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Author(s): Linda M. Prince
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Circumscription and Biogeographic Patterns in the Eastern North American–East Asian Genus *Stewartia* (Theaceae: Stewartieae): Insight from Chloroplast and Nuclear DNA Sequence Data

LINDA M. PRINCE*

Rancho Santa Ana Botanic Garden, 1500 North College Ave., Claremont, California 91711-3157

ABSTRACT

Stewartia is a genus of approximately 26 species which are frequently divided into two subgenera or genera based on the leaf duration. All evergreen species (= *Hartia*) are found in the Old World. The deciduous species (= *Stewartia* sensu stricto) are dominant in the Old World but there are two representatives in the southeastern United States, *Stewartia ovata* and *S. malacodendron*. Maximum parsimony and likelihood data analyses of molecular DNA sequence data from both the nuclear and chloroplast genomes produce similar estimates of phylogeny for the group. All evergreen species sampled are more closely related to each other than to any of the deciduous species. The two New World deciduous species are more closely related to the evergreen species than to the Old World deciduous species. These findings complicate earlier vicariance biogeography hypotheses for the genus and challenge the recognition of *Hartia*, the latter of which disagrees with many published classification systems.

INTRODUCTION

Stewartia sensu lato (s.l.) is a genus of 23–26 species (Ye 1982, Li 1996) and the sole member of tribe Stewartieae in Theaceae. Prince and Parks (2001) confirmed the monophyly of the family and tribe using *rbcL* and *matK* gene sequences of the chloroplast genome. The majority of species are distributed in warm temperate and subtropical forests of the Old World (Figure 1), with only two species (*S. malacodendron* and *S. ovata*) in the southeastern United States. Diagnostic characters by which *Stewartia* can be distinguished from other members of the family Theaceae include: narrowly to broadly winged petioles; sclereids restricted to the petiole and petiole wing (versus throughout the leaf in other members of the family); nearly basal, axile placentation; ascending ovules; a capsular fruit that splits to reveal 2–4 narrowly winged or wingless seeds per locule; fruit lacking a persistent central columella; seeds flattened and containing a small, straight embryo and copious endosperm (Keng 1962).

Stewartia are often divided into two genera, *Hartia*, the evergreen species which are restricted to the Old World, and *Stewartia* sensu stricto (s.s.), the deciduous species which are found in both the Old and New World. The only other deciduous member of the family is the monotypic genus *Franklinia*, an endemic plant of Georgia, United States, which is now extinct in the wild (Bozeman and Rogers 1986). *Hartia* was erected by Dunn in 1902 to recognize a plant collected in Yunnan, China. According to Dunn, characteristics distinctive to the genus were the greater connation of the anther filaments into a staminal tube and the more numerous seeds per locule than is found in *Stewartia*. Wu (1940) expanded the list of characters to include an evergreen habit and the presence of a conspicuously winged or inflated petiole that enclosed the terminal bud or lateral shoot. Keng (1962) recommended merging the

