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Phylogeny and systematics of Crossosomatales as inferred from chloroplast *atpB*, *matK*, and *rbcL* sequences

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ABSTRACT: Crossosomatales is a recently recognized order in the rosid II clade with about 64 species in eight morphologically distinct families that have been previously classified in as many as 15 other orders. Phylogenetic relationships among the families and genera within Crossosomatales were investigated using chloroplast *atpB*, *matK*, and *rbcL* sequences employing maximum parsimony, maximum likelihood, and Bayesian methods. The phylogenetic framework was used to examine the patterns of morphological evolution and synapomorphies for subclades within Crossosomatales. The combined data with representative species from all genera in the order strongly supported monophyly of Crossosomatales. Strong support was found for the families in the Southern Hemisphere, in which Aphloiaceae is sister to the clade of (Geissolomataceae, (Ixerbaceae + Strasburgeriaceae)). The sister relationship between the Southern Hemisphere clade and families distributed primarily in the Northern Hemisphere was also supported. As in the previous studies, following relationships were found within the Northern Hemisphere clade: Staphyleaceae is sister to a clade of (Guamatelaceae, (Stachyuraceae + Crossosomataceae)). The pattern analysis indicates that evolutionary pattern of morphological characters is complex, requiring multiple changes within Crossosomatales. Several reproductive traits, such as inflorescence, aril, stigma, and conspicuous protrusion from pollen aperture, corroborate the molecular phylogeny.

Keywords: Crossosomatales, Phylogeny, chloroplast DNA, morphology

Introduction

Crossosomatales is a small order in the rosid II clade with about 64 species in eight families (Table 1), and characterized by a number of reproductive traits, including presence of hypanthium, imbricate sepals with the outermost smaller than the inner, shortly stalked gynoecium, postgenitally united carpel tips, and lignified multilayered seed coats (Stevens, 2001 onwards; Matthews and Endress, 2005; Oh and Potter, 2006). Members of Crossosomatales are morphologically diverse ranging from tall deciduous and evergreen trees to microphyllous shrubs, and distributed in various regions across the Northern and Southern Hemispheres occupying different biomes including temperate forest, tropical rainforest, and North American deserts. Families in Crossosomatales show narrow endemism, occurring only one geographic region, with the exception of Staphyleaceae, which is broadly distributed in the Northern Hemisphere.

Current concept of Crossosomatales has been established very recently. Crossosomatales was first used to include only Crossosomataceae (Takhtajan, 1987, 1997; Reveal, 1993), and the families transferred to Crossosomatales (Aphloiaceae, Geissolomataceae, Guamatelaceae, Ixerbaceae, Stachyuraceae, Staphyleaceae, and Strasburgeriaceae) were previously classified in as many as 15 orders, such as Rosales, Celastrales, and Violales. Inclusion of the seven families in Crossosomatales has been primarily suggested by a number of molecular phylogenetic studies of angiosperms over the past decade (Nandi et al., 1998; Savolainen et al., 2000; Soltis et al., 2000, 2007; Cameron, 2003; Sosa and Chase, 2003; Oh and Potter, 2006). The expanded circumscription of Crossosomatales is recognized by current classification systems of angiosperms (Thorne and Reveal, 2007; APG III, 2009).

Because Crossosomatales is a newly recognized group a more detailed study is needed. While monophyly of Crossosomatales has been strongly advocated by morphology (Matthews and Endress, 2005), all of previous molecular studies have shown very low or even no support for the group. In addition, phylogenetic relationships among the families and genera within Crossosomatales have remained unclear. The most recent phylogenetic study of Crossosomatales based on chloroplast *rbcL*, *atpB*, and *matK* genes (Oh and Potter, 2006) strongly supported the monophyly of the families distributed in the Northern Hemisphere, including

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Table 1. Taxa of Crossosomatales and distribution.

Family	Genus	No. of species	Distribution
Crossosomataceae	*Crossosoma*	2	western North America and Mexico
	Apacheria	1	Arizona and New Mexico
	Glossopetalon	4	western North America
	Velascoa	1	central Mexico
Guamatelaceae	*Guamatela*	1	southern Mexico, Honduras, and Guatemala
Stachyuraceae	*Stachyurus*	ca. 10	eastern Asia
Staphyleaceae	*Staphylea*	10	Europe, eastern Asia, and North America
	Euscaphis	1	eastern Asia
	Turpinia	ca. 30	tropical eastern and southeastern Asia, and Central and South America
Aphloiaceae	*Aphloia*	1	East Africa, Madagascar, Comoros, Mascarenes, and Seychelles
Geissolomataceae	*Geissoloma*	1	South Africa
Ixerbaceae	*Ixerba*	1	New Zealand
Strasburgeriaceae	*Strasburgeria*	1	New Caledonia

Crossosomataceae, Stachyuraceae, Guamatelaceae, and Staphyleaceae. However, phylogenetic relationships among the Northern Hemisphere clade and the rest of the four Southern Hemisphere families were uncertain in the study. Oh and Potter (2006) is the only study that included all of genera in Crossosomatales including *Guamatela* Donn. Sm., but it has many missing data, in particular, for the *matK* gene from the families in the Southern Hemisphere. Phylogenetic analysis of Strasburgeriaceae based on *rbcL*, *atpB*, 18S genes (Cameron, 2003) and that of Crossosomataceae using the *rbcL* data (Sosa and Chase, 2003), both of which also sampled other members of Crossosomatales, produced similar results: low bootstrap support for Crossosomatales and uncertain relationships of the families in the Southern Hemisphere.

