box genes showed strong signals in floral parts, bracts, and fruits (fig. 6; also table 2). *TraFUL1*, *TraAG1*, *TraAG2*, *TraMADS1*, and *TraMADS2* all show expression in leaves for 35-cycle RT-PCR.

We also examined the expression of the *LFY* homologue in *Trochodendron*, *TraLFY*, which is restricted to stamens (fig. 6) and has signals in pedicels, "carpels," and fruits under 35-cycle reaction. No signal of *TraLFY* was detected in leaves or bracts.

Morphological Observations

The inflorescence of *Trochodendron* develops at the shoot apex (fig. 1), and the flowering buds are very similar to the vegetative buds in outer appearance. The inflorescence is embedded in a series of bracts (fig. 1*A*). They are greenish when young, turn yellowish to white when mature, and drop before the maturation of flowers. These bracts enclose a series of thinner and slightly transparent bracts, which are the

Table 2

Summary of Expression Patterns of A-, B-, C-, and E-Class Homologues Identified from *Trochodendron* Based on 30-Cycle Reverse Transcriptase PCR

	Leaves	Bracts	Pedicels	Stamens	Carpels	Fruits
TraFUL1	+	+[a]	++	++	++	++
TraFUL2	−	+/−[a]	+++	+++	+++	+[a]
TraPI1	−	−[a]	+/++/+++	+++	+/++/+++	−[b]
TraPI2	−	++/−[a]	++	++	++	+
TraAP3	−	−[a]	−[b]	++	−[a]	+[a]
TraAG1	+	++/−[a]	++	+[a]	+++	+++
TraAG2	−[a]	−[a]	++	+[a]	++	++
TraMADS1	++/−[a]	−[a]	++	+++	+++	+++
TraMADS2	−[b]	+[a]	+[a]	++	++/++/−[a]	++
TraMADS3	−	−[b]	+[a]	+[a]	+[a]	+[a]
TraMADS4	+/+/−[b]	+/+/−[b]	+/−[b]/−[b]	+++	++	++/++/+

Note. Expression levels are indicated as follows: minus sign = undetectable; plus sign = barely detectable; two plusses = intermediate in terms of detection; three plusses = strongly expressed, similar to *TraActin* expression. Multiple symbols separated by slashes indicate inconsistent results from repeated reactions.

[a] Signals with intermediate strength in 35-cycle reactions.

[b] Signals weakly in 35-cycle reactions.

subtending bracts of the flowers, here named floral bracts or pherophylls (*PE* in fig. 1).

The terminal flower has some large scalelike structures, which are metaxyphylls (fig. 7*A*). A pair of prophylls can be seen at the base of each flower (*PR*; fig. 1*B*; fig. 7*B*–7*I*). Sometimes they are elongated to up to twice of the length of a flower (in protogynous flowers; fig. 7*D*), but usually they become shorter toward the distal part of the inflorescence. The prophylls are more distinct in the protogynous flowers than in the protandrous flowers. There are a few scales near the bases of stamens, but the number of scales varies between protandrous and protogynous flowers and among the flowers at different positions within the inflorescence. They are less distinct in the protandrous flowers than in the protogynous flowers (fig. 7*F*–7*I* vs. fig. 7*B*–7*E*). In the basalmost flowers of protogynous individuals, there are eight or more scales (asterisks in fig. 7*E*), whereas the scales are almost invisible in the upper lateral flowers of protandrous individuals (asterisks in fig. 7*I*).

The epidermal cell morphology shows quite interesting patterns (fig. 8). The adaxial epidermal cells on bracts and flower pherophylls are flat at both young and mature stages, and the cells are more regularly arranged compared to the epidermal cells in leaves (fig. 8*A*–8*D*). The abaxial epidermal cells are papillate in bracts and flower pherophylls (insets in fig. 8*B*–8*D*). The papillate morphology becomes more distinct when organs achieve maturity, sometimes showing striation or ridges on the surface of epidermal cells. There are no stomata on either side of the pherophyll surface.

Different types of cuticle modification on epidermal cells are seen among floral organs (fig. 8*E*–8*L*). The epidermal cells of prophylls are conical when young (fig. 8*E*), and the surface striations become more distinct toward maturity (fig. 8*F*). Such irregularly cuticular sculpturing can also be seen all over the epidermal cells on the receptacle area and scales (not shown). Stomata are commonly observed on prophylls and receptacles (fig. 8*E*, 8*F*). In filaments and pedicels, the cuticle is sculptured, with striation parallel to the axis of filaments and pedicels (fig. 8*G*, 8*L*). The epidermal cells of anthers (fig. 8*H*) and gynoecium (fig. 8*I*–8*K*) are also conical with cuticular sculpturing but are more irregular than those of the receptacle.

Discussion

MADS-box Homologues

We obtained two A-class, three B-class, two C-class, and four E-class homologues from *Trochodendron aralioides*, including one published in a recent paper (Kramer et al. 2006). The results of phylogenetic analyses show that these floral organ identity genes group with the respective classes of the MADS-box genes from other angiosperms. In addition, the C-terminal region of each floral organ identity gene from *T. aralioides* contains conserved signature motifs corresponding to A-, B-, C-, and E-class genes (Vandenbussche et al. 2003), suggesting that they might maintain similar functions as in other plants.

A major duplication event for floral ABC-class genes at the base of the core eudicot lineages has been suggested by recent studies (Kramer et al. 1998, 2004; Litt and Irish 2003). Such duplication has been shown to occur after the branching of *Trochodendron*, based on *AP3* gene phylogeny (Kramer et al. 2006). The duplication was immediately followed by a frameshift mutation that occurred in one of the copies that gave rise to the eu*AP3* lineage (Kramer et al. 2006). This study demonstrates that this scenario is also supported by A- and C-class gene phylogenies, and all *Trochodendron* homologues show preduplication forms of floral identity genes, i.e., typical paleotypes of motifs of these gene products (*sensu* Irish 2003; Vandenbussche et al. 2003) (fig. 9). In contrast, there are two *TraFUL* and two *TraAG* homologues identified from *T. aralioides*, and they form sister groups in some of the respective phylogenies. This could reflect an ancient duplication of a partial or whole chromosome within the *Trochodendron* lineage. Indeed, *T. aralioides* has been suggested as a tetraploid of n=19

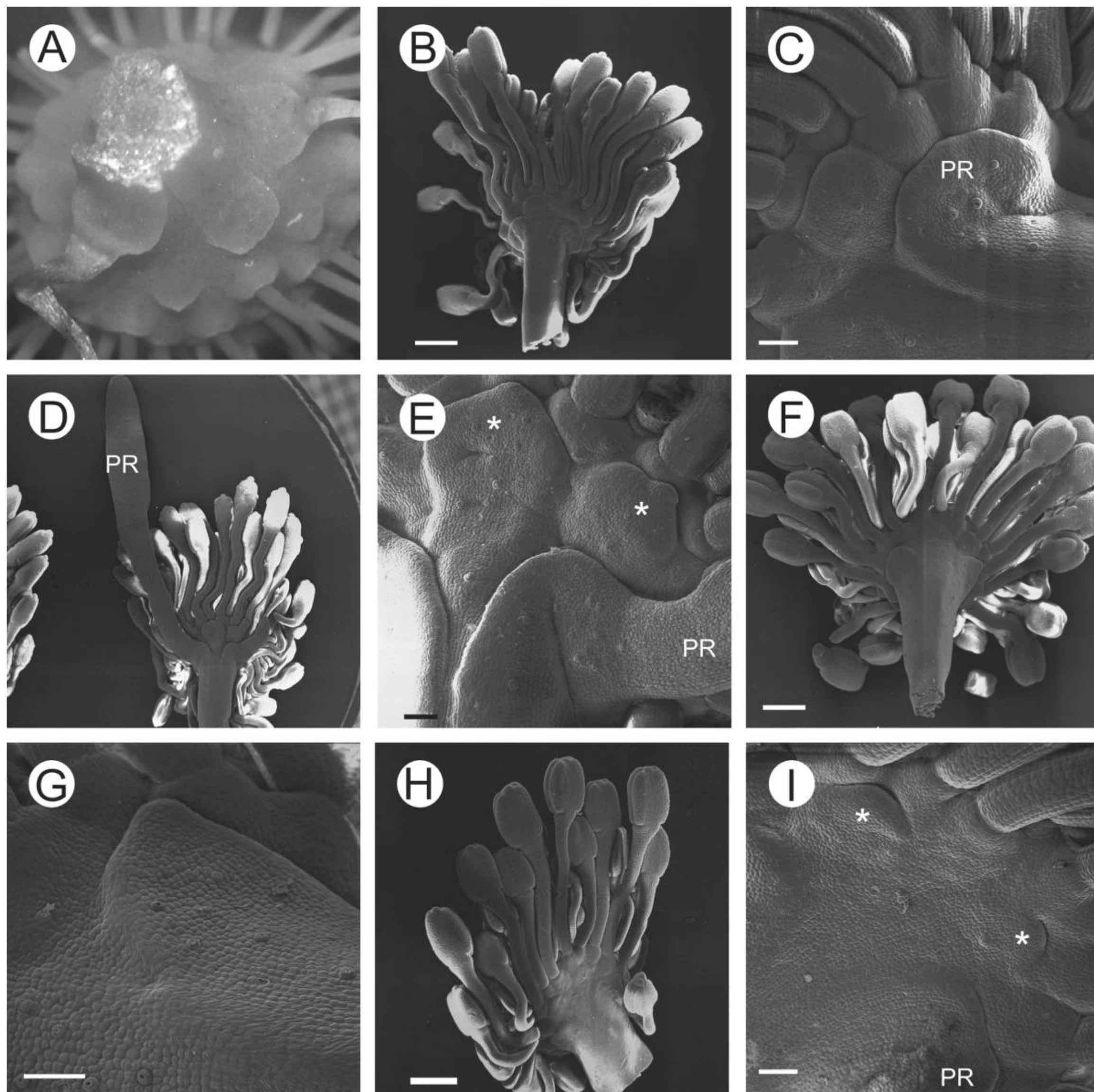

Fig. 7 Morphology of flowers, showing bracts and putative perianth of *Trochodendron*. *A*, Terminal flower viewed from below; numerous bractlike metaxyphylls are seen. *B–E*, Protogynous flowers from an inflorescence bud. *B*, Abaxial view of a lateral flower from upper portion of the inflorescence (bar = 500 μm). *C*, Close-up of *B*, showing a large prophyll (*PR*) in the middle (bar = 100 μm). *D*, Abaxial view of a lateral flower from basalmost part of the inflorescence, showing two large prophylls (*PR*) (bar = 1 mm). *E*, Enlargement of *D*, showing distinct scales (asterisks) between stamens and prophylls (bar = 100 μm). *F–I*, Protandrous flowers from an inflorescence bud. *F*, Abaxial view of a lateral flower from upper portion of the inflorescence, showing two prophylls at the base (bar = 500 μm). *G*, Enlargement of *F*, showing a large prophyll (bar = 100 μm). *H*, Abaxial view of a lateral flower from lower part of the inflorescence (bar = 500 μm). *I*, Enlargement of *H*, showing small residues of scales (asterisks) between stamens and prophylls (*PR*) (bar = 100 μm).

