

Phylogenetics of *Bauhinia* subgenus *Phanera* (Leguminosae: Caesalpinioideae) based on ITS sequences of nuclear ribosomal DNA

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Abstract. The pantropical legume genus *Bauhinia* is currently subdivided into four subgenera. Subgenus *Phanera*, with ca. 150 species, is the largest. A parsimony analysis was conducted on ITS sequence data from 38 species, with representatives from all four subgenera, and species sampled from nine of 11 sections of subgen. *Phanera*, and including two *Cercis* species as the outgroup. The well-resolved strict consensus tree suggests that subgen. *Phanera* and the monotypic subgen. *Barklya* together form a monophyletic group. In the *Phanera*-*Barklya* clade, the African (sect. *Tylosema*), American (sect. *Caulotretus*), and Australasian (subgen. *Barklya*, subgen. *Phanera* sect. *Lysiphyllum*) species are the first (paraphyletic) lineages, while the Asian taxa (except species of sect. *Lysiphyllum*) form a monophyletic group. The ITS tree also revealed that sects. *Phanera* and *Lasiobema* are not monophyletic. Monophyletic groups supported by the phylogenetic analysis include ser. *Fulvae* and ser. *Corymbosae* of sect. *Phanera*, sect. *Tubicalyx*, and the Asian-Australasian sect. *Lysiphyllum*.

Keywords: *Bauhinia*; Caesalpinioideae; ITS; Leguminosae; Phylogenetic analysis; subgen. *Phanera*.

Introduction

Bauhinia L., a pantropical genus of about 300 species (Wunderlin et al., 1987) of trees, shrubs or lianas with bifoliolate, bilobed or simple entire leaves, has always been controversial in its taxonomy, including whether or how it could be divided into small genera.

According to Wunderlin et al. (1981, 1987), the ancestor of the genus gave rise to two main phyletic lines, one to the mostly arborescent or shrubby subgenera *Bauhinia*, *Elayuna* and *Barklya*, the second to subgenus *Phanera*, which mostly comprises lianas. A morphological cladistic analysis of the genus by Zhang (1995) and a survey of the *rpL2* chloroplast intron for 78 species by Lai et al. (1997), however, both yielded results not fully compatible with the conclusions of Wunderlin et al.

Subgenus *Phanera*, comprising about 150 species, is the largest subgenus in *Bauhinia*. Pantropical in distribution, but with most of its species occurring in continental Asia and the Malesia region, the subgenus is subdivided into 11 sections (Wunderlin et al., 1987). Several of these sections are further subdivided into subsections or series.

In their recent phylogenetic analysis of the subfamily Caesalpinioideae based on chloroplast *trnL* intron sequences, Bruneau et al. (2001) suggested that the sub-

genus *Phanera*, as well as the whole genus *Bauhinia*, might not be monophyletic.

Although taxonomic and floristic studies on the genus *Bauhinia* in general, and on subgen. *Phanera* in particular, have helped improve our knowledge of the group (Chen, 1988; Larsen and Larsen, 1973, 1983, 1991, 1996; Larsen et al., 1980, 1984; Zhang, 1993), systematic studies on the genus are still hampered by its large number of species, prevalence of morphological convergence, and difficulties in obtaining material. In this study, we newly sequenced nuclear ITS regions from representative species of *Bauhinia*, with the aim of assessing the monophyly of subgen. *Phanera* as currently defined, and of clarifying the intra-subgeneric relationships within the group.

Materials and Methods

Taxon Sampling

A total of 38 species were included in this molecular study (Table 1). For the ingroup we sampled 40 accessions representing 32 species of subgen. *Phanera*, as well as two species of subgen. *Bauhinia*, one species of the monotypic subgen. *Barklya*, and one species of subgen. *Elayuna*. The ingroup taxa chosen covered all four subgenera recognized by Wunderlin et al. (1987). The 32 species of subgen. *Phanera* represented nine of the 11 sections (Wunderlin et al., 1987). Most of the species chosen were from the two larger sections that are apparently polymorphic, viz., sect. *Lasiobema* and sect. *Phanera*. For some sections, e.g., sects. *Palmatifolia*, *Tubicalyx*, and

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Table 1. Taxa sampled in the molecular study of *Bauhinia* subgen. *Phanera*. Vouchers are deposited at IBSC, with a few samples otherwise from accessioned living collections in several botanic gardens. Taxonomic framework follows Wunderlin, Larsen, and Larsen (1987).