All of large-scale analyses of angiosperms did not include all the major lineages of Crossosomatales, and did not show strong support for the monophyly of Crossosomatales (Nandi et al., 1998; Savolainen et al., 2000; Soltis et al., 2000; Hilu et al., 2003). Wang et al. (2009) generated a phylogeny from 102 members of the rosid clade with two nuclear loci (18S and 26S ribosomal genes), ten chloroplast genes (*aptB*, *matK*, *rbcL*, *ndhF*, *psbBTNH*, *rpoC2*, and *rps4*), and the chloroplast inverted repeat region. The total evidence data suggested that Crossosomatales is a strongly supported monophyletic group and that the families in the Southern Hemisphere forms a clade, sister to the Northern Hemisphere clade with high bootstrap supports. This study, however, did not include Guamatelaceae and Geissolomataceae, thus questions regarding the phylogenetic relationships within Crossosomatales have remained uncertain.

As the families of Crossosomatales were previously classified in diverse orders, morphology in Crossosomatales is heterogeneous. However, little is known about patterns of character evolution within the order. For example, opposite leaves are found in Guamatelaceae, Staphyleaceae, Geissolomataceae, and *Apacheria* in Crossosomataceae, whereas Stachyuraceae, Aphloiaceae, Strasburgeriaceae, and all other genera in Crossosomataceae (*Crossosoma*, *Glossopetalon*, and *Velascoa*) have alternate leaves. Leaves of Ixerbaceae are alternate, opposite, or whorled. Flowers are generally solitary in Crossosomataceae and the four Southern Hemisphere families, but are clustered in Stachyuraceae (spike or raceme), Staphyleaceae (panicle), and Guamatelaceae (raceme).

There are few morphological characters that may define subclades within the order. Morphological characters corroborating the monophyly of the four Northern Hemisphere families are weak. A capitate or flattened stigma may be a synapomorphy for the clade (Stevens 2001 onwards), but the character is also found in Aphloiaceae. Arillate seeds may also be a synapomorphy for the clade, but seeds of *Staphylea* and *Turpinia* do not have an aril and Ixerbaceae and Strasburgeriaceae have rudimentary aril. Conspicuous protrusions of the apertures in pollen (“pollen bud”; Weber and Igersheim, 1994) are found in the Southern Hemisphere families and Guamatelaceae (Oh & Potter, 2006).

This study generated a complete molecular data set of *atpB*, *matK*, and *rbcL* sequences for all representing taxa in Crossosomatales to (1) test the monophyly of Crossosomatales, (2) obtain a maximally robust phylogenetic hypothesis among the genera and families within Crossosomatales, and (3) examine the implications of the phylogenetic framework for the evolution of morphological characters.

Materials and Methods

All 13 genera in Crossosomatales were included in this study (Appendix 1). *Staphylea* was represented by two species (*S. bumalda* and *S. trifolia*), and *Aphloia* by two accessions. Species of *Alvaradoa* and *Picramnia* (Picramniaceae), *Gossypium* (Malvales), *Tapiscia* (Huerteales), and *Citrus* (Sapindales) were included as outgroups (Appendix 1). The outgroups were selected based on the maximum likelihood tree of Wang et al. (2009), in which Crossosomatales was supported as sister to a clade of Picramniaceae plus Huerteales, Brassicales, Malvales, and Sapindales. Brassicales was not represented in this study because it was supported as sister to Huerteales (Christenhusz et al., 2010) or Malvales (Wang et al., 2009; Worberg et al., 2009). All two genera in Picramniaceae were included, as the family formed a sister relationship of a clade of Huerteales, Brassicales, Malvales, and Sapindales.

Nucleotide sequences of three chloroplast regions (*atpB*, *matK*, and *rbcL*) were sampled, widely used for the phylogenetic analysis at higher taxonomic levels. Sequences of all outgroups and 26 of 45 sequences of Crossosomatales were taken from GenBank. Voucher information and GenBank accession numbers for the 19 sequences newly determined for this study were provided in Appendix 1. Fresh leaf materials were collected and dried in the silica gel from field for *Apacheria chiricahuensis, Crossosoma bigelovii, Glossopetalon spinescens var. aridum, Velascoa recondita, Staphylea bumalda,* and *S. trifolia.* DNA material for *Geissoloma marginatum* was obtained from South African National Biodiversity Institute, and those for *Ixerba brexioides* and *Strasburgeria robusta* were provided by DNA Bank at Royal Botanic Garden, Kew. Herbarium material was used for *Aphloia*.

