

PHYLOGENETIC RELATIONSHIPS AND BIOGEOGRAPHY
OF *STEWARTIA* (CAMELLIOIDEAE, THEACEAE)
INFERRED FROM NUCLEAR
RIBOSOMAL DNA ITS SEQUENCES

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ABSTRACT. Sequences of the internal transcribed spacers of nuclear ribosomal DNA were used to estimate phylogenetic relationships within *Stewartia*. Eighteen samples were included representing two species of *Hartia*, seven species of *Stewartia*, and *Franklinia alatamaha*. *Hartia sinensis* and *H. villosa* form a clade that is the sister group of *Stewartia*. Within *Stewartia* the New World and the Old World species form well-supported clades. The subgenera and sections of *Stewartia* proposed by previous authors are not supported by our ITS data. Two clades are recognized within the Old World lineage: *S. serrata* + *S. rostrata* and *S. pseudocamellia* + *S. monadelphica* + *S. sinensis*. Southeastern Asia and China may be a recent center of diversification of *Stewartia* based on the ITS phylogeny and fossil record.

Key Words: biogeography, *Hartia*, nrDNA ITS, phylogeny, *Stewartia*, Theaceae

Stewartia L. comprises 8–21 species (Chang 1998; Li 1996; Spongberg 1974; Yang 1997). Both *S. ovata* (Cav.) Weatherby and *S. malacodendron* L. are native to the eastern United States (Figure 1a; Dove 1981; Kobuski 1951; Wood 1959). Three species are distributed in southern-central Japan, including *S. monadelphica* Siebold & Zucc., *S. serrata* Maxim., and *S. pseudocamellia* Maxim., which is also found in eastern Korea (Hara 1958; Lee 1997), while the rest of the species are distributed in central to southeastern China (Figure 1b). In China the number of species

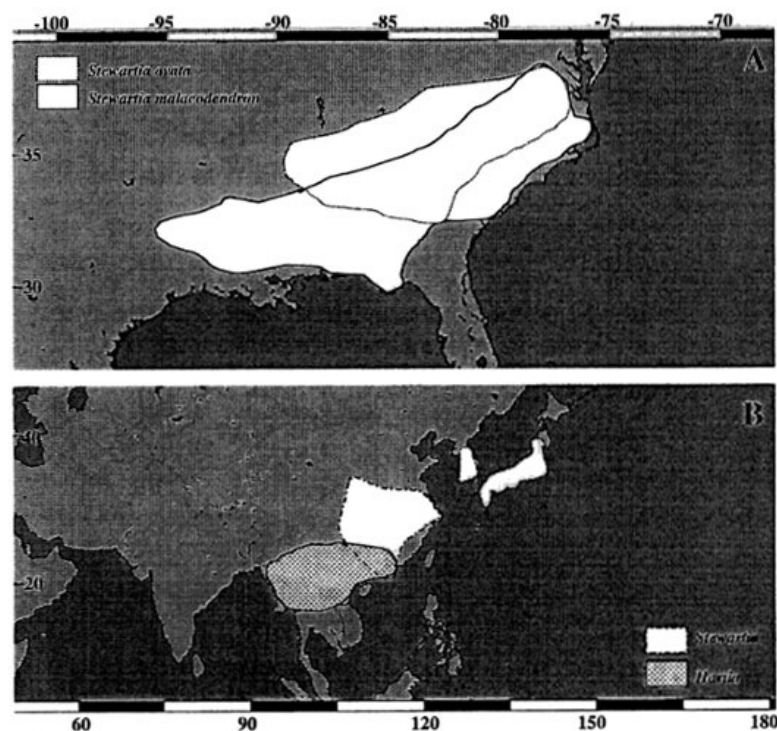


Figure 1. The modern distribution of *Stewartia* and *Hartia* species (Chang 1998; Elias 1980; Hara 1958; Hong 1993; Lee 1997; Li 1996; Spongberg 1974).

of *Stewartia* recognized varies from 3 to 16 (Chang 1998; Chang and Ye 1982; Chien and Cheng 1931; Chiu and Zhong 1988; Li 1996; Spongberg 1974; Yan 1981). *Stewartia rostrata* Spongberg is distributed in Hunan, Jiangxi, and Zhejiang, while *S. rubiginosa* H. T. Chang is endemic to southern Hunan and northern Guangdong. *Stewartia sinensis* Rehder & E. H. Wilson is widespread in central and southern provinces, and its vegetative and floral morphologies are highly variable. Many variants of *S. sinensis* have been described either as species or varieties (Chang 1998; Chang and Ye 1982; Chien and Cheng 1931; Chiu and Zhong 1988; Li 1996; Yan 1981).