(Soltis and Soltis 2000), supporting this ancient polyploidization scenario. However, it remains unclear whether the duplication was due to only one whole-genome duplication event or to multiple gene duplication events (Irish 2006; Kramer and Zimmer 2006). Moreover, the B-class gene phylogeny shows alternative positions of the two identified *TraPI* copies, either as sister groups or in different clades on the branch leading to the core eudicots. One of the copies, *TraPI1*, even grouped with *Houttuynia cordata* (Saururaceae) sequences (*HtcPI1* and *HtcPI2*) with low posterior probability in BI phylogeny (fig. 3). The latter case suggests an even earlier duplication in basal angiosperms, well before the origin of *Trochodendron*. The peculiar positions of *HtcPI1* and *HtcPI2* are further confirmed by additional sequences from *Houttuynia cordata* using *HcPI1* (AB089155) and *HcPI2* (AB089156), both by Ito et al. (obtained directly from GenBank). Nevertheless, these duplicated homologues may have evolved different functions, because there are minor differences of RT-PCR expression patterns between *TraPI1* and *TraPI2* (fig. 6). Southern blot analysis probed by *TraAP3* revealed that there might be more

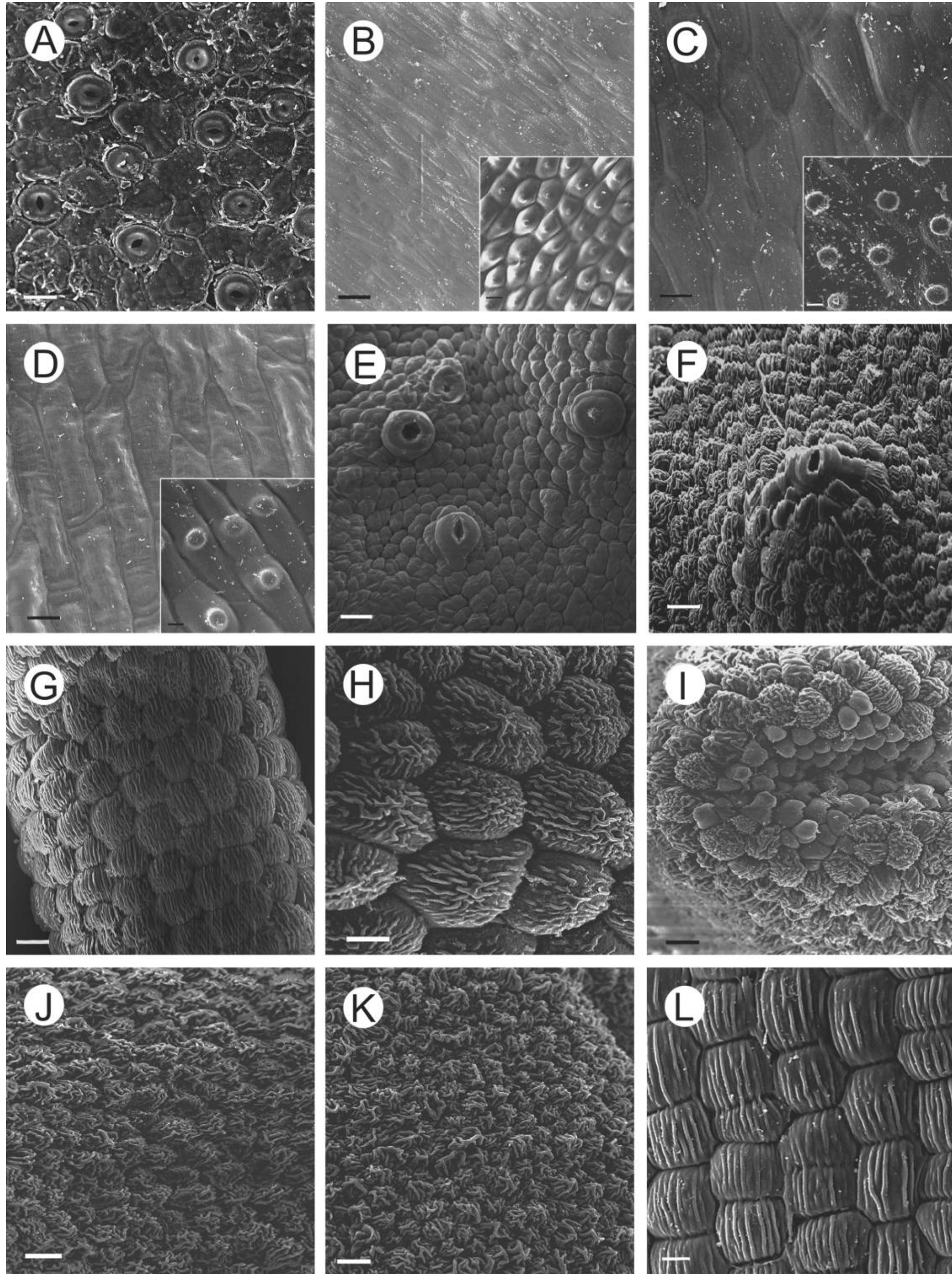

Fig. 8 Epidermal cells of leaves and floral parts of *Trochodendron*. *A*, Abaxial side of a leaf (bar = 20 μm). *B*, Adaxial (bar = 50 μm) and abaxial (inset; bar = 20 μm) epidermal cells of inflorescence bracts at young stage. *C*, Adaxial (bar = 20 μm) and abaxial (inset; bar = 10 μm) epidermal cells of inflorescence bracts at mature stage. *D*, Adaxial (bar = 20 μm) and abaxial (inset; bar = 10 μm) epidermal cells of flower-subtending pherophylls at mature stage. *E*, Epidermal cells of a prophyll from a young protogynous flower (bar = 20 μm). *F*, Epidermal cells of a prophyll from a mature protogynous flower, showing irregular striation (bar = 20 μm). *G*, Portion of a filament, showing parallel striation (bar = 20 μm). *H*, Surface of an anther (bar = 20 μm). *I*, Cells of a stigma (bar = 20 μm). *J*, Surface of a style, showing irregular cuticular sculpturing (bar = 20 μm). *K*, Surface of an ovary, also showing irregular sculpturing (bar = 20 μm). *L*, Surface of a pedicel, showing parallel striation (bar = 20 μm).

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A-lineage
FUL-like  TraFUL1 (Trochodendron)   RGSGGEEDGPRPQNRT-NTLIPPWMLRHMNE
          TraFUL2 (Trochodendron)   RGTGGEDEGSQPHNRT-NTLMPPWMLRHMNE
          OSMADS14 (Oryza)          AAGERIEDVAAGQPQHERIGLPPWMLSHING
          RbFL3 (Ranunculus)        SSGREDE-V-PQTQARPTILMPPWMV???
euFUL     FUL (Arabidopsis)         ERVGGENGGASSLTEP-NSLLPAWMLRPTTTNE
          PhFUL (Petunia)           AGDNGEVEGSSRQQPP-NTVMPPWMLRHLNG
euAP1     AP1 (Arabidopsis)         EDDPMAMR-NDLELTLEPVYNCNLGCFAA
          SQUA (Antirrhinum)        GEGANEDRRNELDLTLDSLYSCHLGCFAA

B-lineage
PI        TraPI1 (Trochodendron)    FAFRLQPMQ----PNLQ----DKK
          TraPI2 (Trochodendron)    FTFRVLPMQ----PNLQ----ERK
          CsPI (Chloranthus)        FIFRVQPIQ----PNLQ----QSK
          PMADS2 (Petunia)          FALRVQPMP----PNLH----ERM
          PI (Arabidopsis)          FGYSVQPIQ----PNLQ----EKIMSLVID
TM6/      TraAP3 (Trochodendron)    FAFRMQPGR----PNLH----DGGGYGSYDLRLA
paleoAP3  AmAP3 (Amborella)         FAYRMRPAE----GNIH----DRG-YGLNDLRLG
          TM6 (Petunia)             YAFRLQTLH----PNLQ----NGGGFGSRDLRLA
euAP3     AP3 (Arabidopsis)         YALRFHQNHHHYYPNHGLHAPSASDIITFHLLE
          DEF (Antirrhinum)         IALRLPTNHH---PTLH--SGGGSDLTTFALLE

C-lineage
AG        TraAG1 (Trochodendron)    FDSRNFLQVNLMEP-NHHYT---RQEQTALQLG
          TraAG2 (Trochodendron)    FDSRNFLQVNLMEP-NHHYS---RQEQTALQLG
          AG (Arabidopsis)          FDSRNYFQVAALQPNNHHYSSAGRQDQTALQLV
          FBP6 (Petunia)            YDARNFLPVNLLEP-NPHYS---RQDQTALQLV
          CsAG1 (Chloranthus)       FDGRNFLPVNLLGS-NHHQFS--HQDQTALQLG

E-lineage
LOFSEP    TraMADS3 (Trochodendron)  VGPDQITV--AAPGQNVNGFIPGWML
          SEP1 (Arabidopsis)        VCSEQITATTQAQAQPGNGYIPGWML
          VvMADS2 (Vitis)           NPAGSSQLSAPSNAQNVNGFIPGWML
SEP3      TraMADS1 (Trochodendron)  -QPD-------APEPSVSNYMPGWLA
          TraMADS2 (Trochodendron)  -QPDQIAV--MAPGPSVNNYMPGWLA
          TraMADS4 (Trochodendron)  -QHDQTAG--MAPGPSGNNYMAGWLA
          SEP3 (Arabidopsis)        -QGQQ-DG--MGAGPSVNNYMLGWLPYDTNSI
          PhSEP3 (Petunia)          -QNDPITV--GGAGPSVNNYMAGWLP
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Fig. 9 Representative predicted amino acid sequences of ABCE genes from *Trochodendron* and selected taxa. Only the C terminus is shown. Conserved motifs are boxed, as defined by previous studies for the *FUL*/*SEP* motif (Litt and Irish 2003), the *PI* and *TM6*/paleo-*AP3* motifs (Kramer et al. 1998), and the *AG* motif II (Kramer et al. 2004).

than five copies in the *Trochodendron* genome (J.-M. Hu, unpublished data), further complicating the situation.

