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PHYLOGENY AND ANDROECIAL EVOLUTION IN SCHISANDRACEAE, INFERRED FROM SEQUENCES OF NUCLEAR RIBOSOMAL DNA ITS AND CHLOROPLAST DNA *trnL*-F REGIONS

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Sequences of nuclear ribosomal DNA ITS and chloroplast *trnL*-F regions were used to construct a phylogeny of Schisandraceae. The results show that there are two major clades in Schisandraceae. One is composed entirely of *Schisandra* species; the other contains a mixture of *Schisandra* and *Kadsura* species. Molecular data place Smith's sect. *Sphaerostema* of *Schisandra* prominently within *Kadsura*, and thus neither *Schisandra* nor *Kadsura* is monophyletic, refuting the traditional division of the family into two genera based on morphological characters of mature fruits. The sister relationship between *S. glabra* (North America) and *S. bicolor* (China) and the monophyly of sect. *Sphaerostema* and sect. *Kadsura* are strongly supported. The *trnL*-F and combined data sets yield phylogenetic trees that are well resolved and concordant with androecial types of staminate flowers; however, they do not support the evolutionary pathways for androecia constructed by previous authors for *Kadsura* and *Schisandra*. The new alignment of species in Schisandraceae indicates that morphological characters traditionally used to construct phylogenetic relationships and evolutionary pathways, e.g., habit (deciduous vs. evergreen or semievergreen), fruit type (bacceta vs. separated apocarps), arrangement of flowers (solitary vs. paired or in glomerules), and pollen (3-colpate vs. 6-colpate), evolved more than once in the family. Preliminary observations suggest that the pentagonal androecium and gynoeceum of *S. glabra* are thermogenic and that heat may play a role in the reproductive biology of *S. glabra* to enhance floral odor or simulate the temperature of brood sites for many types of insects.

Keywords: Schisandraceae, *Schisandra*, *Kadsura*, phylogeny, *trnL*-F region, ITS region, androecial evolution.

Introduction

The first three branches of the angiosperm phylogenetic tree form a "basal grade" including Amborellaceae, Nymphaeales, and Austrobaileyales (Qiu et al. 1999, 2000; Soltis et al. 1999, 2000; Barkman et al. 2000; Graham and Olmstead 2000; Zanis et al. 2002; Soltis and Soltis 2004). Austrobaileyales (monophyletic) is composed of the Austrobaileyaceae, Trimeniaceae, Schisandraceae, and Illiciaceae (Renner 1999; Soltis et al. 2000; Zanis et al. 2002) and is sister to the rest of the extant flowering plants (Soltis et al. 1997; Williams and Friedman 2004).

Schisandraceae consists of *Schisandra* Michx. and *Kadsura* Kaempf. ex Juss. The plants are evergreen or deciduous lianas, bearing small, unisexual flowers with spirally arranged free carpels or stamens (Smith 1947; Law 1996; Saunders 1998, 2000). Estimates of the number of species in the two genera vary, depending on the author (table 1). Recent monographs indicate that there are 23 species in *Schisandra* (Saun-

ders 2000) and 16 in *Kadsura* (Saunders 1998). The taxa are distributed mainly in Asia and grow in temperate and subtropical forests; however, one species of *Schisandra* is native to the southeastern United States (Smith 1947; Saunders 2000) and the Sierra Madre mountains of Mexico (Panero and Aranda 1998).

Traditionally, the shape of the torus in pistillate flowers and its subsequent fruit type were used to distinguish the two genera (Smith 1947; Law 1996; Saunders 1998, 2000). In *Schisandra*, the receptacle becomes elongated and a fruit is formed consisting of widely separated apocarps (Smith 1947; Law 1996; Saunders 2000). In contrast, the fruits of *Kadsura* are bacceta in which the receptacle does not significantly elongate; this results in aggregate fruits (Smith 1947; Law 1996; Saunders 1998).

Another striking morphological feature of many species of *Schisandra* and *Kadsura* is the androecia of staminate flowers. In some species, fusion of filaments or enlargement and elongation of the torus results in staminate flowers with bizarrely shaped androecia (shields, sphere, appendages, etc.; Smith 1947; Saunders 1998, 2000; Liu and Lu 1999; Liu et al. 2001). In contrast, the gynoeceia of pistillate flowers lack the highly variable, elaborate shapes present in staminate flowers (Smith 1947; Saunders 1998, 2000).

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Table 1
Androecial Types, Species, and Classification Systems for Schisandraceae

Androecial type	Description	Species		Classification system		
		Smith 1947	Saunders 1998, 2000	Smith 1947	Law 1996	Saunders 1998, 2000
<i>Schisandra:</i>						
Grandiflora type	Columnar receptacle with numerous spirally arranged free stamens, lacking fused stamens at apex; thecae extrorse to extrorse-lateral	<i>S. grandiflora</i> , <i>S. rubriflora</i> , <i>S. incarnata</i> , <i>S. sphaerandra</i>	<i>S. grandiflora</i> , <i>S. rubriflora</i> , <i>S. incarnata</i> , <i>S. sphaerandra</i>	sect. <i>Pleiostema</i>	subgen. <i>Pleiostema</i>	subgen. <i>Pleiostema</i>
Sphenanthera type	Columnar receptacle with numerous spirally arranged free stamens, with fused stamens at apex; thecae introrse-lateral	<i>S. perulata</i> , <i>S. elongata</i> , <i>S. sphenanthera</i> , <i>S. henryi</i> , <i>S. glaucescens</i> , <i>S. pubescens</i> , <i>S. tomentella</i> , <i>S. neglecta</i> , <i>S. arisanensis</i> , <i>S. lancifolia</i> , <i>S. micrantha</i> , <i>S. viridis</i> , <i>S. wilsoniana</i> , <i>S. gracilis</i>	<i>S. perulata</i> , <i>S. elongata</i> , <i>S. sphenanthera</i> , <i>S. henryi</i> , <i>S. glaucescens</i> , <i>S. pubescens</i> , <i>S. tomentella</i> , <i>S. neglecta</i> , <i>S. arisanensis</i> , <i>S. lancifolia</i> , <i>S. micrantha</i> , <i>S. longipes</i> , <i>S. pubinervis</i>		subgen. <i>Sinoschisandra</i>	subgen. <i>Sinoschisandra</i>
Chinensis type	Columnar receptacle with five free stamens	<i>S. chinensis</i>	<i>S. chinensis</i>	sect. <i>Maximowiczia</i>	subgen. <i>Maximowiczia</i>	subgen. <i>Schisandra</i> , sect. <i>Maximowiczia</i>
Glabra type	Pentagonal synandrous shield with five stamens	<i>S. glabra</i> , <i>S. repanda</i> , <i>S. bicolor</i>	<i>S. glabra</i> , <i>S. repanda</i> , <i>S. bicolor</i>	sect. <i>Euschisandra</i>	subgen. <i>Schisandra</i>	subgen. <i>Schisandra</i> , sect. <i>Schisandra</i>
Propinqua type	Subglobose androecium; introrse thecae borne on a connective that is adjacent to a small depression in the androecial mass	<i>S. propinqua</i> , <i>S. axillaries</i>	<i>S. propinqua</i>	sect. <i>Sphaerostema</i>	subgen. <i>Sphaerostema</i>	subgen. <i>Schisandra</i> , sect. <i>Sphaerostema</i>
Plena type	Subglobose androecium; sessile thecae borne on opposing sides of a cavity in the androecial mass	<i>S. plena</i>	<i>S. plena</i>		subgen. <i>Plena</i>	