Within Theaceae *Stewartia* is generally placed in the taxonomically controversial subfamily Camellioideae. Airy-Shaw (1936), based on morphological and anatomical evidence, revised Mel-

chior's (1925) classification system of the Camellioideae, recognizing two tribes, each with two subtribes. The Gordonieae, to which *Stewartia* belongs, consists of subtribe Gordonieae (*Gordonia* Ellis, *Franklinia* W. Bartram ex H. Marshall, and *Schima* Reinwardt ex Blume) and subtribe Stewartieae (*Stewartia*, including *Hartia* Dunn). Ye (1990) proposed a 5-tribe system for Camellioideae, but also recognized the tribe Stewartieae, consisting of two separate genera, *Stewartia* and *Hartia*.

While some authors have supported the inclusion of *Hartia* in *Stewartia* (Airy-Shaw 1936; Li 1996; Spongberg 1974), others have treated them as separate genera (Chang 1998; Chun 1934; Merrill 1938; Wu 1940; Yan 1981; Ye 1982, 1990). In a recent molecular study of the Camellioideae based on chloroplast DNA sequence data, *Hartia* was found not to be monophyletic (Prince and Parks 1997).

The classification of species within *Stewartia* has also been controversial (Table 1). Gray (1849) recognized two subgenera, the first of which, *Stuartia* (= *Stewartia*), included two species (*S. malacodendron* and *S. monadelphica*). The second subgenus, *Malacodendron*, consisted of a single species, *S. pentagyna* L'Her. (= *S. ovata*). Subgenus *Stewartia* is characterized by united styles (vs. free styles in subgenus *Malacodendron*), subglobose capsules (vs. conical capsules), and unwinged seeds (vs. winged seeds). Szyszylowicz (1893) supported Gray's (1849) groupings but treated them as sections and applied different names (*Synstylia* instead of *Stewartia*, *Dialystyla* instead of *Malacodendron*). Nakai (1950) divided Korean and Japanese *Stewartia* into two sections based on the relative length of sepals and bracts. Section *Pseudocamelliae* has bracts much shorter than the sepals, whereas section *Serratae* possesses bracts subequal to, or longer than, the sepals. Spongberg (1974) did not recognize any of these divisions. In the most recent treatment of *Stewartia* and *Hartia*, Li (1996) recognized *Stewartia* s.l., including *Hartia* and *Stewartia*, and placed the species of *Stewartia* s.s. into two subgenera and 4 of the 5 sections of *Stewartia* s.l. recognized previously.

The objectives of this study were 1) to estimate interspecific relationships of *Stewartia* based on DNA sequence data, 2) to test the monophyly of the subgenera and sections that have been proposed by previous authors, and 3) to provide possible explanations for modern geographic distribution of *Stewartia*. We chose to use sequence data of the internal transcribed spacers (ITS) of

Table 1. Previous taxonomic treatments of *Stewartia* species sampled in this study and their groupings in the ITS trees.