* email address: linda.prince@cgu.edu

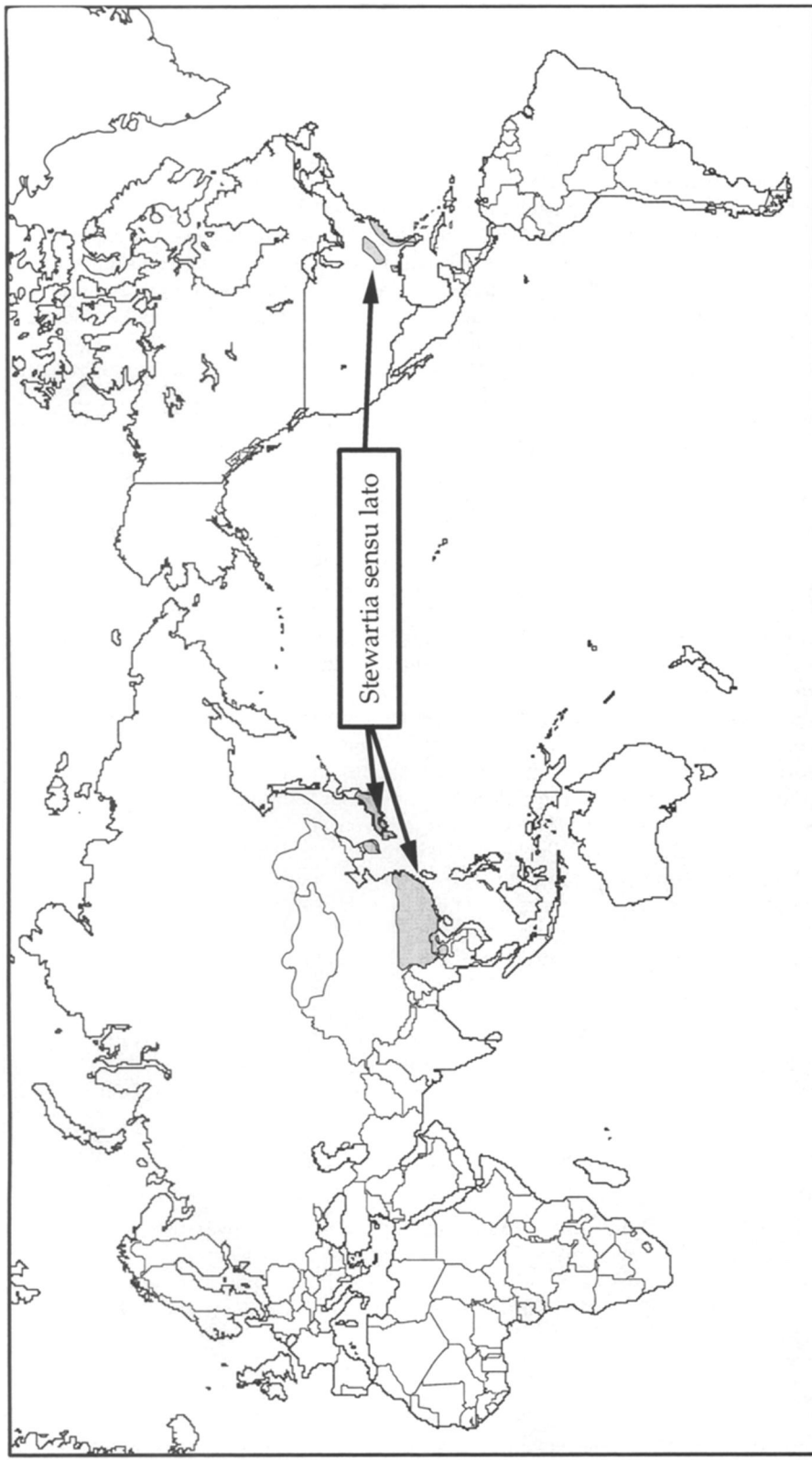

Figure 1. Approximate world distribution of *Stewartia* sensu lato.

two genera based on anatomical data. Spongberg (1974) agreed with the broad circumscription of *Stewartia* as proposed by Keng.

There is no general consensus on whether one or two genera should be recognized in Stewartieae, nor on subgeneric classification. Ye (1982) recognized two genera, *Hartia* with 15 species and *Stewartia* with 12 species. Each genus was further subdivided into three sections based on number of flowers per inflorescence, bract shape, size, and texture, degree of style fusion, and capsule shape. Li (1996) distributed the evergreen species (under the generic name *Stewartia*) into several subgenera based on the degree of style connation, inflorescence type, and bracteole and sepal shape and size. Both classifications are provided in Table 1 along with appropriate author citations which will be omitted from the remainder of the text, figures, and tables. There are as many differences as similarities in the grouping of taxa into sections indicating considerable disagreement on the significance of particular morphological characters. Other classifications of the 20th century include Cheng (1934) and Yan (1981), both of which maintain *Hartia*; and Wu (1940) and Keng (1962), both of which recognize the evergreen species only as a distinct subgenus within *Stewartia*.

The distribution of *Stewartia* s.l. fits the classical eastern Asia–eastern North America vicariance disjunction (see recent reviews by Boufford and Spongberg 1983, Boufford 1998, and Wen 1998). Vicariance biogeography patterns have been attributed to migration and extinction events associated with major climatic and geological changes in evolutionary history, leading to isolation of a once widely distributed species into localized populations which ultimately speciate. Recent wide-spread extinction has been documented in North Temperate latitudes due to glaciation events of the Pleistocene. China appears to have the greatest biodiversity of woody plant species found in all of the North Temperate zone. This higher diversity is probably due to more extensive refugial zones during glaciation due to mountain ranges which formed barriers to limit the advancement of glaciers into southern China, thereby reducing the rate of extinction.

Molecular data are being used to reconstruct phylogenies and to assess the degree of genetic divergence between vicariant "species pairs" or groups (see review by Wen 1998). In some cases it is also possible to estimate approximate divergence times and suggest likely migration routes. Under closer examination, a number of "species pairs" have been shown to be only distantly related [e.g., *Aralia* sect. *Aralia* (Wen et al. 1998), *Eupatorium* (Watanabe et al. 1998), *Gordonia* (Prince and Parks 2001)] emphasizing the importance of broader phylogenetic studies to confirm species relationships prior to hypothesizing vicariant events.