Total DNA from silica gel-dried leaf materials and herbarium material was isolated following the methods described in Oh and Potter (2006). Molecular methods in Oh and Potter (2006) were used to amplify, purify, and sequence the target regions with a slight modification. Phusion High-Fidelity DNA polymerase (New England Biolabs, Ipswich, Massachusetts, USA) was used in this study to amplify the region under the following conditions in 25 μl reactions: initial denaturation at 98°C for 1 min 30 s, 20 cycles of 98°C for 10 s, 60°C for 30 s, and 72°C for 2 min, another 20 "touchdown" cycles of 98°C for 10 s, 60°C with 0.5°C decrease per cycle for 30 s, and 72°C for 2 min, followed by final extension at 72°C for 7 min. All sequences determined in this study were produced at Cornell University Life Sciences Core Laboratories Center, which uses 3730xl DNA Analyzer (Applied Biosystems, Foster City, California, USA). Sequences were edited in Sequencher version 4.8 (Gene Codes Corporation, Ann Arbor, Michigan).

The sequence data of *atpB*, *matK*, and *rbcL* were concatenated and simultaneously analyzed. Phylogenetic analyses of the data were conducted with maximum parsimony (MP), maximum likelihood (ML), and Bayesian methods. All characters were treated as unordered and weighted equally in the MP analyses employed in the program PAUP* version 4.0b10 (Swofford, 2002). Gaps resulted from multiple alignment of indels were treated as missing data. Heuristic searches were used to find the MP trees with 200 replicates of random taxon addition and tree bisection-reconnection (TBR) branch swapping saving all of the best trees at each step (MulTrees). Branches with a minimum length of zero were collapsed using "amb-" option during the searches in the MP analysis (Nixon and Carpenter, 1996). The bootstrap analysis (Felsenstein 1985) with 500 pseudoreplicates was conducted with simple sequence addition and TBR branch swapping. ML analysis was conducted by using the program GARLI version 0.951 (Zwickl, 2006). The best fitting evolutionary model for the data was determined by the hierarchical likelihood ratio test using Modeltest 3.06 (Posada and Crandall, 1998). The selected model, including substitution model, gamma shape parameter, and proportion of invariable sites, was employed to calculate the likelihood value. All other parameters were estimated from the data. Default values were used for genetic algorithm and other settings. ML bootstrap analyses were conducted with 100 pseudoreplicates by using the same parameters in the program GARLI. Bayesian phylogenetic analyses were performed with the program MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). A Markov chain Monte Carlo (MCMC) algorithm was employed for 1,000,000 generations, sampling trees every 200 generations, with four independent chains running simultaneously. The general time-reversal model (GTR; Swofford et al., 1996) with six rate parameters and the gamma distribution was used to estimate the likelihood values. The first 500 trees (100,000 generations) were discarded as "burn-in," and the remaining trees for which the log-likelihood values had reached a plateau were imported in PAUP* to calculate the posterior probability of each clade. The number of burn-in generations was determined by plotting a graph of the log-likelihoods of each generation vs. generation numbers (Huelsenbeck and Ronquist, 2001) in Microsoft Excel, and the posterior probability was computed by generating a 50% majority-rule consensus tree.

Evolutionary patterns of selected morphological characters (Table 2) were analyzed by using the program MacClade version 4.08 (Maddison and Maddison, 2000). Ancestral character states were reconstructed using parsimony optimizations for each character on the ML tree. *Alvaradoa* and *Picramnia* were used as outgroups, and *Citrus*, *Gossypium* and *Tapiscia* were excluded from the tree prior to the analysis, as the character states for the distantly related outgroups may not represent accurate state for the orders. Character states were coded based on literature (Dikison, 1981, 1986, 2007; Sosa and Chase, 2003; Matthews and Endress, 2005; Oh and Potter, 2006; Forest, 2007; Kubitzki, 2007; Schneider, 2007) and observation made by the author. Polymorphism found within a taxon was coded as such. For example, leaf arrangement for *Aphloia* was coded as polymorphic, as the taxon has alternate, opposite, and verticillate leaves. Inflorescence type for *Stachyurus* was coded as polymorphic, as it has both spike and raceme.

Results

The final alignment of the combined *atpB*, *matK*, and *rbcL* data set included 4,494 sites. Statistics of several components of data

Table 2. Morphological characters for the pattern analysis.

Character	State	Description
Leaf arrangement	0	Alternate
	1	Opposite
	2	Verticillate
Inflorescence position	0	Terminal
	1	Axillary
Inflorescence type	0	Solitary
	1	Spike
	2	Raceme
	3	Panicle
Fruit type	0	Follicle
	1	Berry
	2	Capsule
Stigma	0	Capitate
	1	Punctiform
Ovule number per carpel	0	1 – 2
	1	3 – 10
	2	> 10
Aril	0	Absent or rudimentary
	1	Present
Pollen bud	0	Absent
	1	Present

Table 3. Summary statistics of the data matrix and phylogenetic analyses of the combined *atpB*, *matK*, and *rbcL* data.