Species	Gray (1849)	Szyszyłowicz (1893)	Nakai (1950)	Ye (1982)	Li (1996)	This Study
<i>S. ovata</i>	Subg. <i>Malacodendron</i>	Sect. <i>Dialystyla</i>	N/A	Sect. <i>Dialystyla</i>	Subg. <i>Dialystyla</i>	New World clade
<i>S. malacodendron</i>	Subg. <i>Stewartia</i>	Sect. <i>Synstyla</i>	N/A	Sect. <i>Stewartia</i>	Subg. <i>Stewartia</i>	New World clade
<i>S. monadelphica</i>	Subg. <i>Stewartia</i>	Sect. <i>Synstyla</i>	Sect. <i>Serratae</i>	Sect. <i>Foliobracteae</i>	Subg. <i>Stewartia</i>	Sinensis clade
<i>S. serrata</i>	N/A	N/A	Sect. <i>Serratae</i>	Sect. <i>Foliobracteae</i>	Sect. <i>Racemosae</i>	Serrata clade
<i>S. sinensis</i>	N/A	N/A	N/A	Sect. <i>Foliobracteae</i>	Sect. <i>Serratae</i>	Sinensis clade
<i>S. rostrata</i>	N/A	N/A	N/A	Sect. <i>Foliobracteae</i>	Subg. <i>Stewartia</i>	Serrata clade
<i>S. pseudocamellia</i>	N/A	N/A	Sect. <i>Pseudocamelliae</i>	Sect. <i>Stewartia</i>	Subg. <i>Stewartia</i>	Pseudocamellia clade

nuclear ribosomal DNA. This is because many studies have shown that sequences of this DNA region are informative in resolving phylogenetic relationships of plants among genera and species (Baldwin et al. 1995; Li et al. 1999; Li, Boufford, and Donoghue 2001; Li, Davis, Donoghue, Kelley, and Del Tredici 2001).

MATERIALS AND METHODS

Plant material. Eighteen plants were sampled in this study, representing seven species of *Stewartia*, two species of *Hartia*, and the monotypic *Franklinia* (Table 2). These samples represent all previously recognized subgenera and sections (Gray 1849; Li 1996; Nakai 1950; Szyszyłowicz 1893; Ye 1982).

Molecular techniques. DNAs were extracted from silica-gel dried leaves using either a standard CTAB DNA extraction method (Doyle and Doyle 1987) or DNeasy Plant Kit (Qiagen Inc., Santa Clarita, CA) following the manufacturer's protocol with minor modifications.

Procedures and protocols for the polymerase chain reactions (PCR), purification of PCR products, and DNA sequencing are described in detail elsewhere (Li and Donoghue 1999). To examine within-individual variation we cloned the ITS regions for *Franklinia alatamaha* W. Bartram ex H. Marshall, *Stewartia ovata*, *S. pseudocamellia*, and *S. sinensis* using standard T-A tail cloning techniques according to manufacturers' instructions. The pGEM®-T Easy Vector System (cat.# A1360, Promega, Madison, WI) was used to ligate ITS PCR products into pGEM plasmids, which were then transformed into Epicurian Coli® XL1-Blue strain competent cells (cat.# 200249, Stratagene, La Jolla, CA). Three white colonies for each species were picked and cultured for 17 hours at 37°C, and their plasmids were prepared using a Miniprep Kit (Qiagen, Santa Clarita, CA). A small amount of the prepared plasmid (1 µL) was then digested using GibcoBRL EcoRI restriction enzyme (Life Technologies, Rockville, MD) to check the presence of the ITS inserts.

Phylogenetic analysis. Sequences were edited using Sequencher 3.0 (Gene Codes Corp., Inc., Ann Arbor, MI) to verify

Table 2. Species used in this study. Acronyms are as follows: Arnold Arboretum (AA), Jamaica Plain, MA; National Arboretum (NA), Washington DC; Kunming Institute of Botany (KUN), Kunming, China; Smith College (SC), Northampton, MA; Quarryhill Arboretum (QA), CA; University of British Columbia (UBC), Vancouver, Canada.