The ABCDE model was derived from studies in two model systems, *Arabidopsis* and *Antirrhinum* (Coen and Meyerowitz 1991; Theissen and Saedler 2001). If the ABCDE model were to apply to *Trochodendron*, the lack of a perianth in *Trochodendron* might be due to the null function of A-class homologues. Unfortunately, evidence of true A-class function has never been demonstrated except in *Arabidopsis* (*AP1* and *AP2*) and *Antirrhinum* (*LIP1* and *LIP2*) (Keck et al. 2003; Ferrario et al. 2004). In other model plants, such as rice and *Petunia*, A function was not found for *AP1*/*FUL* homologues (Ferrario et al. 2004), thus limiting inference of A function in homologues from nonmodel organisms like *Trochodendron*. For this reason, even though we have successfully obtained the *AP1* homologues *TraFUL1* and *TraFUL2* from *Trochodendron*, we do not infer A function for them. Another A-function candidate, *AP2*, was not included in this study but could be crucial in the future research. In contrast, B and C functions are more conserved among B- and C-class gene homologues in angiosperms (Kramer and Irish 2000; Ferrario et al. 2004), so we expect at least some copies of *TraPI*, *TraAP3*, and *TraAG* to carry out B and C functions, respectively. The RT-PCR results show that *TraAP3* followed expected expression patterns, whereas *TraAG1* and *TraAG2* have extended expression from expected regions (stamens and carpels). Nonetheless, since genetic experiments are very difficult to conduct in woody perennials, evidence for functions of the identified ABCE genes in *Trochodendron* will rely indirectly on complementation or overexpression experiments in an *Arabidopsis* mutant background in the future.

Similar to the B-class gene evolution (Kramer et al. 2006), it has been proposed that a major duplication event occurred near the base of the core eudicots for the *AP1* lineage and gave rise to the *euAP1* and *euFUL* lineages in the core eudicots (Litt and Irish 2003). Although *euAP1* is consistently expressed in sepals and petals (Irish 2003), expression of *euFUL* homologues is usually restricted to carpels and bracts (Mandel and Yanofsky 1995; Gu et al. 1998; Müller et al. 2001). *TraFUL1* is expressed in all floral organs as well as in leaves, similar to the patterns of the *FUL*-like homologues from the basal angiosperms *Eupomatia* and *Magnolia* (Kim et al. 2005*a*, 2005*b*). *TraFUL2* is expressed in bracts, pedicels, stamens, and carpels. This indicates that restricting expression of *AP1*/*FUL* homologues to the outer two whorls of floral organs occurs only in the core eudicot lineage (Kim et al. 2005*b*).

TraAP3 is the only gene that has restricted expression in stamens, mostly consistent with the prediction from the ABCDE model. In contrast, *TraPI1* and *TraPI2* have expression extended to pedicels and carpels or bracts (*TraPI2*). All three genes show expression in 35-cycle RT-PCR among floral parts, suggesting that they might play a common role in these organs. According to previous studies, B-class genes of core eudicots are stably expressed in petals and stamens, but the B-class genes of basal eudicots and basal angiosperms sometimes do not follow this rule (Zahn et al. 2005*b*). For instance, *AP3* and *PI* homologues from Ranunculaceae are expressed throughout the petaloid perianth and the stamens, but some homologues are also expressed in carpels (Kramer et al. 2003). In *Eupomatia*, a member of the Eupomatiaceae (Magnoliales), the *AP3* homologues are also weakly expressed in the calyptras and leaves, while in *Magnolia*, the *AP3* homologue is expressed in spathaceous bracts (Kim et al. 2005*b*). The actual role of B-class homologues expressed outside of petals and stamens requires further study. In addition, it would be best to examine the protein expression patterns, because it is known that RNA expression patterns do not always correlate with protein expressions (e.g., Tzeng and Yang 2001). On the other hand, the role of B-class genes in upregulating genes for specifying conical and/or papillate cell formation might be the reason B-class homologues are expressed throughout all floral organs and bracts in *Trochodendron*. This is discussed in "Perianth in *Trochodendron*."

The *AG*-like homologues are usually expressed exclusively in stamens and carpels and play a specific role for C function (De Bodt et al. 2003; Irish 2003). However, the RT-PCR results showed that *TraAG1* and *TraAG2* from *Trochodendron* were also expressed in pedicels and that *TraAG1* is expressed in bracts and leaves. Based on the results, *TraAG1* and *TraAG2* may have redundant functions, but the purpose for the presence of *TraAG1* transcripts in leaves and bracts is unknown.

Based on the reconstructed phylogeny of E-class genes in this study, *TraMADS1*, *2*, and *4* belong to the *SEP3* clade (Malcomber and Kellogg 2005) or the *AGL9* clade (Zahn et al. 2005*a*). All three copies have similar expression patterns, which differ minorly in leaves, bracts, and pedicels. *TraMADS3* belongs to the *LOFSEP* clade (Malcomber and Kellogg 2005) (or the AGL2/3/4 clade; Zahn et al. 2005*a*)

and is expressed mostly in stamens and carpels, which is consistent with homologues in other species. We found products of two different sizes on some RT-PCRs for *TraMADS4*. Both products were sequenced, but the coding sequences of the two products are identical; they differ only in the 3′ UTR. Our results are consistent with the general finding of E-class homologues that are usually present in multiple copies in flowering plants and may have redundant or very diverse functions, as described in recent reviews (Malcomber and Kellogg 2005; Zahn et al. 2005*a*).

We detected many gene expressed in the "carpel" and fruit portions of the RNA preps. The "carpel" portion, indeed, not only contains carpels but also includes prophylls and the scalelike structure, the putative reduced perianth. Therefore, the expression of B-class genes (*TraPI1* and *TraPI2*) in the "carpel" is not unexpected. Since those parts are not separable for RT-PCR, the exact expression pattern will require further work of *in situ* hybridization.

The expression of *TraLFY* is present only in stamens and is weak in carpels, which is reasonable because *LFY* has been shown to be the upstream gene of B- and C-class genes in *Arabidopsis* (Busch et al. 1999; Lamb et al. 2002). RT-PCR showed that *TraLFY* might be weakly expressed in pedicels but that the peculiar expression pattern is not uncommon. Expression patterns of *LFY* homologues are quite diverse in flowering plants. The expression of the *LFY* orthologues *FLO* of *Antirrhinum majus* and *HbLFY* of the rubber tree (*Hevea brasiliensis*) are confined to floral primordia and are usually not present in vegetative tissues (Coen et al. 1990; Dornelas and Rodriguez 2005). In comparison, the orthologous gene *NFL* of *Nicotiana* is expressed constitutively in both leaf and floral primordia (Kelly et al. 1995), whereas *EcFLO* of the California poppy (*Eschscholzia californica*) is expressed exclusively in the shoot apical meristem (Busch and Gleissberg 2003).

Taken together, our findings demonstrate that the genes corresponding to the floral organ identity genes are retained in *Trochodendron* despite its reduced floral morphology. The expression patterns of the identified genes are limited comparable to the model organisms. The duplicated paralogous genes in *T. aralioides* are likely functionally divergent because they all have different expression patterns. Further functional studies would help to elucidate the roles they play in *Trochodendron*.

Frameshift Mutation of TraAP3

During the screening of *TraAP3*, a shortened transcript was found that showed a premature stop codon on exon 7 in five of the 15 clones screened. After careful examination of the clones from genomic sequences, it was found that the two transcripts are formed by a slippage splicing at the exon 6 and 7 junction (fig. 10). There are two AG dinucleotide sequences at the end of the intron 6 and the beginning of exon 7 that might have caused the confusion in RNA splicing. In a normal transcription, the first AG is recognized, but alternatively, the second AG is recognized and causes the transcript to be four nucleotides short at the junction, consequently resulting in a premature stop codon in the downstream sequence. The misspliced product was found in five independent clones and lacks the majority of the C terminus in the deduced amino acid sequences, but the significance of this product is unknown.

Perianth in Trochodendron

The residual scales found at the base of the stamens are distinct from the two prophylls in terms of shape and location, especially in the protogynous *Trochodendron* flowers. This is congruent with the previous observation that there is a lag in the development (plastochron) between the formation of the two prophylls and of the residual phyllotaxis (Endress 1986). The number of scales are higher on the lower lateral flowers than on the upper lateral flowers, similar to the observation by Endress (1986). In addition, we found more scales between prophylls and stamens in protogynous flowers (more than five) than in protandrous flowers (usually zero to two). The size and shape of the scales can be variable, from as large as the smaller prophylls to as small as a small, bumplike scale. The morphology of the residual scales on the receptacles of protogynous flowers is quite stable among individual flowers,

Fig. 10 Alternative splicing in *TraAP3* transcripts found in the cDNA screening. Partial genomic sequences of exons 6 and 7 and the intron between them are shown in the middle. The normal *TraAP3* transcript (top) is spliced using the first underlined AG. The alternative *TraAP3* transcript (bottom) is spliced using the second underlined AG, which causes a premature stop codon in the downstream sequence.

although the number of scales is usually variable. In contrast, the scales are usually fewer than five in protandrous flowers. Therefore, there might be a correlation between the presence of residual scales and the protogynous flower type.

In general, the conical or papillate epidermal cells are often restricted to petals and stigma in flowering plants, and their function is likely to trap incident light and scatter emergent light, consequently making petals brighter or darker (Kay et al. 1981), or to affect the microenvironment by enhancing scent or intrafloral temperature (Glover and Martin 2002). A special cuticular accumulation on the conical cell surface is commonly present in plants, and it has been suggested that this striation structure can also enhance light reflection (Kay et al. 1981). It has been demonstrated in *Antirrhinum* that the MIXTA protein, which belongs to the MYB transcription family, directly controls the formation of the conical epidermal cells on the inner surface of the petal (Glover and Martin 1998; Martin et al. 2002). *AmMYBML1* (*Antirrhinum majus MYB MIXTA LIKE1*), another MYB transcription family gene, has been shown to be involved in conical cell formation in the hinge region (Perez-Rodriguez et al. 2005). Furthermore, *MIXTA* and *AmMYBML1* promoters have been suggested as being under the direct control of B-class genes (Perez-Rodriguez et al. 2005). We found the conical and/or papillate cell type to be present in all floral organs as well as in bracts of *Trochodendron*; this might explain why B-class homologues of *Trochodendron* (*TraPI1* and *TraPI2*) have extended expression in carpels and bracts/pherophylls.