Earlier studies (Prince and Parks 2001) utilized chloroplast protein coding regions which were not variable enough to determine with significant power relationships within Stewartieae. This research employs noncoding, variable regions of the nuclear (internal transcribed spacer region [ITS]) and chloroplast (*trnE-T* intergenic spacer [IGS], *rpl*16 intron) genome to address relationships in Stewartieae including: 1) are either *Hartia* or *Stewartia* s.s. monophyletic, 2) does the molecular phylogeny support either of the two most recent classifications, 3) are the two North American species more closely related to each other or to Asian species; and 4) what are the biogeographic implications of the molecular phylogenetic data.

MATERIALS AND METHODS

Taxon Sampling

Sequences for 16 ingroup taxa representing 11 species of Stewartieae (3 *Hartia*, 8 *Stewartia*) were included, plus two outgroup representatives, *Apterosperma oblata* (Theeae) and *Gordonia brandegeei* (Gordonieae). A list of the taxa included in this study and complete collection, author citation, voucher information, and GenBank Accession Number for DNA sequences is provided in Table 2.

Molecular Methods

Total genomic DNAs were extracted using a minor modification of Doyle and Doyle (1987) CTAB method. The aqueous phase was extracted with 24 parts chloroform: 1 part

Table 1. Alternative classifications for *Stewartia* sensu lato (Theaceae: Stewartieae). Evergreen species (=*Hartia*) are boldface in Li 1996

Ye 1982	Li 1996
Hartia Dunn	*Stewartia* L.
Sect. *Rotundisepala* C.X. Ye	Subg. *Dialystyla* (Szy.) Li & Ming
H. micrantha Chun	*Stewartia ovata* (Can.) Weatherby
H. yunnanensis Hu	*Stewartia yunnanensis* Chang
H. tonkinensis Merr.	Subg. *Stewartia*
H. nitida Li	Sect. *Racemosae* (Wu) Li et Ming
H. sichuanensis Yan ex Ye	***Stewartia crassifolia* (Yan) Li & Ming**
Sect. *Inflorescentia* C.X. Ye	*Stewartia densivillosa* (Hu ex Chang & Ye) Li & Ming
H. obovata Chun ex Chang	***Stewartia laotica* (Gagnep.) Li & Ming**
H. laotica Gagnep.	*Stewartia monadelpha* Sieb. & Zucc.
H. crassifolia Yan	***Stewartia obovata* (Chun ex Chang) Li & Ming**
H. racemosa Chang et Ye	***Stewartia sinii* (Wu) Sealy**
Sect. *Hartia* (Wu) C.X. Ye	***Stewartia tonkinensis* (Merr.) C.Y.Wu**
H. villosa (Merr.) Merr.	Sect. *Stewartia*
H. nankwanica Chang et Ye	*Stewartia malacodendron* L.
H. densivillosa Hu ex Ye	Sect. *Serratae* Nakai
H. sinii Wu	*Stewartia rostrata* Spong.
H. cordifolia Li	*Stewartia serrata* Maxim.
H. sinensis Dunn	*Stewartia sinensis* Rehd. et Wils. [including *S. gemmata* Chien & Cheng]
Stewartia L.	Sect. *Pseudocamellia* Nakai
Sect. *Stewartia* (Gray) C.X. Ye	***Stewartia calcicola* Ming & Li**
S. rubiginosa Chang	*Stewartia damingshanica* Li & Ming
S. pseudocamellia Maxim.	***Stewartia micrantha* (Chun) Sealy**
S. malac[h]*odendron* L.	*Stewartia pseudocamellia* Maxim.
Sect. *Foliobractea* Ye	*Stewartia rubiginosa* Chang
S. serrata Maxim.	***Stewartia sichuanensis* (S.Z. Yan) Li & Ming**
S. rostrata Spong.	Sect. *Pteropetiolatae* J. Li & Ming
S. sinensis Rehd. & Wils. [including *S. gemmata* Chien & Cheng]	***Stewartia cordifolia* (L.) Li & Ming**
S. monadelpha S. et Z.	***Stewartia medogensis* Li & Ming**
S. nanlingensis Yan	***Stewartia pteropetiolata* Cheng**
S. ×Henayae Li	***Stewartia villosa* Merr.**
S. shensiensis Chang	
Sect. *Dialystyla* Szyszylowicz	
S. ovata (Cavanilles) Weatherby	
S. yunnanensis Chang	