Species	Source and Origin	GenBank #
<i>Stewartia sinensis</i> Rehder & Wilson	AA 373-76A; Lushan, Jiangxi, China	AF431932
<i>S. sinensis</i>	AA 431-34B; Lushan, Jiangxi, China	AF431933
<i>S. sinensis</i>	AA 691-94; Wudangshan, Hubei, China	AF431936
<i>S. pseudocamelia</i> Maxim.	QA 89.071; Japan	AF431937
<i>S. pseudocamelia</i>	AA 11440A; Korea	AF339863
<i>S. monadelpha</i> Siebold & Zucc.	AA 653-74A; Japan	AF431934
<i>S. monadelpha</i>	NA 40211; Yakushima, Japan	AF431938
<i>S. rostrata</i> Spongberg	AA 769-36A; Lushan, Jiangxi, China	AF431935
<i>S. rostrata</i>	Yang 991005; Lushan, Jiangxi, China	AF431939
<i>S. rostrata</i>	AA 761-69A; Lushan, Jiangxi, China	AF431941
<i>S. serrata</i> Maxim.	UBC Bot. Gard.	AF431940
<i>S. malacodendron</i> L.	NA 63252; Accomac, VA	AF431943
<i>S. ovata</i> (Cav.) Weatherby	SC 07190; Cape Cod, MA	AF431944
<i>S. ovata</i> (Cav.) Weatherby f. <i>grandiflora</i> (Bean) Kobuski	AA 18847A, Highlands, NC	AF431942
<i>Hartia sinensis</i> Dunn	AA 18244C, Highlands, NC	AF339861
<i>H. villosa</i> (Merr.) Merr. var. <i>serrata</i> Hu	Yang 98913; KUN	AF431946
<i>Franklinia alatamaha</i> Bartram ex Marshall	Yang 98924; Jinxiu, Guangxi, China	AF431945
	AA 2428-2A; Alatamaha, GA	AF431947

base callings from overlapping sequences and chromatograms generated using different primers. Edited sequences were imported into the computer program PAUP* (version 4.0b3; Swofford 2000) and aligned manually. Characters were weighted equally and their states were unordered. Maximum parsimony (MP) analyses were conducted using both gaps scored as missing data and as a fifth character state. Heuristic tree search options included simple sequence addition, TBR branch swapping, Mulpars on, and steepest decent off. Bootstrap analyses for 300 replicates were conducted to evaluate relative support for individual clades (Felsenstein 1985). All of these analyses were conducted using PAUP*. *Franklinia alatamaha* was included for rooting purposes since several analyses have shown it to be closely related to the clade containing *Stewartia* and *Hartia* (Prince and Parks 1997; Tsou 1998; Ye 1990).

Maximum likelihood ratio test. To test whether ITS sequences in *Stewartia* and *Hartia* evolved in a clockwise fashion, we conducted maximum likelihood (ML) ratio tests using the HKY85+G model, implemented in PAUP* following Baum et al. (1998). ML analyses included the following options: as-is sequence addition, TBR (tree-bisection-reconnection) branch-swapping, and steepest descent option off.

RESULTS

Sequence characteristics. Sequences of the entire ITS region of all samples ranged from 646–657 base pairs (bp) in length, excluding *Franklinia alatamaha*, whose ITS region was 626 bp long. In *Stewartia* the lengths of the ITS-1 and ITS-2 were 246–267 bp and 221–229 bp, respectively. In *Franklinia* the lengths of the ITS-1 and ITS-2 were 242 and 223 bp, respectively. In all samples the sequences of the 5.8S gene were 161 bp in length.

The alignment of all sequences produced a data matrix of 678 characters, 65 of which were parsimony informative. Sequence divergence of the ITS-1 ranged from 0–7.3% (mean, or \bar{x} = 4.4%) among species of *Stewartia*, from 4.9–8.4% (\bar{x} = 6.7%) between species of *Stewartia* and *Hartia*, from 14.6–21.8% (\bar{x} = 16.5%) between species of *Stewartia* and *Franklinia*, and from 17.2–17.7% (\bar{x} = 17.5%) between species of *Hartia* and *Franklinia*.

Sequences of the ITS-2 diverged from 0.0–8.2% (\bar{x} = 4.3%) among *Stewartia* species, from 2.7–8.2% (\bar{x} = 5.1%) between species of *Stewartia* and *Hartia*, from 16.5–19% (\bar{x} = 17.3%) between *Stewartia* species and *Franklinia*, and from 14.8–15.7% (\bar{x} = 15.3%) between *Hartia* species and *Franklinia*. All sequences have been submitted to GenBank (Table 2), and the data matrix and trees are available from the first author upon request and in TreeBASE (<http://www.herbaria.harvard.edu/treebase>).