This hypothesis is questionable because *TraAP3* expression is absent from organs outside stamens. B-class gene products usually form heterodimers for proper function in eudicots (Winter et al. 2002; Kaufmann et al. 2005). The absence of *TraAP3* from other floral organs will limit this inference, unless TraPIs can form homodimers for proper function. However, this is not impossible, because homodimerization of PI homologues have been demonstrated in lily and *Gnetum* (Winter et al. 2002). The exact time for the occurrence of obligate heterodimerization of B-class proteins in angiosperms is still unclear, but it has been demonstrated that in basal eudicot *Akebia trifoliata*, AP3 and PI homologues do not form homodimers (Shan et al. 2006). Another possibility is that there is an uncharacterized *AP3* homologue in *Trochodendron* that contributes to specifying other parts of floral organs. This is plausible since there appeared to be more copies in the genome based on Southern blot data (J.-M. Hu, unpublished data). It would be helpful to determine the protein-protein interaction profiles in *Trochodendron*, as well as in other basal eudicots.

In addition, the papillate cell type on bracts is distinct from that on the floral organs. The papillate cells on the bracts form at an early stage, without cuticular accumulation. In contrast, the conical cells of other floral organs form only at a mature stage, with varying degrees of cuticular deposition. One explanation is that the genetic control of the papillate cells of bracts is different from that of the conical epidermal cells of floral organs.

The absence of a distinct perianth in *Trochodendron* associated with abundant pollens in sedimentary fossils has led to interpretations that the plant is wind pollinated. However, it has been reported that several kinds of insects are attracted to the flowers and serve as pollinators (Endress 1986; Chaw 1992). Furthermore, the nectary on the abaxial side of the gynoecium is thought to attract pollinators (Endress 1986), and it is clearly the reward for some flower visitors of *T. aralioides*, such as *Ctenacroscelis* sp. (J.-M. Hu, personal observation). It is possible that *Trochodendron* uses the whole flower and inflorescence as an attracting agent by distributing conical and papillate cells on all floral organ surfaces instead of using a distinct perianth. Such a strategy might be a compensation for lacking showy petals. The approach of using a whole inflorescence as an attracting unit can be found in many other plants, such as *Medinilla* (Melastomataceae) and *Chione* (Rubiaceae) (Taylor 2002), that show colorful sepals and pedicels. However, this cannot explain the function of papillate cells on the bracts of *Trochodendron*. The papillate cells appear in all bracts, but the inflorescence bracts usually drop before anthesis and thus cannot serve as attractants for pollinators. Papillate epidermis without clear function is also found in several species, e.g., on the adaxial side of a mature leaf and anther surface of *Pentadiplandra braszeana* (Pentadiplandraceae) (Ronse de Craene 2002) and the anther filament of *Trichocline* (Asteraceae). In contrast, papillate cells can be secretory, as demonstrated in Acoraceae (Buzgo and Endress 2000) and *Cyrilla racemiflora* (Cyrillaceae) (Dute et al. 2004). Further examinations are required into the significance of the papillate epidermis in *Trochodendron*.

In conclusion, the floral homeotic gene phylogenies all showed that *Trochodendron* is very close to the base of core eudicots and that the sequences are paleotypes from before the major duplications of ABC-class genes. The results thus support the interpretation that the perianth of *Trochodendron* was secondarily lost rather than primitive. The data also suggest that some aspects of petal identity, e.g., papillate cells, have been retained and might be important for pollinator attraction.

Acknowledgments

We thank Cheng-Chiang Wu and the Hu lab members for helping with collections of plant materials and Nicole Maturen for help in editing the manuscript. We also thank Wann-Neng Jane for help with SEM observation. We would like to thank three anonymous reviewers for their comments on the manuscript. J.-M. Hu is supported by a grant from National Science Council, Taiwan (NSC 92-2621-B-002-022).

Appendix

Table A1

Voucher Information for A-, B-, C-, and E-Class Genes Used in This Study

Taxa	*AP1* lineage	*PI* lineage	*AP3* lineage	*AG* lineage	*SEP* (*AGL2/3/4*) lineage
Gymnosperms:[a]					
Coniferales/Pinaceae:					
Picea abies (L.) H. Karst.	*DAL1* (X80902; Tandre et al. 1995)	*PrDGL* (AF120097; Mouradov et al. 1999)		*DAL2* (X79280; Tandre et al. 1995)	
Gnetales/Gnetaceae:					
Gnetum gnemon L.	*GGM11* (AJ132217; Winter et al. 1999)	*GGM2* (AJ132208; Winter et al. 1999)		*GGM3* (AJ132219; Winter et al. 1999)	
Angiosperms:					
Basal angiosperms/monocots:					
Alismatales/Alismataceae:					
Sagittaria montevidensis Cham. & Schltdl.	NA	*SmPI* (AF230712; Kramer and Irish 2000)	*SmAP3* (AF230705; Kramer and Irish 2000)	NA	NA
Amborellales/Amborallaceae:					
Amborella trichopoda Baill.	N/A	*AmPI* (AB154842; Aoki et al. 2004)	*AmAP3* (AB154845; Aoki et al. 2004)	*AmAG* (AY936231; Kim et al. 2005*b*)	*AmtrAGL2* (AY936232; Kim et al. 2005*b*) *AMtrAGL9* (AY850178; Zahn et al. 2005*a*)
Arecales/Arecaceae:					
Elaeis guineensis Jacq.	*EgSQUA1*, *EgSQUA2*, *EgSQUA3* (AF411840–AF411842; Adam et al. 2006)	*EgGLO1*, *EgGLO2* (AF227195, AF411848; Adam et al. 2006)	*EgDEF1* (AY739700; Adam et al. 2006)	*EgAG1*, *EgAG2* (AY739698, AY739699; Adam et al. 2006) *EgMADS12* (AJ581463; Alwee et al. 2006)	*EgAGL2-1*, *EgAGL2-2*, *EgAGL2-3*, *EgAGL2-4*, *EgAGL2-5* (AF411843–AF411847; Adam et al. 2006)
Asparagales/Asparagaceae:					
Asparagus officinalis L.	NA	*AOGLOA*, *AOGLOB* (AB103465, AB103466; Park et al. 2004)	*AODEF* (AB094964; Park et al. 2003)	NA	*AoAOM1* (AY382400; Caporali et al. 2000) *AoAOM4* (AY383560; Losa et al. 2004)
Canellales/Winteraceae:					
Drimys winteri J. R. Forst. & G. Forst.	NA	*DrwPI-1*, *DrwPI-2* (AY436727, AY436728; Stellari et al. 2004)	*DrwAP3-1*, *DrwAP3-2*, *DrwAP3-3*, *DrwAP3-4* (AY436722–AY436725; Stellari et al. 2004)	NA	NA
Chloranthales/Chloranthaceae:					
Chloranthus spicatus (Thunb.) Makino	*CsAP1* (AY316311; Li et al. 2005)	*CsPI* (AF230710; Kramer and Irish 2000)	*CsAP3* (AF230701; Kramer and Irish 2000)	*CsAG1*, *CsAG2* (AY464100, AY464099; Kramer et al. 2004)	*CsSEP3* (AY397763; Li et al. 2005)
Dioscoreales/Taccaceae:					
Tacca chantrieri André	NA	*TcPI* (AF230713; Kramer and Irish 2000)	*TcAP3* (AF230706; Kramer and Irish 2000)	NA	NA

Table A1

(*Continued*)

Taxa	*AP1* lineage	*PI* lineage	*AP3* lineage	*AG* lineage	*SEP* (*AGL2/3/4*) lineage
Austrobaileyales/Illiciaceae:					
Illicium floridanum J. Ellis	NA	*Il.fl.PI* (AY936224; Kim et al. 2005*b*)	*Il.fl.AP3.1*, *Il.fl.AP3.2*, *Il.fl.AP3.3* (AY936225–AY936227; Kim et al. 2005*b*)	*Il.fl.AG* (AY936229; Kim et al. 2005*b*)	NA
Laurales/Lauraceae:					
Persea americana Mill.	NA	*Pe.am.PI* (AY337738; Kim et al. 2004*b*)	*Pe.am.AP3* (AY337748; Kim et al. 2004*b*)	NA	*PEamAGL9.1*, *PEamAGL9.2* (AY850185, AY850186; Zahn et al. 2005*a*)
Liliales/Liliaceae:					
Lilium longiflorum Thunb.	NA	*LilGLO1* (DQ437527; X. Wu et al., unpublished manuscript)[b]	*LMADS1* (AF503913; Tzeng and Yang 2001) *LfMADS1* (AY829227; Liu et al. 2004)	*LLAG1* (AY500376; Benedito et al. 2004)	*LfMADS3* (AY826062; Liu et al. 2004)
Magnoliales/Magnoliaceae:					
Magnolia praecocissima Koidz.	*MpMADS15* (AB050657; M. Ito et al., unpublished manuscript)[b]	*MAprPI* (AY821779; Kim et al. 2005*a*)	*MAprAP3* (AY337752; Kim et al. 2005*a*)	*MpMADS2* (AB050644; M. Ito et al., unpublished manuscript)[b] *MpMADS11* (AB050653; M. Ito et al., unpublished manuscript)[b]	*MpMADS13* (AB050655; M. Ito et al., unpublished manuscript)[b]
Nymphaeales/Nymphaeaceae:					
Nymphaea sp.		*NymPI* (AY436744; Kramer and Irish 2000)	*NymAP3* (AY436748; Kramer and Irish 2000)	*NymAG1*, *NymAG2*, *NymAG3* (AY464104, AY464103, AY464102; Kramer et al. 2004)	
Nuphar advena (Aiton) W. T. Aiton	*NuadAP1* (AY936223; Kim et al. 2005*b*)		*NuadAP3-1* (DQ004464; Kim et al. 2005*b*)	*Nu.ad.AG* (AY936230; Kim et al. 2005*b*)	*NuadAGL2* (AY850183; Zahn et al. 2005*a*)
Piperales/Saururaceae:					
Houttuynia cordata Thunb.	*HcAP1* (AB089153; M. Ito et al., unpublished manuscript)[b]	*HtcPI-1*, *HtcPI-2* (AY436707, AY436746; Stellari et al. 2004)	*HtcAP3* (AY436745; Stellari et al. 2004)	*HtcAG* (AY464096; Kramer et al. 2004)	*HcSEP1*, *HcSEP2*, *HcSEP3* (AB089157–AB089159; M. Ito et al., unpublished manuscript)[b]
Poales/Poaceae:					
Oryza sativa L.	*OsMADS14*, *OsMADS15* (AF058697, AF058698; Moon et al. 1999*b*) *OsMADS18* (AF091458; Fornara et al. 2004) *OsMADS28* (AJ011675; L. Columbo, unpublished manuscript)[b]	*OsMADS2*, *OsMADS4* (L37526, L37527; Chung et al. 1995)	*OsMADS16* (AF077760; Moon et al. 1999*a*)	*OsMADS3* (L37528; Chung et al. 1995)	*OsMADS5*, *OsMADS7/45*, *OsMADS8/24* (U78890–U78892; Chung et al. 1995)
Eudicots:					
Apiales/Apiaceae:					
Daucus carota L.	*DcMADS1* (AJ271147; Linke et al. 2003)	*MADS2* (AJ271148; Linke et al. 2003)	*MADS3* (AJ271149; Linke et al. 2003)	*DcMADS4* (AJ271150; Linke et al. 2003)	
Aquifoliales/Aquifoliaceae:					
Ilex aquifolium L.		NA	*IxaAP3*, *IxaTM6* (DQ479356, DQ479357; Kramer et al. 2006)	NA	NA