isoamyl alcohol. DNA was resuspended in TE buffer following isopropyl alcohol precipitation. Amplification of ITS was accomplished for most taxa using ITS4 and ITS5 primers (White et al. 1990). ITS amplifications for some recalcitrant samples were completed in two pieces using ITS4 with ITS3G (5′-GCATCGATGAAGAACGTAGT-3′ Kyle J. Williams, Duke University) and ITS2 (White et al. 1990) with ITS5. The chloroplast *rpl*16 intron region was amplified with rpl71F and rpl1516R (Jordan, Courtney, and Neigel 1996, Kelchner and Clark 1997) and the *trnE-T* IGS with trnE and trnTr (Doyle et al. 1992). All amplifications used Gibco BRL Taq DNA polymerase according to the manufacturers directions with annealing temperatures of 54–58°C. Amplified products were purified using the PEG precipitation protocol (Johnson and Soltis 1995) with the products sequenced directly using automated sequencing methodology of the ABI Prism™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Products were cleaned in Sephadex G-50 (fine) Centri-Sep spin columns (Princeton Separations P/N 901), dried under vacuum, and run on an ABI 377 Automated Sequencer at the Smithsonian Institution's Laboratory for Molecular Systematics. Raw sequences were

Table 2. Taxa included in analyses of systematic relationships of *Stewartia* and *Hartia* based on nuclear ITS and plastid *rpl*16 intron and *trnE-T* intergenic spacer DNA sequence data

Taxon	Extr. #[1]	Voucher/source[2]	GenBank accession numbers		
			ITS	*rpl*16	*trnET*
STEWARTIEAE					
Hartia villosa	LP206	*Prince 97-251,* NCU	AY070307	AY070289	AY070277
Hartia sinensis	LP169/LP205	*Sun 96061,* KUN	AY070308	AY070290	AY070278
Hartia sinensis	LP207	*Prince 97-241,* NCU	AY070309	AY070291	–
Hartia sinensis	LP209	*Prince 98-262,* NCU	AY070310	AY070292	–
Hartia yunnanensis	LP148/LP204	*Sun 96060,* KUN	AY070311	AY070293	AY070279
Stewartia gemmata	LP297	*Prince s.n.,* NCU	AY070312	AY070294	–
Stewartia malacodendron	LP294	*Prince 97-242,* NCU	AY070313	AY070295	AY070280
Stewartia malacodendron	LP124	*Prince 97-250,* NCU	AY070314	AY070296	–
Stewartia monadelpha	Li2	*Prince 97-248,* NCU	AY070315	AY070297	AY070281
Stewartia ovata	LP200	*Prince 97-245,* NCU	AY070316	AY070298	AY070282
Stewartia ovata	LP295	*Prince 97-244,* NCU	AY070317	AY070299	–
Stewartia pseudocamellia	LP298	*Prince s.n.,* NCU	AY070318	AY070300	AY070283
Stewartia pseudocamellia	LP122	*Prince 95-217,* NCU	AY070319	AY070301	–
Stewartia rostrata	LP296	*Prince 97-243,* NCU	AY070320	AY070302	AY070284
Stewartia serrata	LP174/LP299	*Parks J-2*	AY070321	AY070303	AY070285
Stewartia sinensis	LP070	*Prince 95-253,* NCU	AY070322	AY070304	AY070286
GORDONIEAE					
Gordonia brandegeei	LP307	*Kress 94-4584,* US	AY070324	AY070305	AY070287
THEEAE					
Apterosperma oblata	LP513	*Tsou 1079,* HAST	AY070325	AY070306	AY070288

[1] L.M. Prince extraction number.
[2] Herbarium abbreviations follow Index Herbariorum; Parks J-2 from living collection of Clifford R. Parks, Chapel Hill, North Carolina, USA.

assembled and edited using Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, Michigan), and manually aligned in Se-Al 2.0a3 (Rambaut 2000).

Computational Methods

All analyses and tests were conducted in PAUP* Version 4.0b8 (beta test version; Swofford 2001) and run to completion unless otherwise noted. Maximum Parsimony (MP) used Fitch (1971) equal weight, branch and bound analyses. Parsimony bootstrap analyses (Felsenstein 1985) used 10 random addition replicates, TBR branch swapping, saving a maximum of 10 trees for 1,000 bootstrap replicates. The partition homogeneity tests were conducted with 10,000 replicates of 1 random addition (TBR branch swapping, saving one tree per replicate). Combined data matrix analyses were conducted as described for the individual data sets above.

Maximum likelihood (ML) scores were calculated for one of the MP trees under one of the simpler models, the Jukes-Cantor (Jukes and Cantor [JC] 1969) model, and the more complex model, the General Time Reversible model ([GTR] with gamma rate estimation and proportion of invariant sites estimation; Lanavae et al. 1984, Rodríguez et al. 1990) for each data partition with subsequent successive approximation analyses (Swofford et al. 1996).