	Value
No. of taxa	20
No. of characters	4,494
No. of variable characters	1,122 (25.0%)
No. of parsimony-informative characters	515 (11.5%)
No. of MP trees	3
Length of MP trees	1,660
CI, excluding autapomorphy	0.6465
RI	0.7483
Best-fitting model selected	GTR + I + G
Alpha	0.7752
Pinvar	0.3180
- ln *L* for ML tree	15,278.637

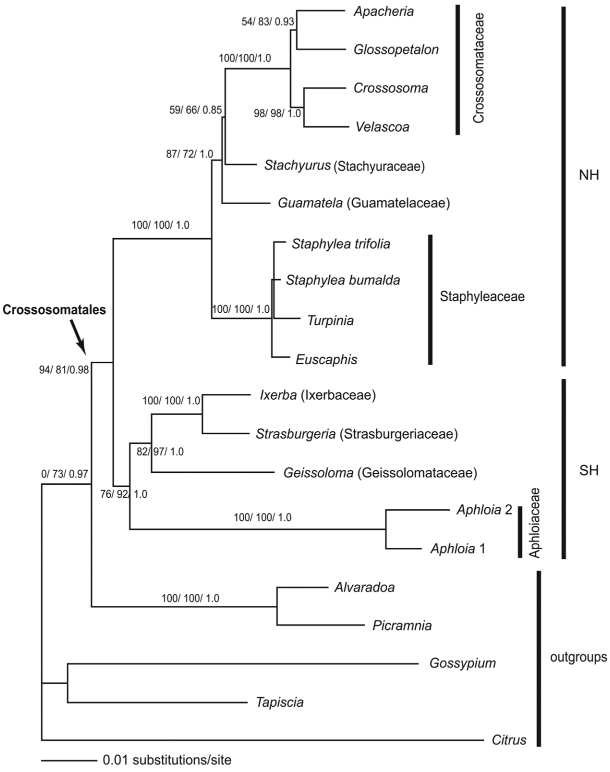

Fig. 1. The ML phylogram from the phylogenetic analysis of the combined *atpB*, *matK*, and *rbcL* data. One of the three MP trees is identical to the ML tree. Bootstrap values greater than 50% under MP, ML, and Bayesian posterior probability, each in that order separated by a slash, are indicated above or below branches. NH indicates the Northern Hemisphere clade, and SH means the Southern Hemisphere clade.

matrix and phylogenetic analyses, such as number of parsimony-informative sites, consistency index, likelihood score for the ML tree, were provided in Table 3.

Phylogenetic analyses using different methods (MP, ML, Bayesian) consistently indicated that Crossosomatales is a strongly supported monophyletic group (Fig. 1). Relationship among the families within Crossosomatales was also congruent across the analyses. MP analysis produced three optimal trees, which differed by the branching order within Staphyleaceae, and one of MP trees resolved *Euscaphis* as sister to *Staphylea* and *Turpinia*, a relationship also supported in the ML and Bayesian analyses (Fig. 1).

Families in the Southern Hemisphere were strongly supported as a monophyletic group (hereafter refers as to SH clade), sister to a clade of the Northern Hemisphere families (NH clade). Aphloiaceae, represented by two accessions in this study, was confirmed as the first diverging lineage among the Southern Hemisphere families, and Geissolomataceae as a sister to the Ixerbaceae and Strasburgeriaceae clade. Bootstrap values and posterior probability for the relationships among the four families were high. In the NH clade, Staphyleaceae was strongly supported as sister to a clade of Guamatelaceae, Stachyuraceae, and Crossosomataceae. Guamatelaceae was placed as a sister to the Crossosomataceae and Stachyuraceae clade.

Figs. 2 and 3 represent ancestral character reconstructions over the ML tree using the parsimony optimization method. Branches of Picramniaceae were pruned in the figures to show the patterns within Crossosomatales. Evolution of the morphological characters was considerably homoplasious with complex patterns of state gains and losses. Each fruit type evolved multiple times within the order: follicles evolved three times, capsules twice, and berries at least three times (Fig. 2A). Evolutionary pattern of ovule number was also complex, particularly within the NH clade (Fig. 2B). Ambiguity for the ancestral state within the NH was derived from the parsimonious optimization option. When the DELTRAN option was selected, which delays the early change of a state favoring changes at the descendant nodes, the state of 1-2 ovules per carpel was inferred as the ancestral state for the internal nodes in the NH clade. On the other hand, the state of more than 10 ovlues per carpel was suggested as the ancestral condition for the NH clade under the ACCTRAN option, which prefers to change a state at the earliest nodes possible resulting in multiple losses of the state at later nodes. Inflorescence type might be a good indicator for the relationship among the families. Panicles evolved twice independently, once in Staphyleaceae and the other in Ixerbaceae (Fig. 2C). Solitary flower also evolved twice,