Kadsura:

Coccinea type	Columnar receptacle with numerous spirally arranged free stamens and sterile apical appendages	<i>K. coccinea</i> , <i>K. ananosma</i> , <i>K. calophylla</i>	<i>K. coccinea</i>	sect. <i>Cosbaea</i>	subgen. <i>Cosbaea</i>	subgen. <i>Cosbaea</i>
Longipedunculata type	Columnar receptacle with a sterile apical cap and closely appressed stamens; the adjacent thecae are contiguous	<i>K. induta</i> , <i>K. heteroclita</i> , <i>K. longipedunculata</i> , <i>K. renchangiana</i> , <i>K. oblongifolia</i> , <i>K. japonica</i> , <i>K. angustifolia</i> , <i>K. philippinensis</i> , <i>K. interior</i> , <i>K. polysperma</i> , <i>K. paucidenticulata</i> , <i>K. matsudai</i>	<i>K. induta</i> , <i>K. heteroclita</i> , <i>K. longipedunculata</i> , <i>K. renchangiana</i> , <i>K. oblongifolia</i> , <i>K. japonica</i> , <i>K. angustifolia</i> , <i>K. philippinensis</i>	sect. <i>Eukadsura</i>	subgen. <i>Kadsura</i>	subgen. <i>Kadsura</i> , sect. <i>Kadsura</i>
Scandens type	Columnar receptacle with closely appressed stamens, lacking a sterile apical cap; the adjacent thecae are not contiguous	<i>K. verrucosa</i> , <i>K. marmorata</i> , <i>K. scandens</i> , <i>K. celebica</i> , <i>K. lanceolata</i> , <i>K. borneensis</i> , <i>K. clemensiae</i> , <i>K. ultima</i>	<i>K. verrucosa</i> , <i>K. marmorata</i> , <i>K. scandens</i> , <i>K. celebica</i> , <i>K. lanceolata</i> , <i>K. borneensis</i> , <i>K. clemensiae</i>	sect. <i>Sarcocarpon</i>		subgen. <i>Kadsura</i> , sect. <i>Sarcocarpon</i>

In the first monograph on *Schisandra*, Smith (1947), using traditional methods, established four sections based on the shape of androecia (table 1). Subsequently, Law (1996) further divided the genus into six subgenera based on the structure of the androecium (distinguishing six types) and the fusion of filaments in certain taxa (table 1). Saunders (2000), using a cladistic approach and morphological characters, revised *Schisandra* and incorporated features of previous workers (table 1).

In *Kadsura*, Smith (1947) recognized three types of androecia and accordingly established three sections. Subsequently, Law (1996) and Saunders (1998) also recognized three types of androecial structures but created new taxonomic hierarchies (table 1). In this article, we define six types of androecia in *Schisandra* and three in *Kadsura* (tables 1, 2).

Recent DNA-based phylogenies of Schisandraceae constructed with nuclear ribosomal ITS regions (Liu et al. 2000) and another study that combined the ribosomal ITS regions and morphological characters (Hao et al. 2001) concluded that neither *Schisandra* nor *Kadsura* is monophyletic. The taxonomic history of the family reflects the early inconclusive delimitation of various taxa (Wallich 1824; Baillon 1869), as do the various views of modern systematists. In this study, nucleic acid sequences of chloroplast *trnL-F* and nuclear ri-

bosomal ITS regions were used to construct phylogenetic trees for Schisandraceae. Morphological classification systems and evolution of androecial types are discussed in comparison to the molecular phylogeny.

Material and Methods

Plant Materials and DNA Extraction, Amplification, and Sequencing

Eleven species of *Schisandra* and six species of *Kadsura* were sampled to represent all subgenera and sections of the three classification systems and nine types of androecia in both genera (table 1). Seventeen *trnL-F* and two ITS sequences were newly generated in this study (table 2). Previous studies of Illiciaceae and Schisandraceae indicate that these two families are closely related (Renner 1999; Soltis et al. 2000), and recent molecular phylogenetic studies of Schisandraceae used species of *Illicium* as outgroups (Liu et al. 2000; Hao et al. 2001). The outgroup in our study was *Illicium fargesii*.

Leaf material of *Schisandra* and *Kadsura* was dried in silica gel and ground in liquid nitrogen. Total DNA was isolated from the leaves using the CTAB (cetyltrimethylammonia