RESULTS

Statistics for all data partitions and analyses are summarized in Table 3. In general, tree topologies were identical for MP and ML analyses, regardless of the ML model selected. The only qualification to this statement is in reference to rooting of the ingroup relative to outgroup taxa. Alignment of the ITS matrix for outgroup taxa was ambiguous in several

Table 3. Summary statistics for data matrices and analyses for *Stewartia* and *Hartia*

	Data Partition			
	ITS	***rpl*16 intron**	***trnET***	**combined**
Data Matrices				
Total # characters	732	1113	904	2749
# invariant characters	519	1036	844	2409
# parsimony uninform.	122	50	44	218
# parsimony inform.	91	27	16	124
raw sequence length (complete sequences only)				
ingroup	696–699	1044–1095	830–850	
outgroup	671, 697	1010, 1028	791, 850	
Maximum parsimony analyses				
# trees	120	3	1	2
Length	141	29	19	176
Consistency index	0.7376	0.9310	0.8421	0.7784
Retention index	0.8432	0.9718	0.8636	0.8274
Rescaled consistency index	0.6219	0.9048	0.7273	0.6441
Maximum likelihood General Time Reversible analyses				
−ln	2293.53362	1960.71838	1537.22633	6003.23897
# subst. types	6	6	6	6
base frequencies				
A	0.19865	0.41919	0.36669	0.34285
C	0.30916	0.14989	0.13841	0.18879
G	0.29528	0.15802	0.14284	0.19069
T	0.19691	0.27290	0.35206	0.27767
Prop. Inv. Sites	0.163604	none	0.541253	0.435919
Gamma approx.				
Alpha	0.659941	300	300	0.81939
# rate categ.	4	4	4	4
# distinct data patterns	196	124	65	230

regions. Alternative alignments and exclusion of some characters shifted rooting within the ingroup, as did the use of alternative outgroup sequences. Rooting of the tree was also sensitive to the ML model selected for data analysis. Correct rooting is an extremely important issue for each of the questions this study wishes to address. Instability of the root in initial exploratory MP analyses (Prince 2000) prompted sequencing of the less variable chloroplast DNA regions included in this paper.

ITS

Branch and bound MP analyses of the 734 characters of the aligned ITS data matrix produced 120 shortest trees. The strict consensus tree is shown in Figure 2A with bootstrap values for branches $\geq$ 50% indicated. Strongest support was for the sister relationships of all *Hartia* samples (99%), and for the monophyly of *Stewartieae* (100%). There was moderate support for a *Stewartia gemmata* + *S. monadelpha* + *S. serrata* + *S. sinensis* clade (88%), and for the sister relationship of *S. ovata* and *S. malacodendron* to all *Hartia* samples (80%). Two of the sequences were incomplete, *S. malacodendron* [LP124] and *S. ovata* [LP295], but the missing data did not result in an unexpected placement of the partially sequenced taxa.

ML analyses under the JC model produced a tree of −ln = 2427.21469 which was similar in topology to the MP tree (not shown), but with higher resolution. The JC model rooted