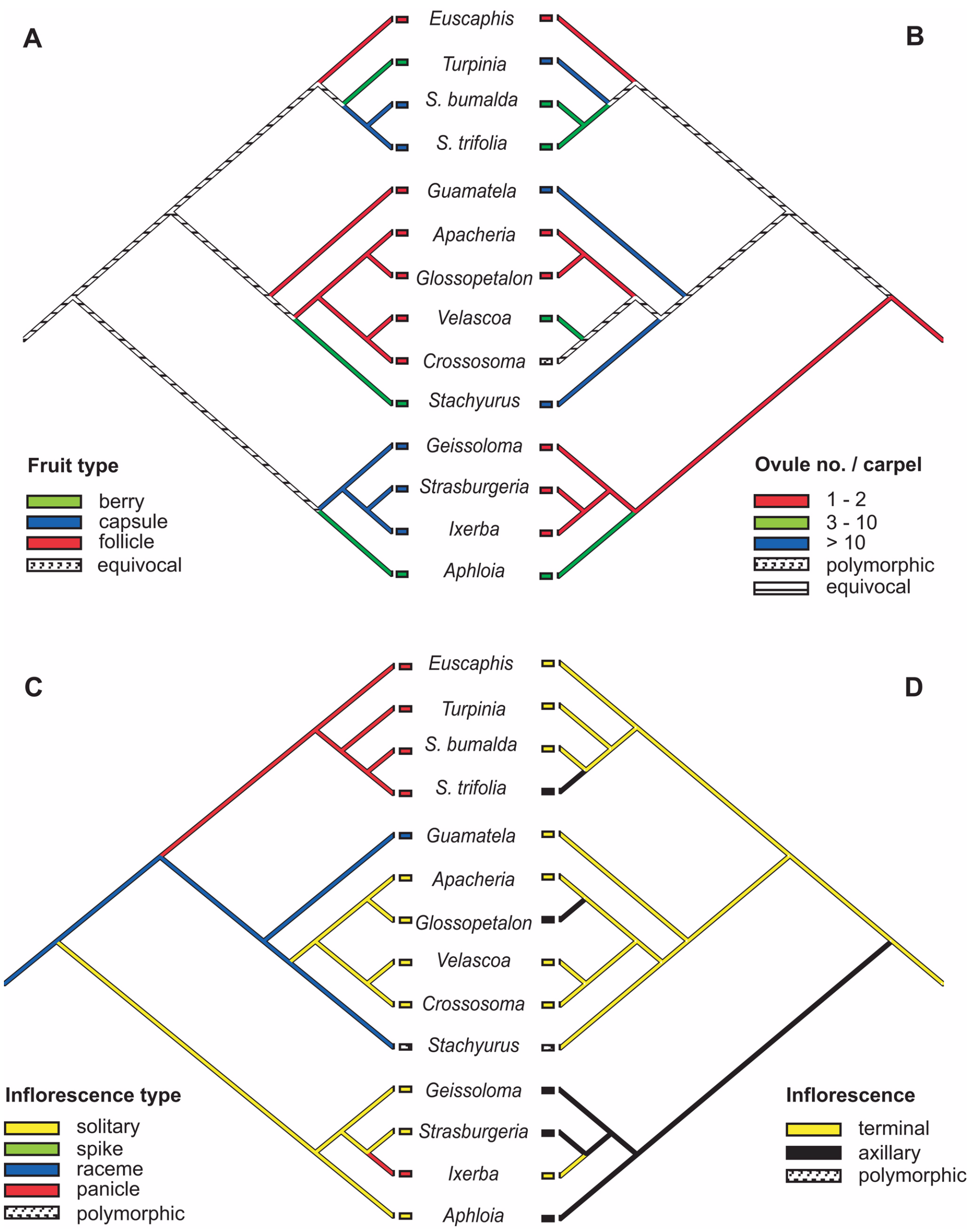

Fig. 2. Parsimony reconstruction of ancestral states. A. fruit type; B. number of ovules per carpel; C. inflorescence type; D. inflorescence position.