Taxon	Source/Voucher	GenBank accession number
<i>Bauhinia</i> aff. <i>didyma</i> L. Chen	Guangxi, China; R.-J. Wang 5	AY258390
<i>B. apertilobata</i> Merr. et Metc.	Guangdong, China; D.-X. Zhang 923	AY258397
<i>B. aurea</i> Levl.	Guangxi, China; S.-J. Li and X.-X. Huang 4	AY258410
<i>B. bohniana</i> L. Chen	Yunnan, China; Y.-F. Deng 14097	AY258403
<i>B. carcinophylla</i> Merr.	Guangxi, China; S.-J. Li and X.-X. Huang 14	AY258389
<i>B. carronii</i> F. v. Muell.	Royal Botanical Garden, Sydney, Australia (living collection); P. Weston 2445	AY258400
<i>B. championii</i> (Benth.) Benth. 1	Guangxi, China; R.-J. Wang s.n.	AY258377
<i>B. championii</i> (Benth.) Benth. 2	Guangdong, China; D.-X. Zhang s.n.	AY258386
<i>B. championii</i> (Benth.) Benth. 3	Guangxi, China; S.-J. Li and X.-X. Huang 24	AY258388
<i>B. clemensiorum</i> Merr.	Guangxi, China; D.-X. Zhang s.n.	AY258391
<i>B. corymbosa</i> Roxb. ex DC. var. <i>corymbosa</i>	Hongkong, China; D.-X. Zhang s.n.	AF286357
<i>B. corymbosa</i> Roxb. ex DC. var. <i>longispes</i> Hosokawa	Hainan, China; R.-J. Wang s.n.	AY258376
<i>B. didyma</i> L. Chen	Guangdong, China; Y.-F. Deng 14470	AY258383
<i>B. fassoglensis</i> Schweinfurth	Fairchild Botanic Garden, USA (living collection); Benoit Jonckheere supplied	AY258393
<i>B. gilva</i> (F.M. Bailey) A.S. George	Royal Botanical Garden, Sydney, Australia (living collection); P. Weston 2446	AY258401
<i>B. glabra</i> Jacq.	Fairchild Botanic Garden, USA (living collection); Benoit Jonckheere supplied	AY258409
<i>B. glauca</i> (Wall. Ex Benth.) Benth. ssp. <i>glauca</i>	Hong Kong, China; D.-X. Zhang s.n.	AY258384
<i>B. glauca</i> Benth. ssp. <i>hupehana</i> (Craib) T. Chen	Guangxi, China; R.-J. Wang 1	AY258380
<i>B. hainanensis</i> Merr. et Chun	Hainan, China; P. Zou and R.-L. Han 16	AY258407
<i>B. integrifolia</i> Roxb	Singapore Botanical Garden (living collection); Ruth Kiew supplied	AY258396
<i>B. japonica</i> Maxim.	South China Botanic Garden (living collection)	AF286358
<i>B. jenningsii</i> P. Wils. ex Britton	Fairchild Botanic Garden, USA (living collection); Benoit Jonckheere supplied	AY258411
<i>B. khasiana</i> Baker	Guangxi, China; R.-J. Wang s.n.	AY258381
<i>B. kockiana</i> Korth.	Singapore Botanical Garden (living collection); Ruth Kiew supplied	AY258394
<i>B. longistipes</i> T. Chen	Yunnan, China; Y.-F. Deng 14078	AY258379
<i>B. nervosa</i> Craib	Yunnan, China; D.-X. Zhang s.n.	AY258399
<i>B. paucinervata</i> T. Chen	Guangxi, China; S.-J. Li and X.-X. Huang 21	AY258387
<i>B. pyrroclada</i> Drake	South China Botanic Garden (living collection)	AF286359
<i>B. reticulata</i> DC.	Singapore Botanical Garden (living collection); Ruth Kiew supplied	AY258404
<i>B. roxburghiana</i> Voigt	Fairchild Botanic Garden, USA (living collection); Benoit Jonckheere supplied	AY258392
<i>B. scandens</i> L. var. <i>horsfieldii</i> (Watt. Ex Prain) K. et S. S. Larsen	Hainan, China; G.-A. Fu 6052	AY258408
<i>B. semibifida</i> Roxb.	Singapore Botanical Garden (living collection); Ruth Kiew supplied	AY258395
<i>B. sp.</i> (ser. <i>Fulvae</i>)	Guangxi, China; S.-J. Li and X.-X. Huang 5	AY258385
<i>B. strychnifolia</i> Craib	Dongphahuan Botanical Garden, Thailand; C. Niyomdham supplied	AY258405
<i>B. strychnoidea</i> Prain	Narathiwat, Thailand; C. Niyomdham s.n.	AY258406
<i>B. syringifolia</i> (F. v. Muell.) Wunderlin	Royal Botanical Garden, Sydney, Australia (living collection); D.-X. Zhang s.n.	AY258398
<i>B. touranensis</i> Gagn.	Guangxi, China; R.-J. Wang 4	AY258382
<i>B. variegata</i> L.	Guangdong, China; D.-X. Zhang s.n.	AY258378
<i>B. winitii</i> Craib	Bankok, Thailand; C. Niyomdham s.n.	AY258402
<i>B. yunnanensis</i> Franch	Yunnan, China; Y.-F. Deng 14050	AF286360
<i>Cercis chinensis</i> Bunge	Jiangsu, China; Y.-F. Deng 13289	AF286351
<i>C. glabra</i> Pamp.	Yunnan, China; L.-X. Guo s.n.	AF286356