Table 2

Materials Used in This Study

Taxon	Collector	Locality	GenBank accession no. (ITS, <i>trnL-F</i>)
<i>Schisandra</i> :			
Sphenanthera type:			
<i>S. henryi</i>	Zhong Liu 97169	Dujiangyan, Sichuan	AF263435, ^a DQ342261
<i>S. pubescens</i>	Qi Lin 984	Nanchuan, Chongqing	AF263436, ^a DQ342260
<i>S. sphenanthera</i>	Zhong Liu 97183	Maowen, Sichuan	AF263437, ^a DQ342256
<i>S. viridis</i>	Qi Lin 975	Hengshan, Hunan	AF263438, ^a DQ342257
<i>S. glaucescens</i>	Zhong Liu 99076	Nanchuan, Chongqing	AF263439, ^a DQ342258
Grandiflora type:			
<i>S. grandiflora</i>	Zhong Liu 99083	Nanchuan, Chongqing	AF263440, ^a DQ342259
Chinensis type:			
<i>S. chinensis</i>	Zhong Liu 99087	Mentougou, Beijing	AF263441, ^a DQ342262
Glabra type:			
<i>S. glabra</i>	Leonard B Thien s.n.	Mississippi	DQ342254, DQ342263
<i>S. bicolor</i>	Liangchen Yuan	Xinning, Hunan	DQ342255, DQ342264
Propinqua type:			
<i>S. propinqua</i>	Qi Lin 985	Nanchuan, Chongqing	AF263444, ^a DQ342265
Plena type:			
<i>S. plena</i>	Zhong Liu 99053	Jinghong, Yunnan	AF263443, ^a DQ342266
<i>Kadsura</i> :			
Coccinea type:			
<i>K. coccinea</i>	Qi Lin 972	Guangzhou, Guangdong	AF263445, ^a DQ342271
<i>K. ananosma</i>	Zhong Liu 99012	Mengla, Yunnan	AF263446, ^a DQ342272
Longipedunculata type:			
<i>K. heteroclita</i>	Zhen-Yu Li 11138c	Nanchuan, Chongqing	AF263447, ^a DQ342267
<i>K. longipedunculata</i>	Qi Lin 971	Guangzhou, Guangdong	AF263448, ^a DQ342268
<i>K. japonica</i>	Gang Hao 379	Kunming, Yunnan	AF163712, ^b DQ342269
Scandens type:			
<i>K. scandens</i>	Kebun Raya, Bogor XVI. E125	Indonesia	AF163719, ^b DQ342270
<i>Illicium</i> :			
<i>Illicium fargesii</i>	Zhong Liu 99079	Nanchuan, Chongqing	AF263449, ^a DQ342273

Note. Specific classification according to Smith (1947).

^a ITS sequences from Liu et al. (2000).

^b Sequences from Hao et al. (2001).

bromide) method of Rogers and Bendich (1988); however, 1 vol isoacetone was used to precipitate the DNA.

The *trnL*-F region (including the *trnL* intron, the *trnL* 3' exon, and the *trnL*-F intergenic spacer) can be amplified in both directions using the polymerase chain reaction (PCR) with the forward primer c plus the reverse primer f (Taberlet et al. 1991; Zhang et al. 2003). The total PCR mixture (25 μ L) contained 2.5 μ L buffer (15 mmol/L), 2.5 μ L dNTPs (0.5 mmol/L), 1.25 μ L (5 pmol/L) for either c or f, 0.15 μ L Taq polymerase (5 U/ μ L) (product of China Agriculture University), 5 μ L template DNA, and a relevant quantity of sterile ddH₂O.

The PCR reactions were conducted with a 9600 automatic thermocycler (PerkinElmer). The following protocol was used: (1) 94°C denaturing for 3 min; (2) 94°C denaturing for 1 min, 48°C annealing for 1 min, 72°C extending for 2 min, 32 cycles; (3) 72°C extending for 7 min. The PCR products were purified (separated) using a DNA purification kit. The protocol for the sequencing reactions on the 9600 automatic thermocycler were as follows: denature for 10 s at 94°C, anneal for 5 s at 52°C, and then extend for 4 min at 60°C for 25 cycles (total volume 10 μ L). The *trnL*-F regions were sequenced on an ABI 377 automated DNA sequencer (Applied Biosystems).

The entire ITS region (including ITS1, 5.8S, and ITS2) was amplified using a pair of primers, P1 and P4 (Takaiwa et al. 1985; White et al. 1990). The total volume of the PCR mixture was 50 μ L, consisting of 50 mmol/L Tris-HCl (pH 8.3), 2 mmol/L MgCl₂, 1.5 U Taq polymerase (product of China Agriculture University), 0.2 mmol/L dNTPs, 10–20 ng template DNA, and 2.5 pmol each of P1 and P4. The PCR was performed on a 9600 automatic thermocycler. The following protocol was used: (1) 70°C for 4 min; (2) 94°C for 1 min, 55°C for 20 s, 72°C for 50 s, two cycles; (3) 94°C for 20 s, 55°C for 20 s, 72°C for 50 s, 38 cycles; (4) 72°C for 5 min. The PCR products were purified using agarose gel electrophoresis and then sequenced using the protocols listed above for the *trnL*-F region.

Sequence Alignment and Phylogenetic Analysis

After alignment by hand, the sequences were used to construct phylogenetic relationships with PAUP* 4.0 (Swofford 2000) using maximum parsimony (MP). For each DNA region (*trnL*-F and ITS), separate analyses were performed, followed by a combined analysis of the two data sets. The most parsimonious trees were generated using heuristic search with TBR branch swapping and MULTREES and STEEPEST DESCENT options, with 1000 replicates and random additions. Gaps were treated as missing data, and each character was equally weighted and its state treated as unordered. Reliability of each branch was obtained using bootstrapping analysis (1000 replicates) with the heuristic search option. Saunders's system of classification of the family (table 1) was used to describe the trees in this article.

The congruence between *trnL*-F and ITS data set was tested by performing the incongruence length difference test (ILD; Farris et al. 1994, under partition homogeneity difference analysis in PAUP* 4.0). This was performed with 1000 replicates, each with 10 random additions using TBR branch swapping. Some authors have suggested that heterogeneity among data partitions is not significant if the *P* value is greater than 0.01 (Sullivan 1996; Cunningham 1997) and that under these

circumstances, combining the data can improve or at least does not reduce phylogenetic accuracy. Thus, we chose 0.01 as a significance threshold in this study. Combinability of the two data sets before phylogenetic analysis was also assessed by visual comparison of the trees derived from the separate data partitions.

Floral Heat Measurement and Flower Visitor Observation

The temperature of the pentagonal androecium of staminate flowers and the gynoecium of pistillate flowers of *Schisandra glabra* was recorded using a portable battery-operated digital thermometer (Omega Engineering, Stamford, CT). The instrument used copper-constantan thermocouples (0.3 mm in diameter) accurate to $\pm 0.01^\circ\text{C}$. The temperature for staminate flowers was taken by inserting the sensor between androecium and inner tepals, and for pistillate flowers, temperatures were taken between inner tepals and torus at both the top and the base. The ambient temperature was measured using the same thermometer by placing the sensor in the air, within ca. 1 cm of the flower. The temperatures of three staminate and two pistillate flowers were taken from one plant growing in a garden in New Orleans (David Heikamp), and those of 11 staminate flowers were taken from at least three separate plants in a natural population of *S. glabra* at Camp McCain, Mississippi. No pistillate flowers could be found in the natural population, and it was difficult to determine the number of plants because the lianas formed a dense cover over bushes in a large area. The garden plant was grown to maturity from a seedling taken from a natural population at Tunica Hills, Mississippi.