Asterales/Asteraceae: *Gerbera hybrida*	*GSQUA1* (AJ009727; Yu et al. 1999)	*GGLO1* (GHY9726; Yu et al. 1999)	*GDEF1*, *GDEF2* (AJ009724, AJ009725; Yu et al. 1999)	*GAGA1*, *GAGA2* (AJ009722, AJ009723; Yu et al. 1999)	*GRCD1* (AJ400623; Kotilainen et al. 2000)
Brassicales/Brassicacaeae: *Arabidopsis thaliana* (L.) Heynh.	*AP1* (Z16421; Mandel et al. 1992) *CAL* (L36925; Kempin et al. 1995) *FUL* (AY072463; Mandel and Yanofsky 1995)	*PI* (D30807; Goto and Meyerowitz 1994)	*AP3* (D21125; Jack et al. 1992)	*AGAMOUS* (X53579; Yanofsky et al. 1990) *SHP1/AGL1*, *SHP2/AGL5* (M55550, M55553; Ma et al. 1991) *STK/AGL11* (U20182; Rounsley et al. 1995)	*SEP1*, *SEP2* (M55551, M55552; Ma et al. 1991) *SEP3* (AF015552; Mandel and Yanofsky 1998) *SEP4* (U81369; Huang et al. 1995)
Buxales/Buxaceae: *Pachysandra terminalis* Siebold & Zucc.	*PatFL1*, *PatFL2* (AY306164, AY306165; Litt and Irish 2003)	NA	*PtAP3-1*, *PtAP3-2* (AF052870, AF052871; Kramer et al. 1998)	NA	*PatSEP1* (AY306166; Litt and Irish 2003)
Caryophyllales/Caryophyllaceae: *Silene latifolia* Poir.	*SLM4*, *SLM5* (X80491, X80492; Hardenack et al. 1994)	*SLM2* (X80489; Hardenack et al. 1994)	*SLM3* (X80490; Hardenack et al. 1994)	*SLM1* (X80488; Hardenack et al. 1994)	*SlSEP1*, *SlSEP3* (AB162019, AB162020; Matsunaga et al. 2004)
Cornales/Hydrangeaceae: *Hydrangea macrophylla* (Thunb.) Ser.		*HmPI* (AF230711; Kramer and Irish 2000)	*HmAP3*, *HmTM6* (AF230702, AF230703; Kramer and Irish 2000)	NA	NA
Cucurbitales/Cucurbitales: *Cucumis sativus* L.		*CUM26* (AF043255; Kater et al. 2001)	*CsMADS1* (AY944060; H. T. Gu et al., unpublished manuscript)[b]	*CAG1*, *CAG2*, *CAG3* (AF022377–AF022379; Perl-Treves et al. 1998) *CUM1*, *CUM10* (AF035438, AF035439; Kater et al. 1998)	*CAGL2* (AF135962; F. Q. Liu et al., unpublished manuscript)[b]
Dilleniales/Dilleniaceae: *Dillenia indica* L.	NA	*DiiPI1*, *DiiPI2* (EF549702, EF549703; this study)	*DiiAP3*, *DiiTM6* (EF549704, EF549705; this study)	*DiiAG* (EF549706; this study)	NA
Ericales/Balsaminaceae: *Impatiens hawkeri* Bull.	NA	*IhGLO1* (DQ493931; Geuten et al. 2006)	*IhDEF1*, *IhDEF2* (DQ493930, DQ493929; Geuten et al. 2006)	NA	*IhSEP3* (DQ493928; Geuten et al. 2006)
Fabales/Fabaceae: *Pisum sativum* L.	*PsFUL* (AY884287; Hecht et al. 2005) *PisFUL* (AY306169; Litt and Irish 2003) *PsPIM* (AJ291298; Berbel et al. 2001)	*PsPI* (AY842491; Hecht et al. 2005)	NA	*PsAG* (AY884291; Hecht et al. 2005) *PsSHP* (AY884292; Hecht et al. 2005)	*PsSEP1-2* (AY884290; Hecht et al. 2005) *PsMTF1* (AJ223318; Buchner and Boutin 1998)
Fagales/Betulaceae: *Betula pendula* Roth	*BpMADS3*, *BpMADS4*, *BpMADS5* (X99653–X99655; Elo et al. 2001)	*Bpmads2* (AJ488589; Jarvinen et al. 2003)	NA	*BpMADS6* (AJ252071; Lemmetyinen et al. 2004)	*BpMADS1* (AJ252070; Lemmetyinen et al. 2001)

Table A1

(*Continued*)

Taxa	*AP1* lineage	*PI* lineage	*AP3* lineage	*AG* lineage	*SEP* (*AGL2/3/4*) lineage
Gunnerales/Gunneraceae:					
Gunnera tinctoria (Molina) Mirb.	NA	NA	*GtAP3-1*, *GtAP3-2*, *GtAP3-3*, *GtAP3-4*, *GtAP3-5* (AY337753–AY337757; Kim et al. 2004*b*)	NA	NA
Lamiales/Plantaginaceae:					
Antirrhinum majus L.	*DEFH28* (AY040247; Müller et al. 2001) *SQUA* (X63701; Huijser et al. 1992)	*GLOBOSA* (X68831; Tröbner et al. 1992)	*DEFA* (X62810; Schwarz-Sommer et al. 1992)	*PLENA* (S53900; Bradley et al. 1993) *FARINELLI* (AJ239057; Davies et al. 1999)	*AmDEFH49* (X95467; E.-C. Davies, unpublished manuscript)[b] *DEFH200*, *DEFH72* (X95469, X95468; Zahn et al. 2005*a*) *AmSEP3b* (AY306141; Litt and Irish 2003)
Malpighiales/Salicaceae:					
Populus trichocarpa Torr. & A. Gray	*PtAP1-1*, *PtAP1-1a*, *PtAP1-2* (AY615964–AY615966; A. M. Brunner, unpublished manuscript)[b]	*PtPI* (grail3.0002017601; Cseke et al. 2005)[c]	*PTD* (AF057708; Sheppard et al. 2000)	*PTAG1*, *PTAG2* (AF052570, AF052571; Brunner et al. 2000)	*PtSEP1*, *PtSEP2*, *PtSEP3-1*, *PtSEP3-2*, *PtSEP4* (grail3.0139000303, eugene3.01550021, grail3.0008011201, grail3.0047013501, gw1.VIII.1808.1; Cseke et al. 2005)[c]
	PtFUL-1, *PtFUL-2* (grail3.0042013901, grail3.0155001801; Cseke et al. 2005)[c] *PtMADS16* (LG IV-187530; C. H. Leseberg et al., unpublished manuscript)[b]				
Malvales/Malvaceae:					
Gossypium hirsutum L.	NA	NA	NA	*GhMADS3* (AY083173; Guo et al. 2007); *GhMADS2* (AF538966; S. Zheng et al., unpublished manuscript)[b]	*GhMADS1* (AF538965; Zheng et al. 2004)
Myrtales/Myrtaceae:					
Eucalyptus globulus Labill.	*EugAP1*, *EugAP2L* (AF305076, AF306349; Kyozuka et al. 1997)		NA	NA	
Eucalyptus grandis W. Hill ex Maiden		*EGM2* (AF029976; Southerton et al. 1998)	NA	NA	*EGM1*, *EGM3* (*Eucalyptus grandis*) (AF029975, AF029977; Southerton et al. 1998)
Papaverales/Papaveraceae:					
Eschscholzia californica Cham.	NA	NA	NA	*EScaAG1*, *EScaAG2*, *EScaAGL11* (DQ088996–DQ088998; Zahn et al. 2006)	*EScaAGL2*, *EScaAGL9* (AY850181, AY850180; Zahn et al. 2005*a*)

Proteales/Platanaceae:					
Platanus occidentalis L.	NA	NA	*PloAP3-1*, *PloAP3-2* (AY162881, AY162882; Kramer et al. 2003)	NA	NA
Ranunculales/Lardizabalaceae:					
Akebia quinata (Houtt.) Decne.		*AkqPI* (AY162882; Kramer et al. 2003)	*AkqAP3-1*, *AkqAP3-2* (AY162835, AY162839; Kramer et al. 2003)	*AkqAG* (AY464107; Kramer et al. 2004)	
Akebia trifoliata (Thunb.) Koidz.	*AktFL-1* (AY627632; Shan et al. 2006)				*AktSEP3-1* (AY627628; Shan et al. 2006)
Rosales/Rosaceae:					
Malus domestica Borkh.	*MadMdMADS2* (U78948; Sung et al. 2000)	*MadMdPI* (AJ291490; Yao et al. 2001)	*MadMdTM6* (AB081093; Kitahara et al. 2004)	*MadMdMADS10* (AJ000762; Yao et al. 1999)	*MadMdMADS1* (U78947; Sung and An 1997)
	MadMdMADS5 (AJ000759; Yao et al. 1999)		*MadMdMADS13* (AJ25116; van der Linden et al. 2002)	*MadMdMADS14*, *MadMdMADS15* (AJ251117, AJ251118; van der Linden et al.)	*MadMdMADS3*, *MadMdMADS4* (U78949, U78950; Sung et al. 2000)
	MadMdMADS12 (AJ320187; van der Linden et al. 2002)				*MadMdMADS8*, *MadMdMADS9* (AJ001681, AJ001682; Yao et al. 1999)
Sabiales/Sabiaceae:					
Meliosma dilleniifolia (Wall. *ex* Wight & Arn.) Walp.	NA	*MedMdPI* (AY436712; Stellari et al. 2004)	*MedMdAP3-1*, *MedMdAP3-2*, *MedMdAP3-3* (AY436709–AY436711; Stellari et al. 2004)	*MedMdAG1*, *MedMdAG2* (AY464098, AY464105; Kramer et al. 2004)	NA
Saxifragales/Saxifragaceae:					
Saxifraga careyana A. Gray	NA	NA	*SxcTM6*, *SxcAP3* (DO479368, DO479367; Kramer et al. 2006)	*SxcAG1*, *SxcAG2* (AY464117, AY464116; Kramer et al. 2004)	NA
Solanales/Solanaceae:					
Petunia hybrida Vilm.	*FBP26*, *PFG* (AF176783, AF176782; Immink et al. 1999)	*PMADS2* (X69947; Kush et al. 1993)	*PhTM6* (AF230704; Kramer and Irish 2000)	*FBP6*, *FBP7*, *FBP11* (X68675, X81651, X81852; Angenent et al. 1995)	*PhSEP1*, *PhSEP3* (AY306173, AY306171; Litt and Irish 2003)
	PhFL, *PhFUL* (AY306170, AY306172; Litt and Irish 2003)	*FBP1* (M91190; Angenent et al. 1992)	*GP* (X69946; Kush et al. 1993)	*pMADS3* (X72912; Tsuchimoto et al. 1993)	
Trochodendrales/Trochodendraceae:					
Trochodendron aralioides Siebold & Zucc.	*TraFUL1*, *TraFUL2* (EF436257, EF436258; this study)	*TraPI1*, *TraPI2* (EF436259, EF436260; this study); *TraPI3*[d]	*TraAP3* (DQ453774; Kramer et al. 2006)	*TraAG1*, *TraAG2* (EF436261, EF436262; this study)	*TraMADS1*, *TraMADS2*, *TraMADS3*, *TraMADS4* (EF436263–EF436266; this study)
Vitales/Vitaceae:					
Vitis vinifera L.	*VAP1*, *VFUL-L* (AY538746, AY538747; Calonje et al. 2004)	NA	NA	*VvMADS1*, *VvMADS5* (AF265562, AF373604; Boss et al. 2002)	*VvMADS2*, *VvMADS4* (AF373601, AF373603; Boss et al. 2002)