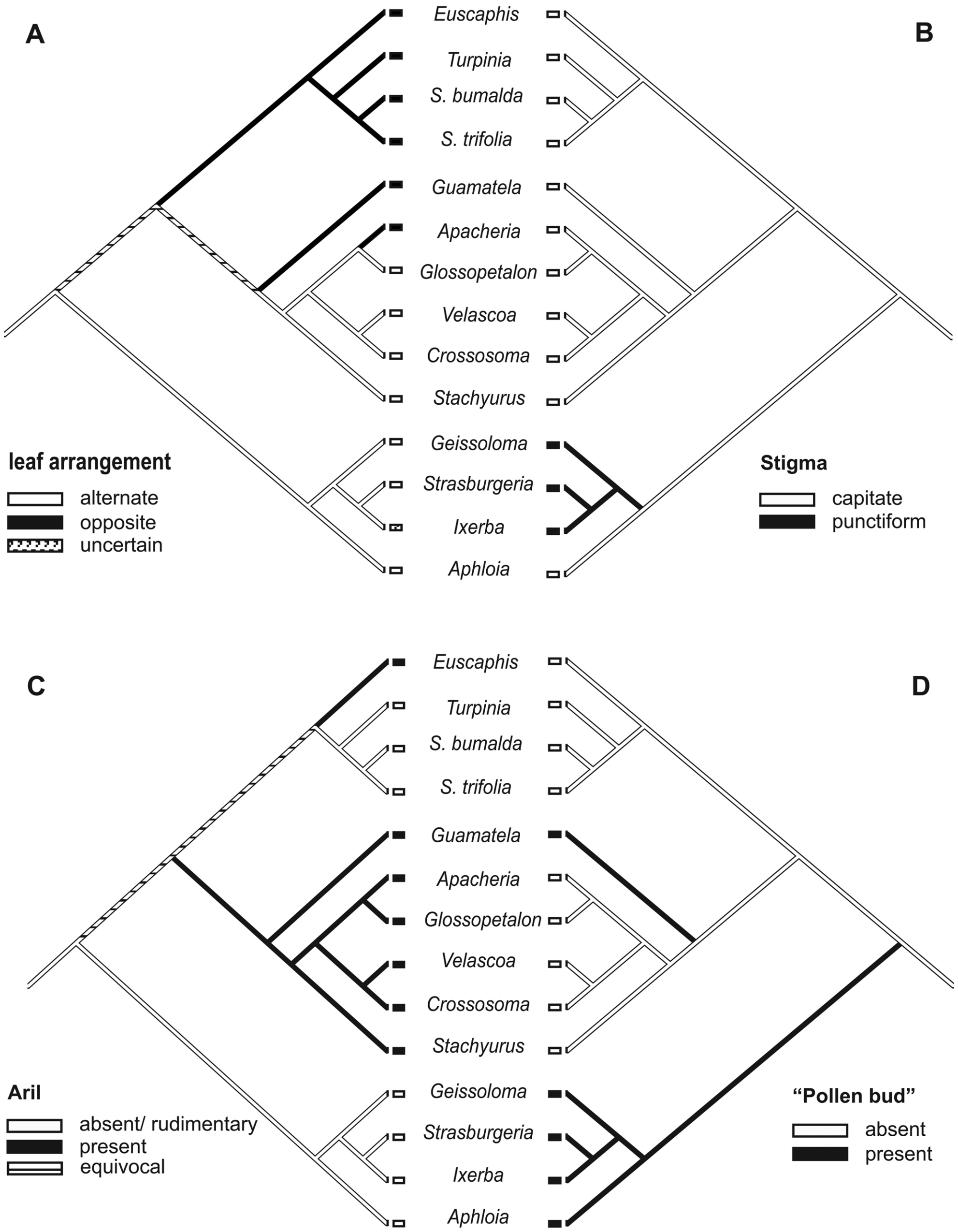

Fig. 3. Parsimony reconstruction of ancestral states. A. leaf arrangement; B. Stigma type; C. Aril; D. Pollen bud.

in Crossosomataceae and in the SH clade, but the position where the flowers are born is different. Solitary flowers in the SH clade are axillary, while those in Crossosomataceae develop at the tip of branches, with the exception of *Glossopetalon* which has axillary flowers. Axillary flowers evolved three times in Crossosomatales with a reversal to terminal inflorescence in Ixerbaceae (Fig. 2D).

Reconstructed patterns of evolution in leaf arrangement, stigma type, presence of aril on seed, and presence of "pollen bud" (Fig. 3) were simple compared with other reproductive traits presented in Fig. 2. Ancestral state of leaf arrangement for the NH clade has uncertainty due to optimization options. Under the DELTRAN option, opposite leaf was indicated as it may have evolved three times: in Staphyleaceae, Guamatelaceae, and *Apacheria*. Under the ACCTRAN option, the parsimony reconstruction inferred the gain of opposite leaf in the NH node, reversal to alternate in the Crossosomataceae with the independent gain of opposite leaf in *Apacheria*, making at least three evolutionary steps within the order. Alternate leaf is the ancestral state for the polymorphic Ixerbaceae, in which opposite and verticillate leaves are also found. Evolution of stigma showed a unique pattern, where punctiform evolved in the most recent common ancestor of the Ixerbaceae, Strasburgeriaceae, and Geissolomataceae clade (Fig. 3B). Aril and pollen bud evolved twice, respectively, within Crossosomatales (Figs. 3C, D).

Discussion

The combined data of *atpB*, *matK*, and *rbcL* genes presented in this study support the monophyly of Crossosomatales. Strong supports for the clade with all genera of Crossosomatales shown here are novel and substantially corroborated with morphology (Matthews and Endress, 2006). The data also produce a well-supported phylogenetic hypothesis among the families within Crossosomatales (Fig. 1). While it is not surprising that the NH clade was strongly supported in this study, as the clade has been identified in previous studies (Cameron, 2003; Sosa and Chase, 2003; Oh and Potter, 2006; Wang et al. 2009), the data provides significant insights into the phylogenetic relationship of the families in the Southern Hemisphere, by confirming a single origin of the families with strong support. It is, however, difficult to find unique morphological characters for the SH clade, as the monotypic families are morphologically distinctive. Conspicuous protrusion of "pollen bud" from the apertures of pollen might be a synapomorphy with an independent evolution in Guamatelaceae in the NH clade (Fig. 3). The pollen character is very rare in angiosperms, only reported from Proteaceae and Rubiaceae (Oh and Potter, 2006). Solitary flowers born in the axils of the leaves (Fig. 2) might also be considered as a synapomorphy for the clade. It requires that an evolution of terminal panicles in *Ixerba* within the SH clade and that the separate evolution of axillary, solitary flower in *Glossopetalon*.

It is noteworthy that Aphloiaceae is sister to all other families in the SH clade with Geissolomataceae sister to the Ixerbaceae and Strasburgeriaceae clade, a relationship that has been uncertain until now. Wang et al. (2009) in their phylogenetic analysis of rosids using the massive amount of chloroplast sequence data supported the close relationship of Ixerbaceae, Strasburgeriaceae, and Aphloiaceae, but relationship of the South African Geissolomataceae was unknown, as the family was not included in that study. The strong phylogenetic signal supporting the relationship within the SH clade in this study should have been derived from the addition of the *matK* data for all Crossosomatales. All previous studies do not sample the gene entirely (Cameron, 2003; Sosa and Chase, 2003) or for the Southern Hemisphere families (Oh and Potter, 2006; Wang et al., 2009). Preliminary analyses of 12 chloroplast genes from Crossosomatales, including the three gene used in this study, and of the nuclear *SHORT ROOT* gene indicate the same relationship of the SH clade as this study (data not shown).