Tylosema, only one or two species were represented. However, these sections are readily identifiable morphologically and by leaf venation patterns (Wunderlin et al., 1987; Zhang, 1994) and have been consistently recognized as natural groups. The three species of sect. *Lysiphyllum* included in this study represented its two subsections. Recent molecular studies (Bruneau et al., 2001) confirmed that *Cercis*, another genus of tribe Cercideae (Wunderlin et al., 1987), has close affinity with *Bauhinia*. We thus chose two species of *Cercis* (*C. chinensis* and *C. glabra*) as the outgroup taxa in this study.

Genomic DNA Extraction, PCR Amplification, and Sequencing

Total DNA was extracted from fresh or silica-dried leaves, following a 2X CTAB method modified from Doyle and Doyle (1987). The entire ITS region was amplified using universal primers "ITS 5" and "ITS 4" (White et al., 1990). PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN). Automated sequencing was conducted using dye terminator on an ABI 377 DNA Sequencer, using at least two of the four primers N18L18 (Wen and Zimmer, 1996), ITS2, ITS3, and ITS4 (White et al., 1990).

Phylogenetic Analysis

The combined sequences of ITS1, 5.8S and ITS2 were initially aligned using Clustal X (Thomson et al., 1997), followed by minor adjustment by eye. Potentially informative and unambiguously assessed indels were scored as separate characters regardless of their length, and added to the data matrix as additional binary or multistate characters (25 such characters in total). Maximum parsimony analysis was performed using PAUP* (Swofford, 1998) on a Macintosh computer. The analysis used heuristic searches with random addition and TBR branch swapping. Clade robustness was evaluated by bootstrap analysis using 1000 replicates of heuristic searches, with simple addition sequence and TBR branch swapping. Sequence divergence values were calculated by the Kimura two-parameter method (Kimura, 1980).

Results

Variation in the ITS Sequences

The length of the entire ITS region in *Bauhinia* species studied ranges from 594 to 626 bp; the 5.8S region is 163 or 164 bp long while the ITS1 and ITS2 regions range between 212 - 247 and 203- 248 bp, respectively. The consensus sequence for the 42 sampled accessions is 689 bp in length. The Kimura two-parameter distance among the ingroup species is estimated as 0.19% to 40.19% (data matrix not shown). If the multiple accessions of *B. championii*, *B. glauca*, and *B. corymbosa* are ignored respectively, the lowest divergence is between *B. didyma* and *B. glauca* (both of subgen. *Phanera*); the highest value is between *B. touranensis* (of subgen. *Phanera*) and *B. variegata* (of subgen. *Bauhinia*).