In a natural population of *S. glabra* in northern Louisiana (Matthew's Brake, south of Alexandria), numerous insects were observed on staminate and pistillate flowers. Insects were captured and identified. In addition, a large number of eggs of various insects and larvae were recorded.

Results

trnL-F Data

After alignment, the length of the *trnL*-F region was 996 bp, of which 90 sites were variable and 34 were parsimony informative. The mean base pair divergence between species within Schisandraceae varied from 0% to 3.3%.

Parsimony analysis produced 78 shortest trees of 96 steps, with a consistency index (CI) of 0.97 and a retention index (RI) of 0.98. The strict consensus tree is shown in figure 1. There are two major clades (A and B) in the tree, which are strongly supported with 94% and 90% bootstrap (BS) values, respectively. Clade A contains subgen. *Sinoschisandra*, subgen. *Pleioestema*, and two sections of subgen. *Schisandra* (sect. *Maximowiczia* and sect. *Schisandra*). Clade B comprises subgen. *Schisandra* sect. *Sphaerostema* of *Schisandra* and all *Kadsura* species.

Within clade A, *Schisandra glabra* (North America) and *S. bicolor* (China) in sect. *Schisandra* form one subclade with 99% BS value. Five species of subgen. *Sinoschisandra* form another subclade with <50% BS value, in which *S. sphenanthera*, *S. henryi*, and *S. pubescens* are united (BS = 57%). *Schisandra grandiflora* of subgen. *Pleioestema* and *S. chinensis* of

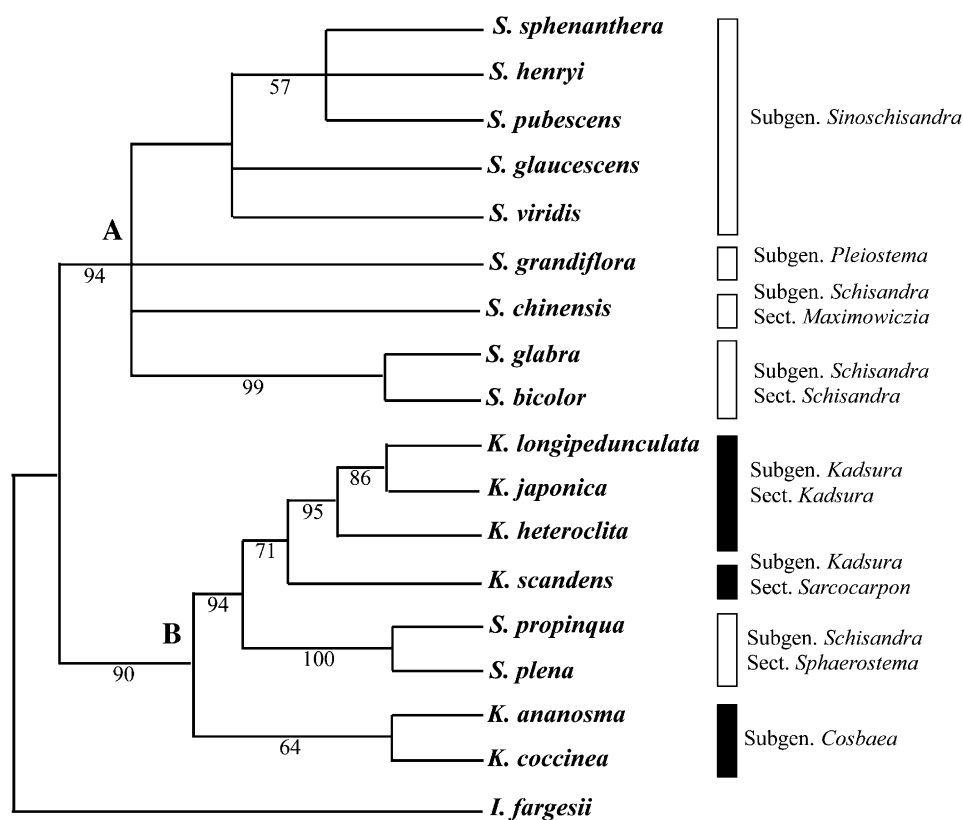


Fig. 1 Consensus tree of the 78 shortest trees with 96 steps based on *trnL-F* sequences; consistency index = 0.97, retention index = 0.98. Numbers below branches are bootstrap values (>50%). Clade names correspond to subgenera and sections of Saunders (1998, 2000).

subgen. *Schisandra* sect. *Maximowiczia* form a polytomy with the above two subclades.

Within clade B, two species, *Kadsura ananosma* and *K. coccinea* of subgen. *Cosbaea*, form one subclade (BS = 64%), which is sister to another subclade (BS = 94%) containing subgen. *Schisandra* sect. *Sphaerostema* of *Schisandra* and subgen. *Kadsura* (including two sections) of *Kadsura*. Section *Sphaerostema* is supported as a monophyletic group (BS = 100%) and sister to the two sections of *Kadsura* (sect. *Kadsura* plus sect. *Sarcocarpon*; BS = 71%). Monophyly of sect. *Kadsura* (including *K. heteroclita*, *K. longipedunculata*, and *K. japonica*) is also supported, with a 95% BS value, as is a sister relationship to *Kadsura scandens*, a representative of sect. *Sarcocarpon*.

ITS Data

The length of the ITS region after alignment was 676 bp. There were 189 variable sites, of which 56 were parsimony informative. The mean base pair divergence between species within Schisandraceae varied from 0% to 6.0%. The strict consensus tree of 72 equally most parsimonious trees with 222 steps (CI = 0.89 and RI = 0.85) is shown in figure 2. The clade composed of *S. bicolor* and *S. glabra* (sect. *Schisandra*) is sister to all other species in the family, but the relationship is poorly supported (BS < 50%). Subgenus *Sinoschisandra*, subgen. *Pleioestema*, and subgen. *Schisandra* sect. *Maximo-*

wiczia of the genus *Schisandra* form a strongly supported clade (BS = 96%), within which subgen. *Schisandra* sect. *Maximowiczia* is sister to the other two subgenera. Subgen. *Sinoschisandra* and subgen. *Pleioestema* are merged, as they are in Smith's sect. *Pleioestema* (see table 1). The relationship is supported with a 76% BS value.