A suite of morphological characters corroborates the clade of Geissolomataceae, Ixerbaceae, and Strasburgeriaceae (Figs. 2, 3). The stigma of these taxa is punctiform with the postgenitally fused tips of the carpels, a derived shared character state for the clade in Crossosomatales (Fig. 3B). The stigma of all other Crossosomatales is capitate or flattened. In addition, other reproductive characteristics, such as capsule and the very small number of ovules per carpel (1 or 2), unite these three families (Figs. 2A, B). Capsules are also found in *Staphylea* (Fig. 2A). But the fruits in *Staphylea* are highly inflated at maturity, and different from those in Ixerbaceae, Strasburgeriaceae, and Geissolomataceae, though the fruit type was coded as the same state in this study.

The close relationship of Ixerbaceae and Strasburgeriaceae, supported in this study and other previous molecular data, is strongly corroborated by morphology (Matthews and Endress, 2005). The two families share a number of unique characteristics, including gland-toothed leaf margins, large flowers, vestigial aril on the ovule, and cells in perianth with thickened mucilaginous inner tangential walls. Both families have the base chromosome number (x) of 25, which may also be a synapomorphy for the clade. But the cytological information in Aphloiaceae and Geissolomataceae should be confirmed to support the proposition. Stevens (2001 onward) recognizes that Strasburgeriaceae includes *Ixerba*. *Ixerba brexioides*, the only species in Ixerbaceae, was classified in Glossulariacaee (Cronquist, 1981) and often placed in Brexiaceae based on the similarity of floral features (Cunningham, 1839; Takhtajan, 1997). It is interesting to note that the name *Ixerba* is an anagram of *Brexia*, and its specific epithet is also a derivative of *Brexia*. Strasburgeriaceae is very distinctive from Ixerbaceae by having spirally arranged leaves with intrapetiolar stipules, 10 stamens, large dry and indehiscent fruits, and extremely high number of chromosome ($2n = 500$), one of the highest in angiosperms (Oginuma et al., 2006).

While the monophyly of the NH clade is strongly supported by the molecular data (Fig. 1), there are few morphological characters that may define this clade. Stevens (2001 onward) suggests that the NH clade may be characterized by these three traits: involute leaves or leaflet, the terminal inflorescence, and more or less expanded stigma. However, exceptions to all of these characters are found within the clade, making it difficult to evaluate the significance of the characters. Leaves of *Guamatela* are not involute, and axillary flowers can be found in *Glossopetalon* and some species of *Staphylea*, while terminal flowers also found in *Ixerba* in the SH clade. All of taxa in the NH clade possess the capitate or flattened stigma, but *Aphloia* has also capitate stigma, making it as an ancestral state in the Crossosomatales (Fig. 3D). Flowers in cluster (i.e., non-solitary flowers) may characterize the clade, but it has to be assumed that reduction to solitary flowers independently evolved in Crossosomataceae (Fig. 2C).

Staphyleaceae needs more comprehensive systematic study. Staphyleaceae is the most diverse family in Crossosomatales in the number of species and distribution (Table 1). The family is easily distinguished from other members of Crossosomatales by having opposite leaves that are often pinnately or tri-foliately compound (a few species of *Turpinia* have simple leaves) and panicle inflorescences (Figs. 2C, 3A). Within the family, fruit type has been used to delimit the three traditionally recognized genera: *Staphylea* species have inflated, bladder-like capsules; *Euscaphis* red, showy follicles; and *Turpinia* berries with flesh or leathery fruit walls. Simmons (2007) proposed a new generic classification based on her preliminary analysis of ITS and cpDNA markers (*trnK*, *rps12*-*rpl20*, 23S-16S, and *trnL*), recognizing two genera in Staphyleaceae. In this arrangement, *Staphylea* includes *Euscaphis*, New World species of *Turpinia*, and the Asian *T. cochinchinensis*; all other Old World species of *Turpinia* are placed in a resurrected genus, *Dalrympelea*. No strong morphological evidence, however, is available to support this rearrangement, specifically the integration of *Euscaphis* and parts of *Turpinia* into *Staphylea*. The taxonomic sampling for Staphyleaceae in this study is too sparse to test Simmons (2007).

The clade of Guamatelaceae, Stachyuraceae, and Crossosomataceae is confirmed in this study (Fig. 1). Arillate seeds may be the synapomorphy for the clade (Fig. 3C). It is likely that the seed character may have evolved independently in *Euscaphis* (Fig. 3C). The monotypic genus *Guamatela*, previously classified in Rosaceae based on the stipulated leaves, pentamerous flowers, and follicular fruits, has been segregated into its own family, Guamatelaceae (Oh and Potter, 2006). The molecular phylogeny in this study (Fig. 1) continues to support the recognition of the Guamatelaceae, as accepted in APG III (2009).