Note. NA = not available.
[a] Used as outgroups in both *AP1/FUL* and *SEP* phylogenies.
[b] Direct submission.
[c] From the *Populus trichocarpa* genomic database (http://genome.jgi-psf.org/Poptr1/Poptr1.home.html).
[d] Alternative spliced form of *TraPI2*.

Literature Cited

Adam H, S Jouannic, F Morcillo, F Richaud, Y Duval, JW Tregear 2006 MADS box genes in oil palm (*Elaeis guineensis*): patterns in the evolution of the *SQUAMOSA*, *DEFICIENS*, *GLOBOSA*, *AGAMOUS*, and *SEPALLATA* subfamilies. J Mol Evol 62:15–31.

Albert VA, MHG Gustafsson, LD Laurenzio 1998 Ontogenetic systematics, molecular developmental genetics, and the angiosperm petal. Pages 349–374 *in* DE Soltis, PS Soltis, JJ Doyle, eds. Molecular systematics of plants II: DNA sequencing. Kluwer, Boston.

Alwee SS, CG Van der Linden, J Van der Schoot, S de Folter, C Angenent, S-C Cheah, MJM Smulders 2006 Characterization of oil palm MADS box genes in relation to the mantled flower abnormality. Plant Cell Tissue Organ Cult 85:331–344.

Angenent GC, M Busscher, J Franken, JN Mol, AJ van Tunen 1992 Differential expression of two MADS box genes in wild-type and mutant petunia flowers. Plant Cell 4:983–993.

Angenent GC, J Franken, M Busscher, A van Dijken, JL van Went, HJM Dons, AJ van Tunen 1995 A novel class of MADS box genes is involved in ovule development in *Petunia*. Plant Cell 7:1569–1582.

Aoki S, K Uehara, M Imafuku, M Hasebe, M Ito 2004 Phylogeny and divergence of basal angiosperms inferred from *APETALA3*- and *PISTILLATA*-like MADS-box genes. J Plant Res 117:229–244.

APG (Angiosperm Phylogeny Group) 1998 An ordinal classification for the families of flowering plants. Ann Mo Bot Gard 85:531–553.

APG 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.

Baum DA 1998 The evolution of plant development. Curr Opin Plant Biol 1:79–86.

Baum DA, BA Whitlock 1999 Plant development: genetic clues to petal evolution. Curr Biol 9:R525–R527.

Benedito VA, PB Visser, JM van Tuyl, GC Angenent, SC de Vries, FA Krens 2004 Ectopic expression of *LLAG1*, an *AGAMOUS* homologue from lily (*Lilium longiflorum* Thunb.), causes floral homeotic modifications in *Arabidopsis*. J Exp Bot 55:1391–1399.

Berbel A, C Navarro, C Ferrándiz, LA Canas, F Madueno, JP Beltran 2001 Analysis of *PEAM4*, the pea *AP1* functional homologue, supports a model for *AP1*-like genes controlling both floral meristem and floral organ identity in different plant species. Plant J 25:441–451.

Boss PK, E Sensi, C Hua, C Davies, MR Thomas 2002 Cloning and characterisation of grapevine (*Vitis vinifera* L.) MADS-box genes expressed during inflorescence and berry development. Plant Sci 162: 887–895.

Bowman JL 1997 Evolutionary conservation of angiosperm flower development at the molecular and genetic levels. J Biosci 22:515–527.

Bradley D, R Carpenter, H Sommer, N Hartley, E Coen 1993 Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the *plena* locus of *Antirrhinum*. Cell 72: 85–95.

Brunner AM, WH Rottmann, LA Sheppard, K Krutovskii, SP DiFazio, S Leonardi, SH Strauss 2000 Structure and expression of duplicate *AGAMOUS* orthologues in poplar. Plant Mol Biol 44:619–634.

Buchner P, JP Boutin 1998 A MADS box transcription factor of the *AP1*/*AGL9* subfamily is also expressed in the seed coat of pea (*Pisum sativum*) during development. Plant Mol Biol 38:1253–1255.

Busch A, S Gleissberg 2003 *EcFLO*, a *FLORICAULA*-like gene from *Eschscholzia californica*, is expressed during organogenesis at the vegetative shoot apex. Planta 217:841–848.

Busch MA, K Bomblies, D Weigel 1999 Activation of a floral homeotic gene in *Arabidopsis*. Science 285:585–587.

Buzgo M, PK Endress 2000 Floral structure and development of Acoraceae and its systematic relationships with basal angiosperms. Int J Plant Sci 161:23–41.

Calonje M, P Cubas, JM Martinez-Zapater, MJ Carmona 2004 Floral meristem identity genes are expressed during tendril development in grapevine. Plant Physiol 135:1491–1501.

Caporali E, A Spada, A Losa, G Marziani 2000 The MADS box gene *AOM1* is expressed in reproductive meristems and flowers of the dioecious species *Asparagus officinalis*. Sex Plant Reprod 13:151–156.

Chang S, J Puryear, J Chirney 1993 A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 11:113–116.

Chase MW, DE Soltis, RG Olmstead, D Morgan, DH Les, BD Mishler, MR Duvall, et al 1993 Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Ann Mo Bot Gard 80:528–580.

Chaw SM 1992 Pollination, breeding syndromes, and systematics of *Trochodendron aralioides* Sieb. & Zucc. (Trochodendraceae), a relictual species in eastern Asia. Pages 63–77 *in* CI Peng, ed. Phytogeography and botanical inventory of Taiwan. Vol 12. Institute of Botany, Academia Sinica, Taipei.

Chung YY, SR Kim, HG Kang, YS Noh, MC Park, D Finkel, GH An 1995 Characterization of two rice MADS box genes homologous to *GLOBOSA*. Plant Sci 109:45–56.

Coen ES, EM Meyerowitz 1991 The war of the whorls: genetic interactions controlling flower development. Nature 353:31–37.

Coen ES, JM Romero, S Doyle, R Elliott, G Murphy, R Carpenter 1990 *Floricaula*: a homeotic gene required for flower development in *Antirrhinum majus*. Cell 63:1311–1322.

Cseke LJ, SB Cseke, N Ravinder, LC Taylor, A Shankar, B Sen, R Thakur, DF Karnosky, GK Podila 2005 *SEP*-class genes in *Populus tremuloides* and their likely role in reproductive survival of poplar trees. Gene 358:1–16.

Davies B, P Motte, E Keck, H Saedler, H Sommer, Z Schwarz-Sommer 1999 *PLENA* and *FARINELLI*: redundancy and regulatory interactions between two *Antirrhinum* MADS-box factors controlling flower development. EMBO J 18:4023–4034.

De Bodt S, J Raes, YV Van de Peer, G Theissen 2003 And then there were many: MADS goes genomic. Trends Plant Sci 8:475–483.

Dornelas MC, APM Rodriguez 2005 The rubber tree (*Hevea brasiliensis* Muell. Arg.) homologue of the *LEAFY*/*FLORICAULA* gene is preferentially expressed in both male and female floral meristems. J Exp Bot 56:1965–1974.

Dute RR, DR Folkerts, JE Watkins, ME Saxon, RS Boyd 2004 Floral anatomy and pollination biology of *Cyrilla racemiflora* (Cyrillaceae). Southeast Nat 3:309–326.

Elo A, J Lemmetyinen, M-L Turunen, L Tikka, T Sopanen 2001 Three MADS-box genes similar to *APETALA1* and *FRUITFULL* from silver birch (*Betula pendula*). Physiol Plant 112:95–103.

Endress PK 1986 Floral structure, systematics, and phylogeny in Trochodendrales. Ann Mo Bot Gard 73:297–324.

——— 1990 Patterns of floral construction in ontogeny and phylogeny. Biol J Linn Soc 39:153–175.

——— 1994 Floral structure and evolution of primitive angiosperms: recent advances. Plant Syst Evol 192:79–97.

——— 2006 Angiosperm floral evolution: morphological developmental framework. Pages 1–61 *in* DE Soltis, J Leebens-Mack, PS Soltis, eds. Developmental genetics of the flowers. Advances in botanical research, no 40. Academic Press, San Diego, CA.

Ferrario S, RG Immink, GC Angenent 2004 Conservation and diversity in flower land. Curr Opin Plant Biol 7:84–91.

Fornara F, L Pařenicová, G Falasca, N Pelucchi, S Masiero, S Ciannamea, Z Lopez-Dee, MM Altamura, L Colombo, MM Kater 2004 Functional characterization of *OsMADS18*, a member of the *AP1/SQUA* subfamily of MADS box genes. Plant Physiol 135: 2207–2219.

Frohlich MW, DS Parker 2000 The mostly male theory of flower evolutionary origins: from genes to fossils. Syst Bot 25:155–170.

Geuten K, A Becker, K Kaufmann, P Caris, T Viaene, G Theissen, E Smets 2006 Petaloidy and petal identity MADS-box genes in the balsaminoid genera *Impatiens* and *Marcgravia*. Plant J 47:501–518.