Section *Sphaerostema* of *Schisandra* and species of *Kadsura* form a clade, as in the *trnL-F* tree, but the BS value of it is only 56%. Subgenus *Schisandra* sect. *Sphaerostema*, subgen. *Cosbaea*, and subgen. *Kadsura* sect. *Kadsura* are all monophyletic, with strong support (BS = 98%, 99%, 95%, respectively); however, the relationships among them are unresolved. They form a polytomy with subgen. *Kadsura* sect. *Sarcocarpon*.

Congruence Analysis

The result of the ILD test indicates that the *P* value is 0.02. There is no significant incongruence between *trnL-F* and ITS at *P* < 0.01. On the other hand, by visual comparison of the trees (figs. 1, 2), we found that difference in the topologies is mainly on the position of *S. grandiflora* and the *S. bicolor*-*S. glabra* clade. In the ITS tree (fig. 2), *S. grandiflora* and five species of subgen. *Sinoschisandra* form a clade with a moderate support (BS = 76%); the *S. bicolor*-*S. glabra* clade is sister to the rest of Schisandraceae species sampled in this study, but the relationship is poorly supported

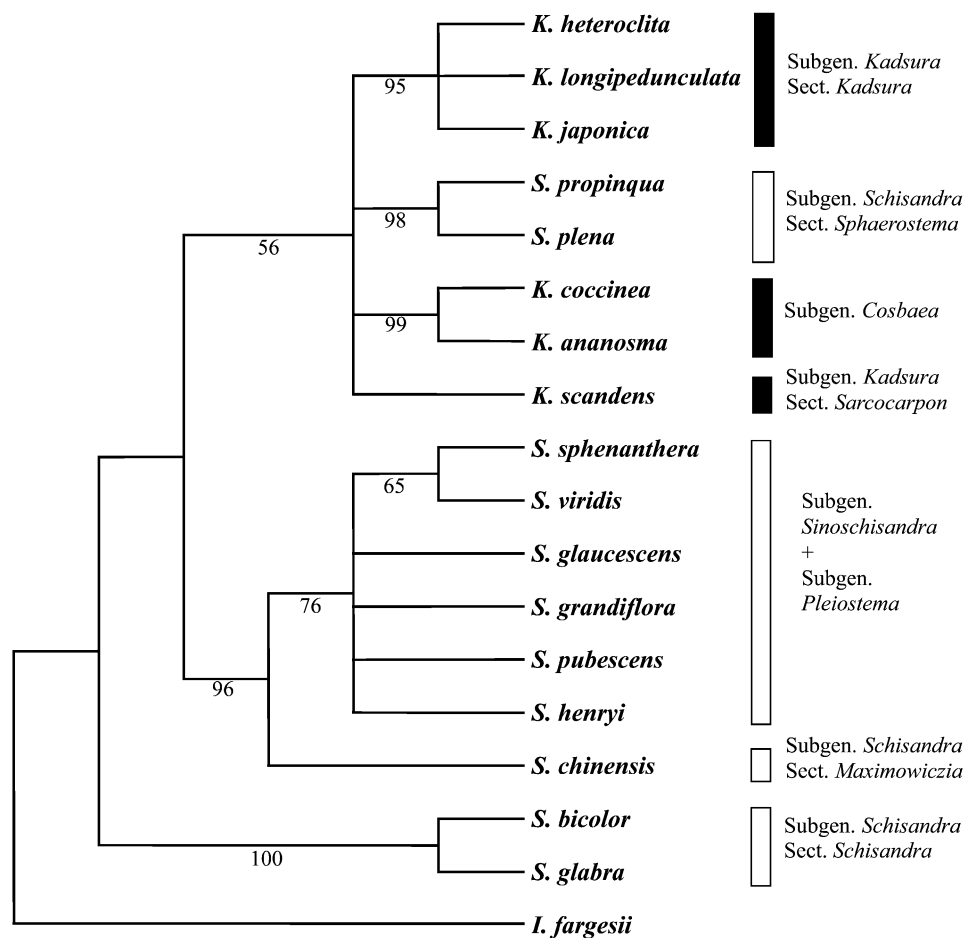


Fig. 2 Consensus tree of the 72 shortest trees with 222 steps based on ITS sequences; consistency index = 0.89, retention index = 0.85. Numbers below branches are bootstrap values (>50%). Clade names correspond to subgenera and sections of Saunders (1998, 2000).

(BS < 50%). In the *trnL-F* tree (fig. 1), however, *S. grandiflora* and the species of subgen. *Sinoschisandra* do not form a clade. In contrast, *S. bicolor* and *S. glabra* membership in clade A is highly supported by the *trnL-F* data. Therefore, only one of the alternative positions for *S. grandiflora* or the *S. bicolor*–*S. glabra* clade can receive strong or moderate support from one of the two data sets. This suggests that the differences reflect not only slight heterogeneity between the data sets but also insufficient informative sites of each set for resolving all of the relationships in the family. When *S. grandiflora* and/or *S. bicolor*–*S. glabra* were deleted from the data sets, the relationships of other taxa remain constant in the trees generated by separate and combined analyses.

Combined Data

After alignment, the combined data set contained 1672 bp, of which 180 sites were variable and 90 were parsimony informative. The strict consensus tree of 36 shortest trees with 325 steps (CI = 0.90 and RI = 0.89) and the nine types of androecia corresponding to clades in the tree are shown in figure 3. The two major clades (A and B) were recognized, as in the *trnL-F* tree, but the BS support for each clade is weaker. Clade A (BS = 80%) consists of all *Schisandra* taxa except for

sect. *Sphaerostema*. The *S. bicolor*–*S. glabra* clade (BS = 100%) is strongly supported as sister to all other members of the clade A, as is *S. chinensis* as sister to the clade formed by *S. grandiflora* and subgen. *Sinoschisandra*. Clade B (BS = 70%) consists of all *Kadsura* species and sect. *Sphaerostema* of *Schisandra*. Subgenus *Cosbaea*, subgen. *Kadsura* sect. *Kadsura*, and subgen. *Schisandra* sect. *Sphaerostema* are supported as monophyletic (BS = 99%, 100%, 100%, respectively). However, the relationships among them are unresolved.