Crossosomataceae is an endemic family in the deserts of North America (Table 1). Species of Crossosomataceae are mostly microphyllous shrubs with small solitary flowers, and they often grow in limestone or rhyolitic rock crevices. *Crossosoma californicum* endemic to the Channel Islands of California and Guadalupe Island in Mexico is an exception: large shrubs of the chaparral with large leaves and flowers. Stachyuraceae is an endemic family in eastern Asia with about ten species with the center of diversity in China. The sister relationship between Crossosomataceae and Stachyuraceae (Fig. 1) implies that there is a disjunctive distribution pattern between western North America and eastern Asia involving these taxa. It needs to further study to infer the origin of Crossosomataceae, whether or not the desert plants were derived from an Asian ancestor, associated with biome shift. The xerophytic traits in Crossosomataceae, such as small leaves, branches often spiny at the tip, and the simple perforation plates (Carlquist, 2007), are derived features within Crossosomatales, as its closest relatives (Stachyuraceae, Guamatelaceae, Staphyleaceae) are all mesophytic. *Crossosoma californicum* may be an intermediate taxon or a relict in the transition to xerophytes. A more detailed phylogenetic study of the Crossosomataceae is underway.

Species delimitation in *Stachyurus* has been highly controversial. Li (1943) recognized 12 species; Chen (1981) reported 16 species and eight varieties; Shan (1999) treated ten species and five varieties from China; and Qiner and Stevens (2007) recognized seven species from China. The phylogeny of the monogeneric Stachyuraceae and its relatives was recently estimated based on sequences of four chloroplast DNA regions (*trnL-trnF*, *rps*16, *ndhF*, and *trnS-trnG*) and the nuclear ITS region (Zhu et al., 2006). *Stachyurus sigeyosii* from Taiwan was previously synonymized as *S. himalaicus*, but Zhu et al. (2006) have shown that it is sister to the morphologically highly distinct *S. praecox* from Japan, and does not form a clade with *S. himalaicus.* Species delimitation and diversification of this eastern Asian endemic genus need to be further analyzed and tested.

The sequence data of *atpB*, *matK*, and *rbcL* genes provide a strong phylogenetic structure among the families within Crossosomatales. The phylogeny provides a significant framework for the pattern analysis, which not only reveals the complexity of the patterns of morphological evolution but also identifies synapomorphies for subclades within the order. Given the distribution of Crossosomatales across the Northern and Southern Hemispheres, further studies with complete sampling of tropical taxa, of which biodiversity is uncertain to date, are necessary to understand the evolution of Crossosomatales.

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Appendix 1. List of taxa used in the phylogenetic analyses of this study and GenBank accession numbers for *atpB*, *matK*, and *rbcL*. Sources for plant or DNA materials, from which sequences were newly determined for this study, were provided. Dashes indicate sequences were not available for this study.

Outgroups. Malvaceae: *Gossypium hirtusum* L., DQ345959 (complete chloroplast genome); **Picramniaceae**: *Alvaradoa amorphoides* Liebm., AJ235387.2, ____, AF123277.1; *Picramnia pentandra* Sw., AJ235559.2, _____, AF127025.1; **Rutaceae**: *Citrus sinensis* (L.) Osbeck, DQ864733 (complete chloroplast genome); **Tapisciaceae**: *Tapiscia sinensis* Oliver, AF209685, EU002190, ____.

Crossosomatales.

Aphloiaceae: *Aphloia theiformis* (Lahl) Benn. (*Aphloia* 1), AF209528, ___, AF206735; *Apholia* 2, HQ680705, HQ680692, HQ680702- *Ranirison 635* (MO).

Crossosomataceae: *Apacheria chiricahuensis* C. T. Mason, HQ680706, HQ680693, HQ680703 (*Oh 316*, BH); *Crossosoma bigelovii* S. Watson, HQ680707, HQ680694 (*Oh 5342*, BH), [*Crossosoma californicum* Nutt., L11179, *rbcL*]; *Glossopetalon spinescens* A. Gray var. *aridum* M. E. Jones, HQ680708, HQ680695, AY101845 (*Oh 5343*, BH); *Velascoa recondita* Calderón and Rzed., HQ680709, HQ680696, AY101846 (*Rubio 2714,* IEB).

Geissolomataceae: *Geissoloma marginatum* (L.) A. Juss., HQ680710, HQ680697, X83990 (Kew DNA Bank #16029).

Guamatelaceae: *Guamatela tuerckheimii* Donn. Sm., DQ443453, DQ443461, DQ443463.

Ixerbaceae: *Ixerba brexioides* A. Cunn., AF209606, HQ680698, AF084475 (Kew DNA Bank #18927).

Staphyleaceae: *Euscaphis japonica* (Thunb.) Kanitz, DQ663627, DQ663628, DQ663626; *Staphylea trifolia* L., AJ235611, HQ680699, AJ238406 (*Oh 5116*, BH), *S. bumalda* DC., HQ680711, HQ680700, HQ680704 (*Oh 5117*, BH); *Turpinia paniculata* Vent., DQ443454 DQ443459 AJ403013.

Strasburgeriaceae: *Strasburgeria robusta* (Vieill. ex Panch. and Seb.) Guillaumin, AJ502597, HQ680701, AJ403007 (Kew DNA Bank #22640).