Glover BJ, C Martin 1998 The role of petal cell shape and pigmentation in pollination success in *Antirrhinum majus*. Heredity 80: 778–784.

——— 2002 Evolution of adaptive petal cell morphology. Pages 160–172 *in* QCB Cronk, RM Bateman, JA Hawkins, eds. Developmental genetics and plant evolution. Taylor & Francis, New York.

Goto K, EM Meyerowitz 1994 Function and regulation of the *Arabidopsis* floral homeotic gene *PISTILLATA*. Genes Dev 8:1548–1560.

Gu Q, C Ferrándiz, MF Yanofsky, R Martienssen 1998 The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. Development 125:1509–1517.

Guo YL, QL Zhu, SY Zheng, MY Li 2007 Cloning of a MADS box gene (*GhMADS3*) from cotton and analysis of its homeotic role in transgenic tobacco. J Genet Genomics (forthcoming).

Hardenack S, D Ye, H Saedler, S Grant 1994 Comparison of MADS box gene expressing in developing male and female flowers of the dioecious plant white campion. Plant Cell 6:1775–1787.

Hecht V, F Foucher, C Ferrándiz, R Macknight, C Navarro, J Morin, ME Vardy, et al 2005 Conservation of *Arabidopsis* flowering genes in model legumes. Plant Physiol 137:1420–1434.

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

Hoot SB, S Magallón, PR Crane 1999 Phylogeny of basal eudicots based on three molecular data sets: *atp*B, *rbc*L, and 18S nuclear ribosomal DNA sequences. Ann Mo Bot Gard 86:1–32.

Huang H, M Tudor, CA Weiss, Y Hu, H Ma 1995 The *Arabidopsis* MADS-box gene *Agl3* is widely expressed and encodes a sequence-specific DNA-binding protein. Plant Mol Biol 28:549–567.

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

Huijser P, J Klein, WE Lonnig, H Meijer, H Saedler, H Sommer 1992 Bracteomania, an inflorescence anomaly, is caused by the loss of function of the MADS-box gene *SQUAMOSA* in *Antirrhinum majus*. EMBO J 11:1239–1249.

Immink RGH, DJ Hannapel, S Ferrario, M Busscher, J Franken, MML Campagne, GC Angenent 1999 A petunia MADS box gene involved in the transition from vegetative to reproductive development. Development 126:5117–5126.

Irish VF 2003 The evolution of floral homeotic gene function. BioEssays 25:637–646.

——— 2006 Duplication, diversification, and comparative genetics of angiosperm MADS-box genes. Pages 129–161 *in* DE Soltis, J Leebens-Mack, PS Soltis, eds. Developmental genetics of the flowers. Advances in botanical research, no. 40. Academic Press, San Diego, CA.

Jack T, LL Brockman, EM Meyerowitz 1992 The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. Cell 68:683–697.

Jarvinen P, J Lemmetyinen, O Savolainen, T Sopanen 2003 DNA sequence variation in *BpMADS2* gene in two populations of *Betula pendula*. Mol Ecol 12:369–384.

Kater MM, L Colombo, J Franken, M Busscher, S Masiero, MMV Campagne, GC Angenent 1998 Multiple *AGAMOUS* homologs from cucumber and petunia differ in their ability to induce reproductive organ fate. Plant Cell 10:171–182.

Kater MM, J Franken, KJ Carney, L Colombo, GC Angenent 2001 Sex determination in the monoecious species cucumber is confined to specific floral whorls. Plant Cell 13:481–493.

Kaufmann K, R Melzer, G Theissen 2005 MIKC-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. Gene 347:183–198.

Kay QON, HS Daoud, CH Stirton 1981 Pigment distribution, light reflection and cell structure in petals. Bot J Linn Soc 83:57–84.

Keck E, P McSteen, R Carpenter, E Coen 2003 Separation of genetic functions controlling organ identity in flowers. EMBO J 22:1058–1066.

Kelly AJ, MB Bonnlander, DR Meekswagner 1995 *NFL*, the tobacco homolog of *FLORICAULA* and *LEAFY*, is transcriptionally expressed in both vegetative and floral meristems. Plant Cell 7:225–234.

Kempin SA, B Savidge, MF Yanofsky 1995 Molecular basis of the cauliflower phenotype in *Arabidopsis*. Science 267:522–525.

Kim S, J Koh, H Ma, Y Hu, PK Endress, BA Hauser, M Buzgo, PS Soltis, DE Soltis 2005*a* Sequence and expression studies of A-, B-, and E-class MADS-box homologues in *Eupomatia* (Eupomatiaceae): support for the bracteate origin of the calyptra. Int J Plant Sci 166:185–198.

Kim S, J Koh, MJ Yoo, HZ Kong, Y Hu, H Ma, PS Soltis, DE Soltis 2005*b* Expression of floral MADS-box genes in basal angiosperms: implications for the evolution of floral regulators. Plant J 43:724–744.

Kim S, DE Soltis, PS Soltis, MJ Zanis, Y Suh 2004*a* Phylogenetic relationships among early-diverging eudicots based on four genes: were the eudicots ancestrally woody? Mol Phylogenet Evol 31: 16–30.

Kim S, M-J Yoo, VA Albert, JS Farris, PS Soltis, DE Soltis 2004*b* Phylogeny and diversification of B-function MADS-box genes in angiosperms: evolutionary and functional implications of a 260-million-year-old duplication. Am J Bot 91:2102–2118.

Kitahara K, T Ohtsubo, J Soejima, S Matsumoto 2004 Cloning and characterization of apple class MADS-box genes including a novel AP3 homologue *MdTM6*. J Jpn Soc Hortic Sci 73:208–215.

Kotilainen M, P Elomaa, A Uimari, VA Albert, DY Yu, TH Teeri 2000 *GRCD1*, an *AGL2*-like MADS box gene, participates in the C function during stamen development in *Gerbera hybrida*. Plant Cell 12:1893–1902.

Kramer EM, VS Di Stilio, PM Schlüter 2003 Complex patterns of gene duplication in the *APETALA3* and *PISTILLATA* lineages of the Ranunculaceae. Int J Plant Sci 164:1–11.

Kramer EM, RL Dorit, VF Irish 1998 Molecular evolution of genes controlling petal and stamen development: duplication and divergence within the *APETALA3* and *PISTILLATA* MADS-box gene lineages. Genetics 149:765–783.

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

——— 2000 Evolution of the petal and stamen developmental programs: evidence from comparative studies of the lower eudicots and basal angiosperms. Int J Plant Sci 161(suppl):S29–S40.

Kramer EM, MA Jaramillo, VS Di Stilio 2004 Patterns of gene duplication and functional evolution during the diversification of the *AGAMOUS* subfamily of MADS box genes in angiosperms. Genetics 166:1011–1023.

Kramer EM, H-J Su, C-C Wu, J-M Hu 2006 A simplified explanation for the frameshift mutation that created a novel C-terminal motif in the *APETALA3* gene lineage. BMC Evol Biol 6:30.

Kramer EM, EA Zimmer 2006 Gene duplication and floral developmental genetics of basal eudicots. Pages 353–384 *in* DE Soltis, J

Leebens-Mack, PS Soltis, eds. Developmental genetics of the flowers. Advances in botanical research, no. 40. Academic Press, San Diego, CA.

Kush A, A Brunelle, D Shevell, NH Chua 1993 The cDNA sequence of two MADS box proteins in *Petunia*. Plant Physiol 102:1051–1052.

Kyozuka J, R Harcourt, WJ Peacock, ES Dennis 1997 *Eucalyptus* has functional equivalents of the *Arabidopsis AP1* gene. Plant Mol Biol 35:573–584.

Lamb RS, TA Hill, QK-G Tan, VF Irish 2002 Regulation of *APETALA3* floral homeotic gene expression by meristem identity genes. Development 129:2079–2086.

Lemmetyinen J, M Hassinen, A Elo, I Porali, K Keinonen, H Makela, T Sopanen 2004 Functional characterization of *SEPALLATA3* and *AGAMOUS* orthologues in silver birch. Physiol Plant 121: 149–162.

Lemmetyinen J, T Pennanen, M Lännenpää, T Sopanen 2001 Prevention of flower formation in dicotyledons. Mol Breed 7:341–350.

Li GS, Z Meng, HZ Kong, ZD Chen, G Theissen, AM Lu 2005 Characterization of candidate class A, B and E floral homeotic genes from the perianthless basal angiosperm *Chloranthus spicatus* (Chloranthaceae). Dev Genes Evol 215:437–449.

Li HL, SM Chaw 1996 Trochodendraceae. Pages 504–505 *in* Flora of Taiwan. Vol 2. Editorial Committee of the Flora of Taiwan, Taipei.

Linke B, T Nothnagel, T Borner 2003 Flower development in carrot CMS plants: mitochondria affect the expression of MADS box genes homologous to *GLOBOSA* and *DEFICIENS*. Plant J 34: 27–37.

Litt A, VF Irish 2003 Duplication and diversification in the *APETALA1/FRUITFULL* floral homeotic gene lineage: implications for the evolution of floral development. Genetics 165:821–833.

Liu Q, Y Zhang, Y Zhao 2004 Cloning of flower development associated MADS box gene fragments in *Lilium*. Acta Hortic Sin 31:332–336.

Losa A, E Caporali, A Spada, S Martinelli, G Marziani 2004 *AOM3* and *AOM4*: two MADS box genes expressed in reproductive structures of *Asparagus officinalis*. Sex Plant Reprod 16:215–221.

Ma H, C dePamphilis 2000 The ABCs of floral evolution. Cell 101: 5–8.

Ma H, MF Yanofsky, EM Meyerowitz 1991 *AGL1-AGL6*, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes. Genes Dev 5:484–495.

Maddison DR, WP Maddison 2000 MacClade 4: analysis of phylogeny and character evolution. Sinauer, Sunderland, MA.

Malcomber ST, EA Kellogg 2005 *SEPALLATA* gene diversification: brave new whorls. Trends Plant Sci 10:427–435.

Manchester SR, PR Crane, DL Dilcher 1991 *Nordenskioldia* And *Trochodendron* (Trochodendraceae) from the Miocene of northwestern North America. Bot Gaz 152:357–368.

Mandel MA, C Gustafson-Brown, B Savidge, MF Yanofsky 1992 Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. Nature 360:273–277.

Mandel MA, MF Yanofsky 1995 The *Arabidopsis AGL8* MADS box gene is expressed in inflorescence meristems and is negatively regulated by *APETALA1*. Plant Cell 7:1763–1771.

——— 1998 The *Arabidopsis AGL9* MADS box gene is expressed in young flower primordia. Sex Plant Reprod 11:22–28.