As shown in figure 3, morphological characters, such as habit (deciduous vs. evergreen or semievergreen), aggregate free berries (separated on an elongated receptacle vs. closely appressed on an ellipsoid or clavate receptacle), and arrangement of flowers (solitary vs. paired or in glomerules), have evolved more than once in Schisandraceae. For androecium morphology, the sphenanthera, grandiflora, and chinensis types all have a columnar torus; together they correspond to a strongly supported clade. The above three types and the glabra type with flattened torus are included in clade A. The propinqua and plena types have a subglobose torus; the relationship between them is highly supported. However, they belong to clade B, together with the three androecial types of *Kadsura*. Except for the scandens type, represented by one

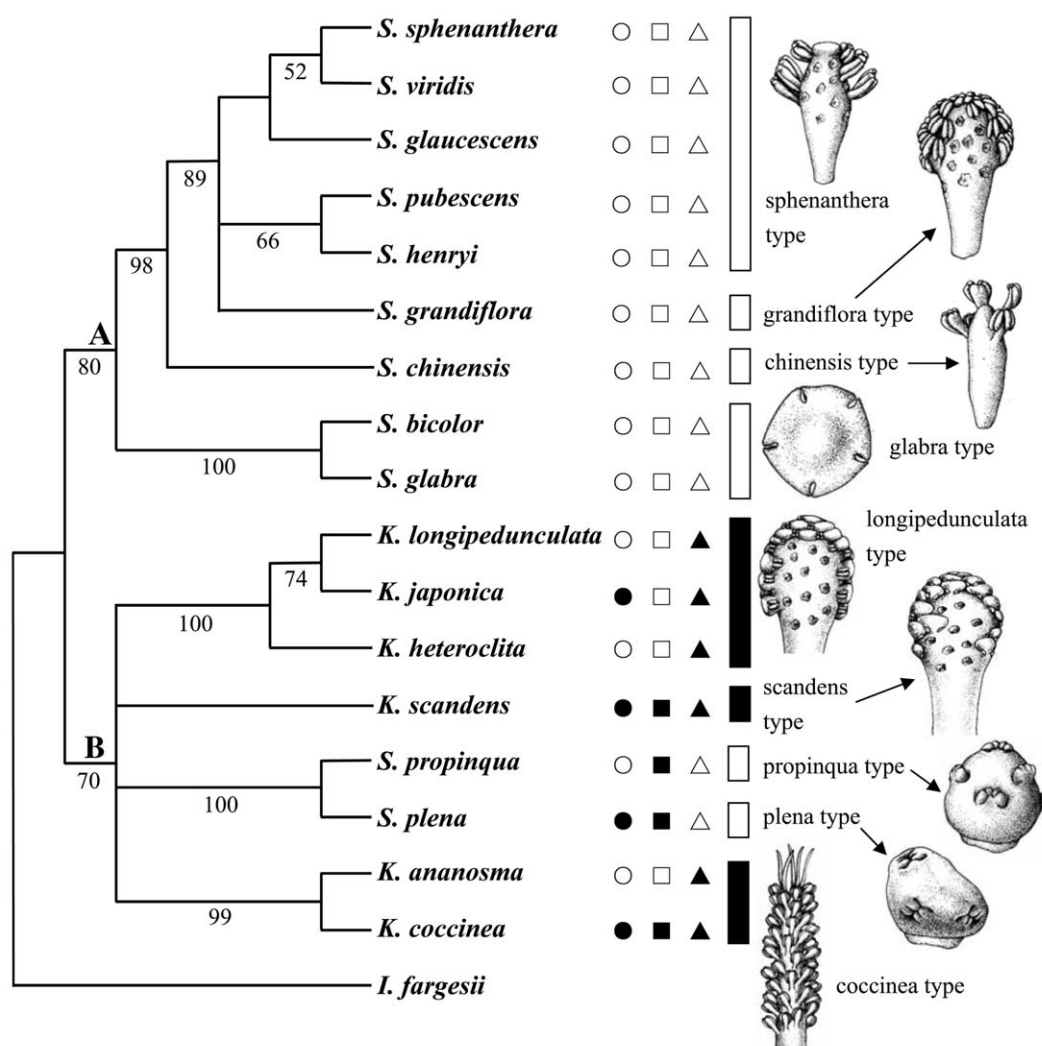


Fig. 3 Consensus tree of the 36 shortest trees with 325 steps based on combined *trnL-F* and ITS sequences; consistency index = 0.90, retention index = 0.89. Numbers below branches are bootstrap values (>50%). Open circle = deciduous; filled circle = evergreen or semievergreen; open square = flowers solitary; filled square = flowers paired or in glomerules, open triangle = separated-apocarps fruit type; filled triangle = bacceta fruit type. Clades (not named) are the same as in fig. 1 and correspond to the nine androecial types of staminate flowers in Schisandraceae. The definition for each androecial type is seen in table 1.

species, the clades corresponding to the *coccinea* and *longipedunculata* types are also strongly supported.

Floral Heat and Flower Visitor Behavior

The temperatures of the pentagonal shield in three staminate flowers grown in the garden in New Orleans were 1.3°, 2.6°, and 2.0°C above ambient air temperature. Temperatures of the gynoecia (small strobilus) in two pistillate flowers were 3.2° and 0.40°C above ambient (top and bottom of the strobilus, respectively) for the first and 3.4° and 1.0°C above ambient (top and bottom, respectively) for the second. The temperatures of the polygonal shield in 11 staminate flowers in the natural population were 1.7°, 2.3°, 0.20°, 1.0°, 0.70°, 1.7°, and 0.10°C above ambient (no above-ambient temperatures were recorded in four flowers, probably because they were in a later flowering stage).