Phylogenetic relationships. Parsimony analyses of the ITS data set generated 3 trees of 175 steps when gaps were treated as missing data. The strict consensus (MP-M, maximum parsimony-missing) tree is shown in Figure 2 (solid branches, CI = 0.83, RI = 0.83). Species of *Hartia* form a strongly supported clade (bootstrap, or b = 99%), which is sister to the clade containing all species of *Stewartia* (b = 77%). Within the *Stewartia* clade, the two North American species, *S. ovata* and *S. malacodendron*, form a clade (b = 83%), which is sister to the clade containing all of the eastern Asian species (b = 74%). *Stewartia serrata* and *S. rostrata* form a well-supported clade (b = 85%), which is sister to the clade containing *S. pseudocamellia*, *S. monadelpha*, and *S. sinensis* (b = 89%). Accessions of *S. pseudocamellia* from Japan and Korea form a clade (b = 100%), which is sister to a clade consisting of *S. monadelpha* and *S. sinensis* (b = 96%). When gaps were treated as the fifth character state, the MP analyses produced a single (MP-F, maximum parsimony-fifth) tree of 254 steps (Figure 2, dashed branches; CI = 0.85, RI = 0.82). The MP-F tree is identical to the MP-M tree except that the three accessions of *S. sinensis* formed a moderately supported clade (b = 70%).

The maximum likelihood ratio test indicated that rates of ITS base substitution in the *Stewartia* and *Hartia* clade are significantly heterogeneous ($P < 0.05$). Thus, we did not attempt to estimate times of divergence for different lineages of *Stewartia*.

DISCUSSION

Monophyly of *Stewartia*. *Hartia* was proposed by Dunn (1902) to accommodate a plant collected from Yunnan province (Spongberg 1974). However, it has been debated whether *Hartia* should simply be included in *Stewartia*. Some authors have main-