Martin C, K Bhatt, K Baumann, H Jin, S Zachgo, K Roberts, Z Schwarz-Sommer, B Glover, M Perez-Rodrigues 2002 The mechanics of cell fate determination in petals. Philos Trans R Soc B 357:809–813.

Matsunaga S, W Uchida, E Kejnovsky, E Isono, F Moneger, B Vyskot, S Kawano 2004 Characterization of two *SEPALLATA* MADS-box genes from the dioecious plant *Silene latifolia*. Sex Plant Reprod 17: 189–193.

Moon YH, JY Jung, HG Kang, GH An 1999*a* Identification of a rice *APETALA3* homologue by yeast two-hybrid screening. Plant Mol Biol 40:167–177.

Moon YH, HG Kang, JY Jung, JS Jeon, SK Sung, G An 1999*b* Determination of the motif responsible for interaction between the rice APETALA1/AGAMOUS-LIKE9 family proteins using a yeast two-hybrid system. Plant Physiol 120:1193–1203.

Mouradov A, B Hamdorf, RD Teasdale, JT Kim, K-U Winter, G Theissen 1999 A *DEF*/*GLO*-like MADS-box gene from a gymnosperm: *Pinus radiata* contains an ortholog of angiosperm B class floral homeotic genes. Dev Genet 25:245–252.

Müller BM, H Saedler, S Zachgo 2001 The MADS-box gene *DEFH28* from *Antirrhinum* is involved in the regulation of floral meristem identity and fruit development. Plant J 28:169–179.

Nast CG, IW Bailey 1945 Morphology and relationships of *Trochodendron* and *Tetracentron* II. Inflorescence, flower, and fruit. J Arnold Arbor Harv Univ 26:267–276.

Park JH, Y Ishikawa, T Ochiai, A Kanno, T Kameya 2004 Two GLOBOSA-like genes are expressed in second and third whorls of homochlamydeous flowers in *Asparagus officinalis* L. Plant Cell Physiol 45:325–332.

Park JH, Y Ishikawa, R Yoshida, A Kanno, T Kameya 2003 Expression of *AODEF*, a B-functional MADS-box gene, in stamens and inner tepals of the dioecious species *Asparagus officinalis* L. Plant Mol Biol 51:867–875.

Parkinson CL, KL Adams, JD Palmer 1999 Multigene analyses identify the three earliest lineages of extant flowering plants. Curr Biol 9:1485–1488.

Perez-Rodriguez M, FW Jaffe, E Butelli, BJ Glover, C Martin 2005 Development of three different cell types is associated with the activity of a specific MYB transcription factor in the ventral petal of *Antirrhinum majus* flowers. Development 132:359–370.

Perl-Treves R, A Kahana, N Rosenman, Y Xiang, L Silberstein 1998 Expression of multiple *AGAMOUS*-like genes in male and female flowers of cucumber (*Cucumis sativus* L.). Plant Cell Physiol 39: 701–710.

Posada D, TR Buckley 2004 Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. Syst Biol 53:793–808.

Qiu Y-L, MW Chase, SB Hoot, E Conti, PR Crane, KJ Sytsma, CR Parks 1998 Phylogenetics of the Hamamelidae and their allies: parsimony analyses of nucleotide sequences of the plastid gene *rbc*L. Int J Plant Sci 159:891–905.

Qiu Y-L, O Dombrovska, J Lee, LB Li, BA Whitlock, F Bernasconi-Quadroni, JS Rest, et al 2005 Phylogenetic analyses of basal angiosperms based on nine plastid, mitochondrial, and nuclear genes. Int J Plant Sci 166:815–842.

Ronse de Craene LP 2002 Floral development and anatomy of *Pentadiplandra* (Pentadiplandraceae): a key genus in the identification of floral morphological trends in the core Brassicales. Can J Bot 80:443–459.

Rounsley SD, GS Ditta, MF Yanofsky 1995 Diverse roles for MADS box genes in *Arabidopsis* development. Plant Cell 7:1259–1269.

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.

Schultz EA, GW Haughn 1991 *Leafy*, a homeotic gene that regulates inflorescence development in *Arabidopsis*. Plant Cell 3:771–781.

Schwarz-Sommer Z, I Hue, P Huijser, PJ Flor, R Hansen, F Tetens, WE Lonnig, H Saedler, H Sommer 1992 Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: evidence

for DNA-binding and autoregulation of its persistent expression throughout flower development. EMBO J 11:251–263.

Shan HY, KM Su, WL Lu, HZ Kong, ZD Chen, Z Meng 2006 Conservation and divergence of candidate class B genes in *Akebia trifoliata* (Lardizabalaceae). Dev Genes Evol 216:785–795.

Sheppard LA, AM Brunner, KV Krutovskii, WH Rottmann, JS Skinner, SS Vollmer, SH Strauss 2000 A *DEFICIENS* homolog from the dioecious tree black cottonwood is expressed in female and male floral meristems of the two-whorled, unisexual flowers. Plant Physiol 124:627–639.

Soltis DE, VA Albert, S Kim, M-J Yoo, PS Soltis, MW Frohlich, J Leebens-Mack, et al 2005 Evolution of the flower. Pages 165–200 *in* RJ Henry, ed. Plant diversity and evolution: genotypic and phenotypic variation in higher plants. CABI, Wallingford.

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

Soltis PS, DE Soltis 2000 The role of genetic and genomic attributes in the success of polyploids. Proc Natl Acad Sci USA 97: 7051–7057.

Southerton SG, H Marshall, A Mouradov, RD Teasdale 1998 Eucalypt MADS-box genes expressed in developing flowers. Plant Physiol 118:365–372.

Stellari GM, MA Jaramillo, EM Kramer 2004 Evolution of the *APETALA3* and *PISTILLATA* lineages of MADS-box-containing genes in the basal angiosperms. Mol Biol Evol 21:506–519.

Sung SK, GH An 1997 Molecular cloning and characterization of a MADS-box cDNA clone of the Fuji apple. Plant Cell Physiol 38: 484–489.

Sung SK, GH Yu, J Nam, DH Jeong, G An 2000 Developmentally regulated expression of two MADS-box genes, *MdMADS3* and *MdMADS4*, in the morphogenesis of flower buds and fruits in apple. Planta 210:519–528.

Swofford DL 2002 PAUP*: phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, MA.

Takhtajan AL 1991 Evolutionary trends in flowering plants. Columbia University Press, New York. 241 pp.

Tandre K, VA Albert, A Sundas, P Engstrom 1995 Conifer homologs to genes that control floral development in angiosperms. Plant Mol Biol 27:69–78.

Taylor DW 2002 A taxonomic revision of the genus *Chione* (Rubiaceae). Syst Geogr Plants 73:171–198.

Theissen G, H Saedler 2001 Floral quartets. Nature 409:469–471.

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.

Tröbner W, L Ramirez, P Motte, I Hue, P Huijser, WE Lonnig, H Saedler, H Sommer, Z Schwarz-Sommer 1992 *GLOBOSA*: a homeotic gene which interacts with *DEFICIENS* in the control of *Antirrhinum* floral organogenesis. EMBO J 11:4693–4704.

Tsuchimoto S, AR van der Krol, N-H Chua 1993 Ectopic expression of *pMADS3* in transgenic *Petunia* phenocopies the *Petunia blind* mutant. Plant Cell 5:843–853.

Tzeng T-Y, C-H Yang 2001 A MADS box gene from lily (*Lilium longiflorum*) is sufficient to generate dominant negative mutation by interacting with PISTILLATA (PI) in *Arabidopsis thaliana*. Plant Cell Physiol 42:1156–1168.

Vandenbussche M, G Theissen, Y Van de Peer, T Gerats 2003 Structural diversification and neo-functionalization during floral MADS-box gene evolution by C-terminal frameshift mutations. Nucleic Acids Res 31:4401–4409.

van der Linden CG, B Vosman, MJM Smulders 2002 Cloning and characterization of four apple *MADS* box genes isolated from vegetative tissue. J Exp Bot 53:1025–1036.

von Balthazar M, PK Endress 2002 Reproductive structures and systematics of Buxaceae. Bot J Linn Soc 140:193–228.

Weberling F 1989 Morphology of flowers and inflorescences. Cambridge University Press, Cambridge. 405 pp.

Winter K-U, A Becker, T Münster, JT Kim, H Saedler, G Theissen 1999 MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. Proc Natl Acad Sci USA 96:7342–7347.

Winter K-U, C Weiser, K Kaufmann, A Bohne, C Kirchner, A Kanno, H Saedler, G Theissen 2002 Evolution of class B floral homeotic proteins: obligate heterodimerization originated from homodimerization. Mol Biol Evol 19:587–596.

Yanofsky MF, H Ma, JL Bowman, GN Drews, KA Feldmann, EM Meyerowitz 1990 The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. Nature 346: 35–39.

Yao JL, YH Dong, A Kvarnheden, B Morris 1999 Seven MADS-box genes in apple are expressed in different parts of the fruit. J Am Soc Hortic Sci 124:8–13.

Yao JL, YH Dong, BAM Morris 2001 Parthenocarpic apple fruit production conferred by transposon insertion mutations in a MADS-box transcription factor. Proc Natl Acad Sci USA 98:1306–1311.

Yu DY, M Kotilainen, E Pollanen, M Mehto, P Elomaa, Y Helariutta, VA Albert, TH Teeri 1999 Organ identity genes and modified patterns of flower development in *Gerbera hybrida* (Asteraceae). Plant J 17:51–62.

Zahn LM, HZ King, JH Leebens-Mack, S Kim, PS Soltis, LL Landherr, DE Soltis, CW dePamphilis, H Ma 2005*a* The evolution of the *SEPALLATA* subfamily of MADS-box genes: a preangiosperm origin with multiple duplications throughout angiosperm history. Genetics 169:2209–2223.

Zahn LM, JH Leebens-Mack, JM Arrington, Y Hu, LL Landherr, CW dePamphilis, A Becker, G Theissen, H Ma 2006 Conservation and divergence in the *AGAMOUS* subfamily of MADS-box genes: evidence of independent sub- and neofunctionalization events. Evol Dev 8:30–45.

Zahn LM, J Leebens-Mack, CW dePamphilis, H Ma, G Theissen 2005*b* To B or not to B a flower: the role of *DEFICIENS* and *GLOBOSA* orthologs in the evolution of the angiosperms. J Hered 96:225–240.

Zheng S-Y, Y-L Guo, Y-H Xiao, M Luo, L Hou, X-Y Luo, Y Pei 2004 Cloning of a MADS box protein gene (*GhMADS-1*) from cotton (*Gossypium hirsutum* L.). Acta Genet Sin 31:1136–1141.