Phylogenetic Analysis

The parsimony analysis generated nine trees of 1,282 steps, with a consistency index (CI) of 0.48, and a retention index (RI) of 0.65. The strict consensus tree is shown in Figure 1. The monophyly of sampled *Bauhinia* species has maximum support (100%). Two species representing subgen. *Bauhinia* (*B. variegata* and *B. jenninsii*), and the one species representing subgen. *Elayuna* (*B. reticulata*), form a clade with weak support (62%), which is the sister to the clade containing subgen. *Phanera* and *Barklya*. For the latter clade, the African species, *B. fassoglensis* of subgen. *Phanera* sect. *Tylosema*, is the first to branch out, followed by the American species, *B. glabra* (representing subgen. *Phanera* sect. *Caulotretus*) and the Australasian *B. syringifolia* (the sole species of subgen. *Barklya*). The remainder of subgen. *Phanera* forms a clade. The above branching patterns receive relatively high bootstrap support (Figure 1). A significant feature in the latter clade is that sect. *Phanera*, the biggest section of subgen. *Phanera*, is highly polyphyletic, including at least five distantly related lineages, although their relationships with other sections are always relatively weakly supported. Sect. *Lasiobema* is also revealed as a heterogeneous assembly of three lineages with the present sampling, with its members scattered throughout the strict consensus tree (Figure 1).

Discussion

Monophyly of Subgen. *Phanera*

As the ITS tree (Figure 1) shows, the genus *Bauhinia* is divided into two branches. The first branch only includes subgen. *Bauhinia* and subgen. *Elayuna*, not *Barklya* as Wunderlin et al. (1981, 1987) otherwise proposed. Subgen. *Phanera* is thus parapatetic since subgen. *Barklya* (*B. syringifolia*) is nested within it. The ITS analysis also revealed that in the subgen. *Phanera* - *Barklya* clade, the African (sect. *Tylosema*), American (sect. *Caulotretus*), and Australasian (subgen. *Barklya*, subgen. *Phanera* sect. *Lysiphyllum*) species are the first to branch out, while the Asian taxa form a monophyletic group. Unfortunately, the monotypic New Guinean section *Austrocercis* and the tropical American section *Schnella* were not sampled in the present analysis, and should be included to test the monophyly of the sections.

Taxonomic Implications and Character Evolution

The monophyletic groups supported by the present phylogenetic analysis based on ITS sequences, e.g., sect. *Phanera* ser. *Fulvae*, ser. *Corymbosae*, sect. *Tubicalyx*, and sect. *Lysiphyllum*, are species groups with very similar flower morphology (Wunderlin et al., 1987), pollen types (Larsen, 1975; Larsen and Larsen, 1983; Ferguson and Pearce, 1986; Schmitz, 1973, 1977) and leaf venation patterns (Zhang, 1994).

Wunderlin et al. (1987) conjectured that the ancestral stock of Cercideae consisted of moderate-sized trees with bifoliolate compound leaves as found in the extant