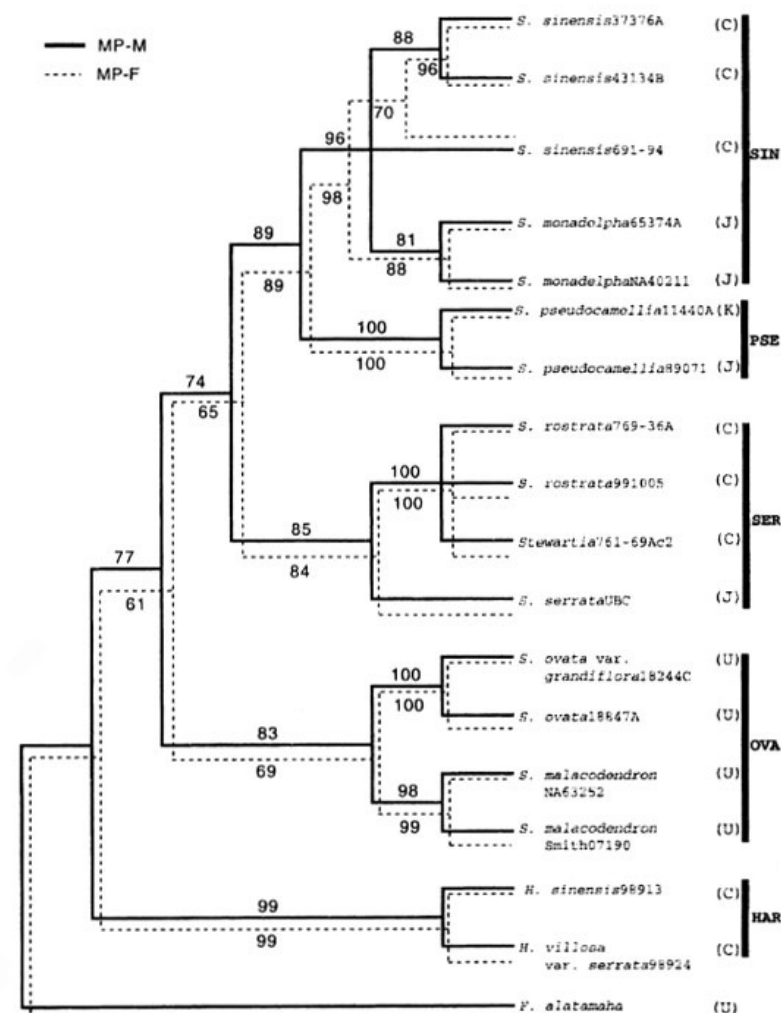


Figure 2. Phylogenetic trees based on maximum parsimony analyses of sequences of nrDNA ITS: strict consensus of 3 trees of 175 steps treating gaps as missing data (MP-M, not dashed), and the single tree of 254 steps treating gaps as the 5th character state (MP-F, dashed). Numbers above and below the branches indicate bootstrap percentages. Clade denotation: SIN, sinensis; PSE, pseudocamellia; SER, serrata; OVA, ovata; and HAR, hartia. Letters in parentheses represent geographic distributions: C for China, J for Japan, K for Korea, and U for the United States.

tained *Hartia* as a separate genus (Chang 1998; Chun 1934; Merrill 1938; Wu 1940; Ye 1982), while others support the inclusion of *Hartia* in *Stewartia* (Cheng 1934; Keng 1962; Li 1996; Sealy 1958; Spongberg 1974). In their phylogenetic study of the Theaceae based on sequences of the chloroplast gene *rbcL*, Prince and Parks (1997) concluded that *Stewartia* might be paraphyletic with *Hartia* nested within it. However, only three species of *Stewartia* and one species of *Hartia* were included in that analysis. In our trees (Figure 2), two species of *Hartia* form a well-supported clade sister to the clade containing species of *Stewartia*. *Hartia* and *Stewartia* have distinct differences in 15 non-molecular characters from morphology, palynology, and wood anatomy (Ye 1982). In addition, *Hartia* and *Stewartia* also differ in chromosome numbers: $n = 15$ in *Stewartia* (Santamour 1963) and $n = 18$ in *Hartia* (Oginuma et al. 1994). Therefore, our results, together with non-molecular data, suggest that both *Hartia* and *Stewartia* are monophyletic genera. Nevertheless, more species of *Hartia* need to be included in the future to further test this hypothesis.

Phylogenetic relationships within *Stewartia*. Although relationships within *Stewartia* have not been explicitly analyzed prior to this study, previous taxonomic treatments are considered as working hypotheses to be tested. Based on fruit, style, and seed characters, *S. ovata* has been separated from the rest of the species as either a monotypic subgenus, *Malacodendron*, or as the section, *Dialystyla* (Gray 1849; Li 1996; Szyszylowicz 1893). This treatment implies that *S. malacodendron*, which is the other North American species and has been placed in the Old World group, is more closely related to the Old World species than it is to *S. ovata*. In our ITS trees (Figure 2) *S. ovata* is linked directly with *S. malacodendron*. That is, the two North American species form a clade that is the sister group to all of the Old World species.

Nakai (1950) recognized two sections mainly based on the relative length of bracts and sepals. *Stewartia pseudocamellia* differs from all the other Asian taxa in having shorter bracts. On this basis it was treated as a monotypic section *Pseudocamelliae*, and the rest of the species were assigned to section *Serratae*. In our ITS trees (Figure 2), *S. pseudocamellia* is not a sister species to a clade containing the remaining *Stewartia* species. In contrast,

it forms a strongly supported clade with *S. monadelpha* and *S. sinensis*.