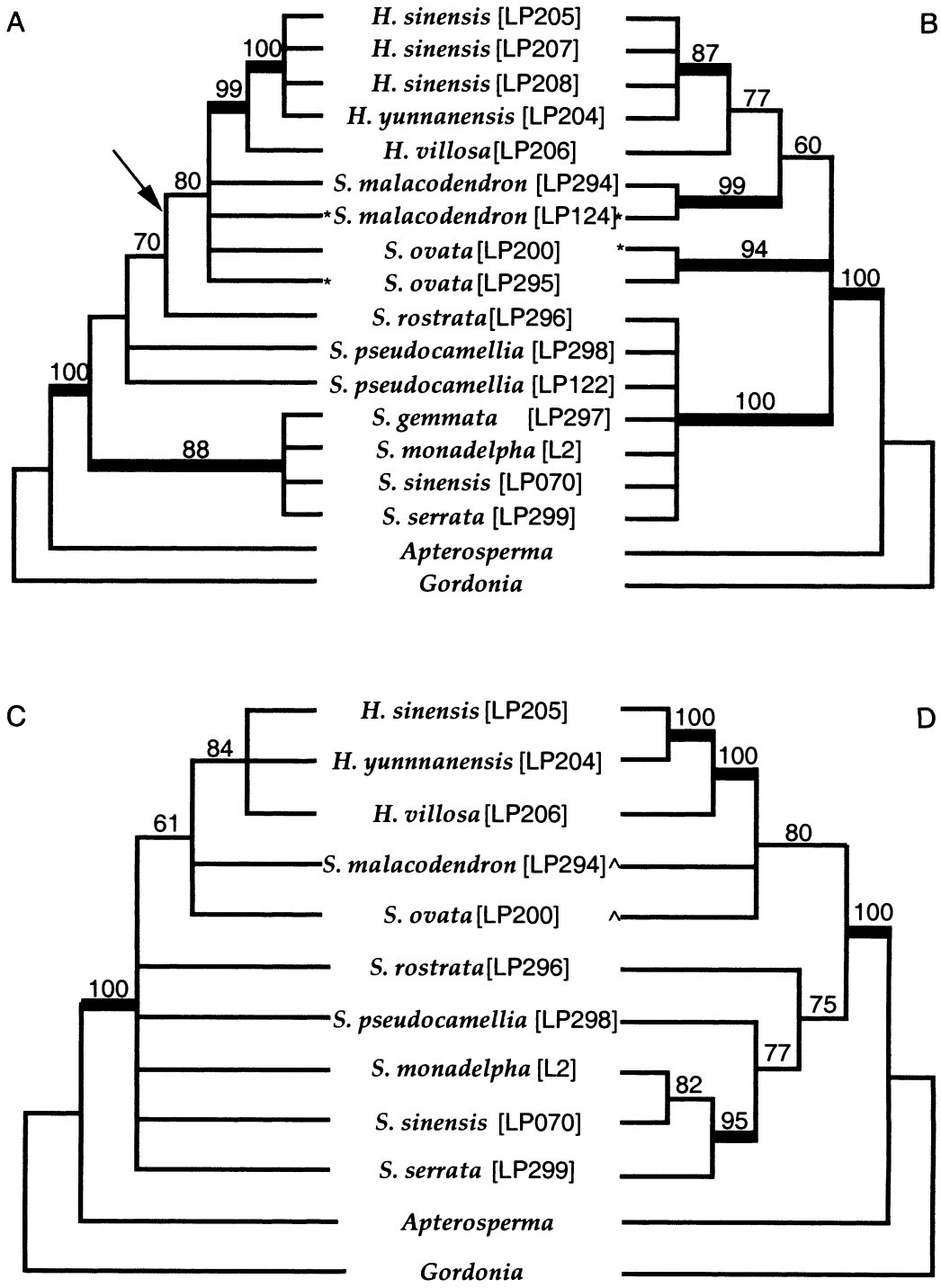

Figure 2. Maximum parsimony strict consensus trees for *Stewartia* and *Hartia*. * partial sequences, ^ composite sequences, arrow indicates root location in maximum likelihood analysis. A: ITS, 120 shortest trees; B: *rpl*16 intron, 3 shortest trees; C: *trnE-T* intergenic spacer, 1 shortest tree; D: combined data sets, 2 shortest trees.

the tree in the same location as the MP analysis. The GTR+G+I model with all parameters estimated from the data (see Table 3 for parameter details) produced a tree of −ln = 2293.53362. The topology was similar to the strict consensus of the MP analysis but with a different root location (see Figure 2). The GTR+G+I analyses divided the ingroup into two similar-sized clades, one of *Hartia* + *S. ovata* + *S. malacodendron* and one of all other *Stewartia* samples.

rpl16 Intron

MP analyses of the *rpl*16 intron data matrix produced 3 shortest trees. The fewer characters produced far fewer trees than the ITS data matrix and higher index values suggesting fewer homoplastic characters in the intron data set. The strict consensus tree is shown in Figure 2B with bootstrap values indicated. This data set found strong support (100%) for the monophyly of *Stewartieae* and also identified a strongly supported (100%) *Stewartia gemmata* + *S. monadelpha* + *S. serrata* + *S. sinensis* + *S. pseudocamellia* + *S. rostrata* clade. The inclusion of two partial sequences (*S. malacodendron* [LP124] and *S. ovata* [LP200]) did not appear to alter tree topology. Alignment of the *rpl*16 intron matrix for all taxa required the inference of few insertion and deletion (INDEL) events. The inferred INDELs were virtually free of ambiguity and the inclusion or exclusion of those characters did not alter rooting or ingroup topology (data not shown).

ML analyses under the JC and GTR+G+I models data (see Table 3) produced a tree of −ln = 2084.98239 and −ln = 1960.71838 respectively. Topologies were identical to the strict consensus of the MP analyses and are not shown. The *rpl*16 intron MP and ML analyses produced similar although less resolved relationships to the ITS ML analyses.

trnE-T Intergenic Spacer

MP analyses of the *trnE-T* data matrix produced a single shortest tree which is shown in Figure 2C with bootstrap values indicated. These data provided only 16 parsimony informative characters for tree building, thus limiting bootstrap support. These data provided strong support (100%) for the monophyly of *Stewartieae*, moderate support (84%) for a *Hartia* clade, and weak support (61%) for a sister relationship of *S. malacodendron* and *S. ovata* to *Hartia*. Alignment of the *trnE-T* matrix for all taxa required the inferrence of few INDEL events. The inferred INDELs were virtually free of ambiguity and the inclusion or exclusion of those characters did not alter rooting or ingroup topology (data not shown).