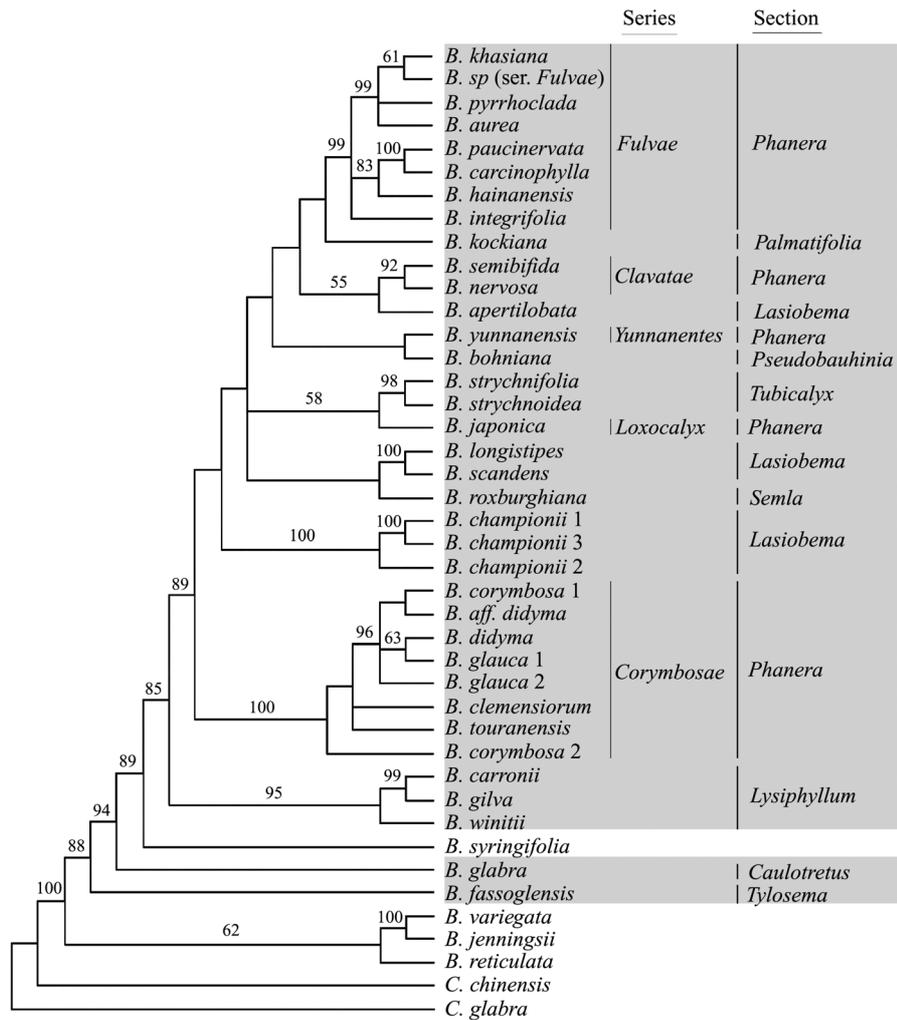


Figure 1. Strict consensus tree of the nine most parsimonious trees from the entire ITS sequences of *Bauhinia* species. Tree length = 1282 steps, CI = 0.48, RI = 0.65. Numbers above lines represent bootstrap values in 1000 replicates. Supraspecific classification follows Wunderlin et al. (1987). Grey areas show taxa from subgen. *Phanera*. Series of sect. *Phanera* are indicated. *B. corymbosa 1* = *B. corymbosa* var. *corymbosa*; *B. corymbosa 2* = *B. corymbosa* var. *longispes*; *B. glauca 1* = *B. glauca* ssp. *glauca*; *B. glauca 2* = *B. glauca* ssp. *hupehana*.

Detarieae (Caesalpinioideae). The arborescent habit—found in all species of subgen. *Bauhinia*, *Elayuna*, and *Barklya*, and some species of subgen. *Phanera*—is presumably the plesiomorphic state in the genus *Bauhinia*. In the *Phanera*-*Barklya* branches in the strict consensus tree (Figure 1), the first clades to branch out, viz., representatives of sects. *Tylosema* and *Caulotretus*, as most of the species in the subgenus, are lianas, while the arborescent taxa, e.g., *B. syringifolia* (subgen. *Barklya*), *B. carronii*-*B. gilva*-*B. winitii* (sect. *Lysiphyllum*), *B. roxburghiana* (sect. *Semla*), and *B. bohniana* (sect. *Pseudobauhinia*), do not form a monophyletic group. A reversal to the arborescent habit has very likely occurred independently in these taxa.