In his review of *Hartia* and *Stewartia*, Ye (1982) recognized three sections within *Stewartia*. The first section, *Stewartia*, characterized by non-foliaceous bracts and orbicular to obovate sepals, included *S. rubiginosa*, *S. pseudocamellia*, and *S. malacodendron*. Although *S. rubiginosa* was not available for this study, the distant relationship between *S. malacodendron* and *S. pseudocamellia* (Figure 2) indicates that section *Stewartia sensu* Ye (1982) is not supported by the ITS sequences. The second section, *Foliobracteae*, comprising *S. monadelpha*, *S. sinensis*, *S. rostrata*, and *S. serrata*, was marked by foliaceous bracts and fused styles. In our ITS trees, species of section *Foliobracteae* form a monophyletic group with *S. pseudocamellia*, which was placed by Ye (1982) in section *Stewartia*. Thus, ITS sequences indicate that section *Foliobracteae sensu* Ye (1982) is not monophyletic. Ye's third section, *Dialystyla*, was unique in having distinct styles and consisted of three species, *S. ovata*, *S. yunnanensis* H. T. Chang, and *S. oblongifolia* Hu ex S. Z. Yan; the latter two species were transferred by Yang (1997) to the distantly related *Pyrenaria* Blume.

Li (1996) included *Hartia* within *Stewartia* and divided *Stewartia s.l.* into two subgenera based on whether the styles are fused (subgenus *Stewartia*) or distinct (subgenus *Dialystyla*). In subgenus *Stewartia*, he recognized five sections. His first section, *Racemosae*, consisted of *S. monadelpha* and six *Hartia* species. In our phylogenetic trees, however, *S. monadelpha* is not directly related to *Hartia*. Li's second section, *Stewartia*, included only one species, *S. malacodendron*. His third section, *Serratae*, contained *S. sinensis*, *S. serrata*, and *S. rostrata*. In our ITS trees (Figure 2), these three species form a clade that also contains *S. pseudocamellia* of section *Pseudocamelliae* (see below) and *S. monadelpha* of section *Racemosae*. The fourth section, *Pseudocamelliae*, consisted of *S. pseudocamellia*, three *Hartia* species, *S. rubiginosa*, and *S. damingshanica* J. Li & T. Ming. The latter five species were not available for this study, so we are unable to assess the monophyly of this section. Li's fifth section, *Pteropetiolatae*, consisted of four *Hartia* species.

When describing the segregate species, *Stewartia rostrata*, Spongberg (1974) hypothesized that it was most closely related to *S. serrata*. Probable synapomorphies of these two lineages in-

clude glabrous ovaries and 2–3 winter bud scales. Recently, Chang (1998) treated *S. rostrata* as a variety of *S. sinensis*. In our ITS trees, *S. rostrata* accessions form a clade with *S. serrata* with strong support ($b = 85\%$). In contrast, *S. sinensis* is distantly related to *S. rostrata*, being most closely related to *S. monadelphica*. A close relationship between *S. sinensis* and *S. monadelphica* also supports Spongberg (1974), who stated that these two species were so closely related that *S. monadelphica* could be considered as a subspecies of *S. sinensis*.

In summary, our results indicate that none of the subgenera and sections of *Stewartia* proposed by previous authors (Gray 1849; Li 1996; Szyszylowicz 1893; Ye 1982) are monophyletic, except possibly for section *Pseudocamelliae* *sensu* Ye (1982), whose monophyly we were unable to assess due to insufficient sampling.

Evolution of morphological characters. In *Stewartia* all species have fused styles except for *S. ovata*, which has five distinct styles. This condition and a single bract enclosing axillary buds have been used to justify the separation of *S. ovata* from the rest of the *Stewartia* species, including the other North American species, *S. malacodendron* (Gray 1849; Li 1996; Szyszylowicz 1893). In our ITS trees (Figure 2), the two North American species form a well-supported clade, which is sister to the clade containing all of the Old World species of *Stewartia*. All species of *Hartia* have fused styles. Styles are occasionally found to be only half fused in *S. sinensis*; this condition also appears to be derived within *Stewartia*. Therefore, having distinct styles may be a derived condition and therefore an autapomorphy of *S. ovata*.

It is interesting to note that the fruits of *Stewartia* are capsules that split from the top to the bottom loculicidally, releasing seeds. It is possible that the free styles of *S. ovata* facilitate the release of winged seeds by avoiding the hindrance from the fused styles during the top-to-bottom splitting of the capsules. Field studies could be conducted to compare the seed dispersal efficiency of *S. ovata* with its sister species *S. malacodendron*, which has fused styles and unwinged seeds.