ML analyses under the JC and GTR+G+I model with all parameters estimated from the data (see Table 3) produced a trees of −ln = 1646.28964 and −ln =1537.22595 respectively. Tree topologies for these analyses were also identical to the strict consensus of the MP analyses and are not shown.

Combined Analyses

Partition homogeneity tests of pruned data matrices produced P-values of 0.5811 (ITS × *rpl*16 intron) to 1.0000 (ITS × *trnE-T* igs) indicating no significant heterogeneity in the data partitions. The combined data set included one representative of each species (composite sequences for *S. malacodendron* and *S. ovata*) for a total of ten ingroup sequences and two outgroup sequences. MP analyses produced 2 shortest trees. The strict consensus tree is shown in Figure 2D along with bootstrap values. As expected, the combined analyses produced a more highly resolved tree with stronger bootstrap support than any of the individual data partition analyses. The only relationship not resolved in the combined analysis is whether *S. ovata* or *S. malacodendron* are sister to each other or whether one of the two is more closely related to all *Hartia* representatives included in this study. The ML tree (not shown) is identical to the MP tree except ML analyses produce a fully resolved tree of −ln = 6242.85764 (JC) and −ln = 6003.23897 (GTR+G+I), with *S. malacodendron* sister to *Hartia*.

Table 4. Phylogenetically informative INDELs within the ingroup for molecular data used in an evaluation of the *Stewartia*/*Hartia* group (Theaceae: Stewartieae)

INDEL #	DNA region	location	description
1	ITS	240	inferred insertion of base A (outgroups mixed, one with an inferred gap, one with a G)
2	ITS	483	inferred deletion of base C
3	ITS	506	inferred insertion of base G
4	ITS	674	inferred insertion of base C or T
5	*rpl*16 intron	387	inferred insertion of base A (outgroup taxa mixed for presence or absence)
6a	*rpl*16 intron	766–785	inferred loss (outgroup sequences not homologous)
6b	*rpl*16 intron	766–775, 777–785	inferred gain
7	*rpl*16 intron	874–896	inferred gain (and subsequent loss)
8	*rpl*16 intron	968–976	inferred gain
9	trnET IGS	251–266	inferred gain
10	trnET IGS	582–584	inferred gain

DISCUSSION

Hartia distinct from Stewartia

Analyses of three data matrices alone and in combination produce highly similar topologies for species of the *Hartia* + *Stewartia* group, with slightly different rooting for some of the ITS analyses. Ambiguity in the ITS alignment and long branches to the outgroup taxa are the most probable cause of this difference in rooting since GTR+G+I ML analysis of the same data partition results in a rooting that is consistent with the other data partitions. In all analyses, *Hartia* representatives form a moderately to highly supported clade, so (based on the limited sampling used here) they are clearly not dispersed throughout the tree. Based on the tree topology, the evergreen habit appears to be a derived rather than primitive condition in this particular tribe.

All of the data partitions include INDELs. Many of the inferred INDELs are not parsimony informative, but several are (see Table 4). Ten parsimony informative INDEL characters are mapped as gains or losses relative to the outgroup taxa on to Figure 3. Several support the rooting indicated by the ITS ML and all other analyses. This rooting indicates *Hartia* is a distinctive clade, but it is embedded within *Stewartia* s.s. Based on the limited sampling here, *Hartia* should not be maintained as a distinct genus.

Current classifications

Two recently published classifications are provided in Table 1. Conclusions regarding classification are somewhat limited since approximately two thirds of the species were not sampled, and sampling across sections was not uniform. With this in mind, neither classification is supported by the data presented here. Ye's 1982 classification splits *Hartia* and *Stewartia* into two genera with several sections in each genus. No conclusions regarding the *Hartia* sectional classification can be drawn due to insufficient sampling. *Stewartia* sectional classification is not supported (see Figure 2D) since *S. pseudocamellia* (section *Stewartia*) is embedded within representatives of section *Foliobractea*, and not with the other member of section *Stewartia* (*S. malacodendron*). The molecular phylogeny also disagrees significantly with Li's 1996 classification for the placement of *S. pseudocamellia* (Sect. *Pseudocamellia*) within Sect. *Serratae* (*S. rostrata*, *S. serrata*, *S. sinensis*) and the placement of *S. ovata* in a separate subgenus.