In a natural population of *S. glabra* in northern Louisiana (Matthew's Brake), insects captured and identified included the following Coleoptera: *Longitarsus* sp. (Chrysomelidae: Alticinae), *Cyphon variabilis* (Helodidae), and *Ormiscus* sp. (Anthribidae), all on staminate flowers. Diptera on the flowers included *Atrichopogon levis* (Chironomidae) and members of the Ceratopogonidae on staminate and pistillate flowers. In a survey of 170 staminate flowers, 33 contained larvae and/or eggs. In another survey of 100 staminate flowers selected at random in the population, 75 flowers contained eggs of various insects. In a survey of 100 pistillate flowers, 25 contained eggs. This suggests that the flowers may function as a brood site for many types of insects. In the staminate flowers, the eggs were laid on the pentagonal shield and on the margins of the shield with the tepals. In the pistillate flowers, the eggs were deposited on the stigma or in the axile of the ovary and strobilus.

Discussion

Phylogeny of the Schisandraceae

Two major clades are present in Schisandraceae, as shown in cladograms constructed with *trnL-F* (fig. 1) and combined *trnL-F* and ITS sequences (fig. 3), although the BS support for them and the resolution within each clade differ, with clade A resolved better in the combined tree and clade B better in the *trnL-F* tree. Clade A is composed entirely of *Schisandra* species; however, clade B in both trees contains a mixture of *Schisandra* and *Kadsura* species (specifically; *S. propinqua* and *S. plena*; figs. 1, 3).

The close relationship between *S. propinqua* and *S. plena* is evident in all data sets (figs. 1, 2, 3). This finding supports Smith's (1947) and Saunders's (2000) concept of sect. *Sphaerostema*; however, Law (1996) placed them into two independent subgenera (table 1). The molecular data place sect. *Sphaerostema* within the traditional *Kadsura* clade (prominently in *trnL-F* tree, with a BS value of 90%; fig. 1). In the ITS and combined trees, however, the BS values of clade B are only 56% and 70%, respectively (figs. 2, 3). This conclusion is very different from those of the main classification systems (Smith 1947; Law 1996; Saunders 1998, 2000), and thus neither *Schisandra* nor *Kadsura* is monophyletic in Schisandraceae. Although in the previous study based on morphological data (Hao et al. 2001) both genera are considered monophyletic, which was consistent with traditional classifications, the resolution was poorly supported, which might be the result of incomplete sampling of the taxa or characters. Therefore, the result of this study may not be a reliable inference of the species' phylogenetic relationships within the family.

Both individual and combined data sets of ITS and *trnL-F* support the close relationship of *S. glabra* and *S. bicolor* (figs. 1, 2, 3), which are disjunct between North America and East Asia, but the position of the *S. glabra*–*S. bicolor* clade is uncertain. The *trnL-F* and the combined trees indicate that *S. glabra* and *S. bicolor* are sister to all other species in clade A, with moderate to high BS support (80% in the *trnL-F* tree, 94% in the combined tree); however, the ITS phylogenetic tree places them as sister to all other species in the family (fig. 2), although the relationship is supported with a BS value of less than 50%, which might in part have resulted from an insufficient number of informative sites of the ITS data. These results demonstrate that the two species having unique staminate flowers with pentagonal synandrous shields and five stamens are distinctive in Schisandraceae.

Previous authors have suggested that *S. grandiflora* is primitive in the genus *Schisandra* (Smith 1947; Law 1996; Saunders 2000). The ITS and combined data sets illustrate that the species has a closer relationship with *S. sphenanthera* and its allies in subgen. *Sinoschisandra* (BS = 76% in the ITS tree, 89% in the combined tree). The results support Smith's section *Pleio-stema*; thus, Saunders's or Law's subgen. *Pleio-stema* and subgen. *Sinoschisandra* could be merged.

The mean base pair divergence of *trnL-F* between species within Schisandraceae varies from 0% to 3.3%. For ITS, it varies from 0% to 6.0%. In Illiciaceae, the sequence divergence in the ITS region among species is 0.31% to 6.53% (Hao et al. 2000). In the Winteraceae, a family with a great

number of ancestral morphological characters, sequence divergence in the ITS region (0%–12.9%) is low compared to that in other angiosperm families (Suh et al. 1993; Baldwin et al. 1995). The *ndhF* gene sequence divergence in Magnoliaceae is 2.45% (1.05% in subfamily Magnolioideae and 0.73% in subfamily Liriodendroideae) (Kim et al. 2001). In addition, in all of these families the DNA phylogenetic trees indicate recent diversification of species (Suh et al. 1993; Hao et al. 2000; Kim et al. 2001). Thus, in Magnoliaceae, Figlar and Nooteboom (2004) utilized DNA-generated phylogenetic trees and subsequent observation of morphological characters to reduce the subfamily Magnolioideae to one genus, *Magnolia* (three subgenera). The taxonomy of *Schisandra* and *Kadsura* must be reevaluated utilizing DNA sequence data.

Structure and Evolution of the Androecia in Schisandraceae

The androecial structures of staminate flowers in *Schisandra* and *Kadsura* exhibit a wide range of bizarre variations formed by stamen filaments, staminodes, and receptacles (Smith 1947; Law 1996; Saunders 1998, 2000). In *Schisandra*, Smith (1947) and Saunders (2000) recognized four basic androecial types, A, B, C, and D, some exhibiting distinct evolutionary lines. Type A is characterized by *S. grandiflora* and *S. sphaerandra*, in which many adjacent stamens are connate; the type B androecium is characterized by a pentagonal shield; type C by a reduced number of stamens occurring only in *S. chinensis*; type D exhibits the most extreme form of synandry in sect. *Sphaerostema* (Smith 1947; Saunders 2000). In contrast, Law (1996) further divided the *Schisandra* androecia into six types that correspond to his six subgenera in *Schisandra* (see table 1 for definition of each type). Based on observations of floral morphogenesis (Tucker and Bourland 1994; Liu and Lu 1999; Liu et al. 2001), we accepted Law's classification of androecia. However, these six types can be further classified into three fundamental categories from the view of the organogenesis of androecia in *Schisandra*: columnar torus, flattened torus, and subglobose torus, corresponding to the grandiflora-sphenanthera-chinensis type, the glabra type, and the propinqua-plena type, respectively (table 1). Each of the *trnL-F*, ITS, and combined trees has three well-supported clades concordant with the three fundamental categories, and these data also support the subdivision of categories into types (fig. 3).