The bark of *Stewartia* species is quite variable. Several species develop smooth, mottled bark on the trunks and limbs, resulting in irregularly arranged, buff- or cinnamon-colored patches. These species include *S. malacodendron*, *S. sinensis*, *S. serrata*, *S. pseu-*

docamellia, and *S. monadelphica*. Our ITS phylogenies imply that the mottled bark probably evolved several times independently in *Stewartia*.

Stewartia seeds develop a narrow wing around the perimeter in all species except for the North American *S. malacodendron* and the Japanese/Korean *S. pseudocamellia*. In addition, species of both *Hartia* and *Franklinia* have winged seeds. Therefore, unwinged seeds appear to have been derived twice within *Stewartia*.

Biogeographic implications. Species of *Stewartia* are distributed disjunctly between eastern Asia and the eastern United States (Figure 1). This interesting disjunction has long attracted attentions from both systematists and biogeographers (Boufford and Spongberg 1983; Gray 1849; Hong 1993; Li 1952; Li et al. 2000; Tiffney 1985; Wen 1999). Previous hypotheses concerning interspecific relationships of disjuncts have sometimes proven to be erroneous (Gould and Donoghue 2000; Li, Davis, Donoghue, Kelley, and Del Tredici 2001; Wen 1999; Wen et al. 1998; this study). As more phylogenetic studies are conducted some congruent patterns are emerging. For example, Xiang, Soltis, and Soltis (1998) have shown that phylogenetic relationships in seven plant taxa, including ferns, conifers, and angiosperms, point to a single phylogenetic split between Old World and New World species with western North American species being most closely related to eastern North American species. Our results, together with several other recent phylogenetic investigations (*Aesculus*, Xiang, Crawford, Wolfe, Tang, and DePamphilis 1998; *Pachysandra*, Cuénoud et al. 2000; *Torreya*, Li, Davis, Donoghue, Kelley, and Del Tredici 2001), are consistent with this pattern.

To further understand the formation of this disjunction, the fossil record of both *Hartia* and *Stewartia* should be consulted. In North America, according to Grote and Dilcher (1989), no fossils have been reliably assigned to *Stewartia* or *Hartia*. In the Old World, Mai (1975) described fruits and seeds of *H. quinqueangularis* Mai from the Upper Miocene of western Europe. Knoblock and Mai (1986) reported fossil fruits and seeds assigned to the modern *Stewartia* from the Upper Cretaceous of Europe. Kirchheimer (1957) and van der Burgh (1978) have found fruits and seeds of *S. beckerana* (Ludwig) Kirchheimer from central European deposits of the Pliocene and Miocene. An amber-embedded

fossil flower was described from the Oligocene deposit in Germany as *S. kowalewskii* by Raüffle and Helms (1970); however, this assignment is questionable (Mai 1971). Neogene floras of Japan contain two species of *Stewartia* based on fruit and leaf remains (Tanai and Suzuki 1972). As summarized by Grote and Dilcher (1989), the European late Tertiary sediments include both *Hartia* and *Stewartia*, but reliable fossils of *Hartia* or *Stewartia* have not been found in either North America or eastern Asia except for Japan. It is possible that species of *Hartia* and *Stewartia* were absent from China through most of the Tertiary and migrated there relatively recently (Grote and Dilcher 1989). Our analyses suggest that the radiation of *Stewartia* in China might have taken place rather recently.

Neither the Chinese nor Japanese *Stewartia* species form their own clades in ITS trees, indicating that there has been continuing population exchange between these two areas throughout the Tertiary. We did not estimate the time of divergence of *Stewartia* lineages since the maximum likelihood ratio tests have shown significant rate heterogeneity of the ITS sequences in *Stewartia* and *Hartia*. In these ITS trees (Figure 2), the first branching is between the Old and New World clades, implying that the time of divergence of the New World *Stewartia* species from the Old World species is earlier than that of the Japanese and Chinese species. In addition, the Japanese islands were not separated from the Asian continents until late Miocene (Tao 1992). Thus, the Old and New World *Stewartia* species had diverged from each other by the late Miocene.

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