The relationships between bifoliate, bilobed, and unifoliate leaves in *Bauhinia* have been a fascination, and several studies had been performed on the topic (Watari, 1934; Pijl, 1951; Cusset, 1966; Owens, 2000). The so-called “split-

ter hypothesis” (e.g., Cusset, 1966, hypothesizing that the ancestral leaf form in Cercideae is unifoliate, from which the bilobed and the bifoliate leaves are derived) and the “fusion hypothesis” (e.g., Pijl, 1951, suggesting that the bifoliate leaf is the ancestral state in the tribe, from which the bilobed and unifoliate leaves are derived) have been proposed. The species with bifoliate leaves (e.g., *B. winitii*, *B. carcinophylla*) and unifoliate leaves (e.g., *B. syringifolia*, *B. strychnifolia*, *B. strychnoidea*, *B. paucinervata*) are scattered on the cladogram among species with bilobed leaves. Our study did not lend credence to either the “splitter hypothesis” or the “fusion hypothesis.” Our only conclusion is that both bifoliate and unifoliate leaves have arisen many times in *Bauhinia*.

The number of fertile stamens is another important character in *Bauhinia* classification. Ten fertile stamens are found in all other genera of *Cercideae*, in subgen. *Elayuna*, *Barklya*, and in most sections of subgen.

Bauhinia. Three fertile stamens are found in most species of subgen. *Phanera* while 10 fertile stamens are found in sects. *Lysiphyllum* and *Caulotretus*, although species with two fertile stamens have been found in sects. *Tylosema*, *Lasiobema* (Zhang, 1993) and *Phanera* (Wunderlin et al., 1987). Judged from the topology of the ITS tree, the 10 fertile stamens very likely represent a plesiomorphic state in the subgenus while the three fertile stamens represent a derived one. The state of two fertile stamens may have independent origins in sects. *Tylosema*, *Phanera*, and *Lasiobema*.

Most of the sections in subgen. *Phanera* as presently circumscribed (Wunderlin et al., 1987) have to be redefined if only monophyletic groups are to be recognized. If *Barklya* is accepted as a subgenus, the subgeneric status of *Caulotretus* and *Tylosema* are also warranted. Sects. *Phanera* and *Lasiobema* may each need redefinition; sect. *Phanera* ser. *Loxocalyx* is best included in sect. *Tubicalyx*, a result similar to that of a previous cladistic analysis of morphological data in the genus *Bauhinia* (Zhang, 1995); ser. *Corymbosae*, with unique leaf venation patterns (Zhang, 1994) and pollen morphology (Larsen, 1975; Zou et al., unpublished data), should be promoted to sectional level. Wunderlin et al. (1987) asserted that a close relationship exists between ser. *Fulvae* and sect. *Palmatifolia* and further suggested that the latter was derived from the former. Our study revealed these two groups to be sister groups. If the sectional status of *Pseudobauhinia* is preserved, a sectional status is also warranted for ser. *Yunnanentes* (with a sole species *B. yunnanensis*). *Semla* is proved to be derived from within a paraphyletic *Lasiobema*. The monophyletic status of sect. *Lysiphyllum*, however, is strongly supported by this study.

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基於 nrDNA ITS 序列的羊蹄甲屬顯托亞屬 (豆科，雲實亞科) 的系統發育分析

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泛熱帶分佈的豆科羊蹄甲屬目前分為 4 個亞屬。約含 150 種的顯托亞屬是其中最大的亞屬。本文報導了對於 38 個種類應用 ITS 區段序列資料進行系統發育分析的結果，其中包括羊蹄甲屬全部 4 個亞屬、顯托亞屬 11 個組中的 9 個組的代表。解析度很高的嚴格一致性分支圖表明顯托亞屬和含單一種類的 *Barklya* 亞屬一起構成一個單系群。在該單系群中，非洲的種類 (*Tylosema* 組)、美洲的種類 (*Caulotretus* 組)、和大洋州的種類 (*Barklya* 亞屬、顯托亞屬的 *Lysiphylum* 組) 是依次最先分支出來的，而亞洲的類群 (*Lysiphylum* 組的種類除外) 形成一個單系。分支圖還表明，顯托組和厚盤組不是單系類群。為系統發育分析結果所支援的單系類群包括顯托組內的黃毛羊蹄甲系 (ser. *Fulvae*) 和傘房花序系 (ser. *Corymbosae*)，筒萼組 (sect. *Tubicalyx*)、以及亞洲—大洋州的 *Lysiphylum* 組。

關鍵詞：羊蹄甲屬；雲實亞科；ITS；豆科；系統發育分析；顯托亞屬。