In *Kadsura*, three morphological types of androecia, the coccinea, longipedunculata, and scandens types, are consistent with Smith's three sections *Cosbaea*, *Eukadsura*, and *Sarcocarpon*, respectively (table 1). As noted by Saunders (1998), the androecium of the coccinea type is highly variable, with up to 70 free stamens on an elongated receptacle with numerous elongate appendages (fig. 3; *K. coccinea* in Saunders 1998). The androecium of the longipedunculata type consists of a rounded head of stamens with thick connectives, and the receptacle may form a sterile cap. The androecium of the scandens type is similar to that of the longipedunculata type, but it differs in the shape of the connectives and the absence of the sterile cap formed by the receptacle (Saunders 1998).

To explain the evolution of floral structures in *Schisandra*, Smith (1947) hypothesized the existence of two evolutionary lines based on the structure of the androecium. The first line commences with *S. grandiflora* (type A androecium), continuing with a progressive decline in the number of free stamens via the sphenanthera type (also type A) to either the glabra (type B) (stamens fused into a shield; fig. 3) or the chinensis type (type C). In *S. grandiflora*, the apical stamens are completely differentiated, while in the sphenanthera type, the upper stamens are not completely differentiated and are fused with the receptacle. In addition, *S. grandiflora* possesses extrorse to extrorse-lateral thecae and 3-colpate pollen grains, whereas the stamens of *S. sphenanthera* and its allies have introrse-lateral thecae and 6-colpate pollen grains (Smith 1947; Pragłowski 1976; Malla et al. 1977; Sandhu et al. 1989; Law 1996; Saunders 2000). Therefore, *S. grandiflora* was regarded as more primitive than *S. sphenanthera* and its allies (Smith 1947). The second evolutionary line in *Schisandra* is formed by sect. *Sphaerostema* (Smith 1947). In *Kadsura*, Smith (1947) and Saunders (1998) considered the coccinea type to be primitive and the longipedunculata and scandens types to be derived from the coccinea type.

Although the evolutionary polarity of androecia of Schisandraceae is still difficult to determine, as implied by the suggestion of Crisp and Cook (2005) that the ancestral state of sister group in an unbalanced tree must be cautiously interpreted, based on the molecular phylogenetic framework in this study, the *trnL-F* and the combined *trnL-F* and ITS data sets support the existence of two different evolutionary lines for the types of androecia in the genus *Schisandra* hypothesized by Smith (1947) (fig. 3; also see fig. 21 in Saunders 2000). However, because all the cladograms (figs. 1, 2, 3) place *Schisandra* sect. *Sphaerostema* in the midst of the *Kadsura* species, we could infer that the subglobose androecium in *S. propinqua* and *S. plena* might share a pathway with columnar androecia in *Kadsura* rather than with those in other species of *Schisandra*.

The new alignment of species in Schisandraceae indicates that morphological characters traditionally used to construct phylogenetic relationships and evolutionary pathways, e.g., torus shape, fruit type (bacceta vs. separated apocarps), deciduous versus evergreen plants, and 3-colpate versus 6-colpate pollen (Smith 1947; Pragłowski 1976; Malla et al. 1977; Sandhu et al. 1989; Law 1996; Sampson 2000; Saunders 2000), need to be reevaluated. In the basal angiosperms, the number of floral parts is often high and variable, and the “ground plan” limits synorganization of parts (Endress 1990). In this regard, the Schisandraceae, Illiciaceae (Hao et al. 2000), Magnoliaceae (Azuma et al. 1999, 2000, 2001; Kim et al. 2001), and Winteraceae (Suh et al. 1993) are examples of basal angiosperm families in which morphological characters have high homoplasy but DNA sequence data can produce robust phylogenetic trees.

Function of Androecia in Schisandraceae and Reproductive Biology of *S. glabra*

The role of heat production in the reproductive biology of *S. glabra* is not known. Explanations for heat production in flowers involve many processes, including enhancement of floral odor and providing an optimum temperature for devel-

opment of insect larvae (Thien et al. 2000). Preliminary observations suggest that heat may play a role in the reproductive biology of *S. glabra* by enhancing the floral odor or simulating the temperature of brood sites. The flowers of some species of plants are utilized by flies as a brood site and in the process are pollinated. Sakai (2002) reported that various species of Diptera oviposited and pollinated the flowers of *Aristolochia maxima*, with subsequent development of larvae in the decaying fallen flowers (some species of *Aristolochia* are thermogenic; Thien et al. 2000). In *Alocasia odora* (Araceae), Yafuso (1993) recorded heat production and cross-pollination by flies (Drosophilidae).

Temperatures of flower parts in *S. glabra* are typical of thermogenic plants, e.g., *Magnolia tamaulipana* and *Illicium floridanum* (Dieringer et al. 1999; Thien et al. 2000). In the Magnoliaceae and Illiciaceae, the floral receptacle is a “hot spot” of heat production. In Magnoliaceae, the top of the strobilus (cone) produces heat that spreads down the strobilus, and eventually the stamens become thermogenic in the male phase of protogyny. In *S. glabra*, Tucker and Bourland (1994) suggest that the central region of the “shield” is derived from the floral apex and is not connate with the filaments (also see Saunders 2000). In many species of *Schisandra* (Liu and Lu 1999; Saunders 2000; Liu et al. 2001) and *Kadsura* (Saunders 1998), the receptacle extends and combines with stamens to various degrees. Thus, we hypothesize that heat production in *S. glabra* plays a role in the reproductive biology of the species and may be widespread in Schisandraceae. Further elucidation of pollination systems is needed to determine whether other species of Schisandraceae use floral heat in their reproductive biology.

Little is known about the pollination biology of *Schisandra* and *Kadsura* (Saunders 1998, 2000). In species of *Kadsura* and *Schisandra*, Saunders (1998) mentions the presence of nectaries (stomata-like pores) (Igersheim and Endress 1997) on the adaxial surface of the inner tepals (Saunders 1998). Willemstein (1987) suggested that members of Schisandraceae were beetle-pollinated because pollen was the primary food in staminate flowers and the stigmas were wet in pistillate flowers. Morphology (Liu and Lu 1999; Liu et al. 2001) and ongoing pollination studies (L. Yuan, Y.-B. Luo, L.B. Thien, and Z.-D. Chen, unpublished manuscript) indicate that members of Schisandraceae are pollinated by insects, and pollen is the main reward in the staminate flowers. Perhaps the bizarre androecial structures in Schisandraceae are associated with various types of pollen dispersal mechanisms. Androecial structures and functions may be the keys to understanding modes of pollination in the family.

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