American taxa

The third question to be addressed by this study concerns the placement of the two American taxa, *S. malachodendron* and *S. ovata*, relative to the Asian species. Figure 3

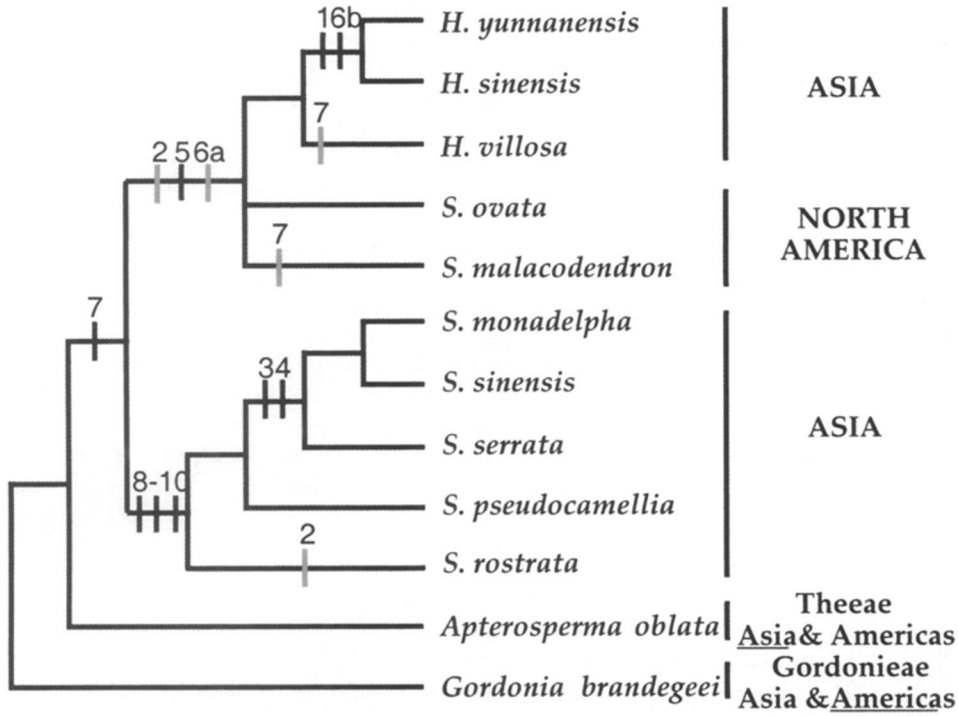

Figure 3. Strict consensus MP tree for the combined data set with INDELs and biogeography mapped onto the tree. Black bars indicate inferred insertion and deletion (INDEL) gains, gray bars indicate inferred INDEL losses. See Table 4 for additional INDEL information.

shows the strict consensus of the combined data analysis with general geographical distribution of the taxa. The North American deciduous species (*S. ovata* and *S. malacodendron*) are most closely related to the Old World evergreen species (*Hartia* spp.), which form a separate clade from all other Old World (deciduous) species. Some of the shortest MP trees place *S. ovata* and *S. malacodendron* sister to each other, but that relationship is not supported in the strict consensus of the MP analyses nor in the ML analyses. There is insufficient evidence to conclude that the two North American species are sister taxa. Although some analyses place *S. malacodendron* sister to *Hartia*, there is also insufficient statistical support to conclude this species is more closely related to the evergreen species.

Biogeographic implications

All but two members of *Stewartia* s.l. are currently distributed in the Old World. Molecular data for the nuclear and plastid genomes produced similar phylogenetic trees in which the only New World species are either sister to each other, or one is sister to all Old World evergreen species. All Old World deciduous species form a separate sister lineage. The genus is most diverse in the Old World, suggesting an Old World origin. A single migration event to the New World is the most parsimonious explanation of the distribution, but the molecular phylogeny cannot confirm or refute that suggestion. Geographical distributions of the outgroup taxa might be useful for polarizing this particular character, but the other two tribes of the family (Theeae and Gordonieae) each include both Old World and New World representatives. It is likely that *Gordonia*, a New World taxon is the basal-most lineage in Gordonieae, and *Apterosperma*, an Old World taxon is basal-most in Theeae (see Prince and Parks 2001), but there is no strong support as to which tribe is most closely related to Stewartieae. Fossil data for Theaceae s.s. (= Theoideae of Cronquist 1981) are abundant with representatives from all regions of the Northern Hemisphere (Grote and Dilcher 1992, Traverse 1994,

Shibuya and Hayashi 1997, Tsukagoshi, Ono, and Hashimoto 1997). The oldest known fossil flowers attributed to *Stewartia* are from the Oligocene (Caspary 1872). Definitive *Stewartia* and *Hartia* fossils are also available from the Miocene from Japan (Suzuki and Hiraya 1989; Suzuki and Terada 1996) and Europe (Mai 1971, Mai and Walther 1985), but have yet to be found in North America. A close examination of morphological characters for extant and fossil taxa would be requried to determine whether fossil taxa can be confidently identified as either deciduous or evergreen, a character which seems critical to unravelling the biogeography of this group. Additional sampling of the extant evergreen taxa is also necessary since it is possible that the evergreen taxa might not represent a monophyletic lineage.

A number of significant conclusions may be drawn from this study. It is possible that the two New World representatives (*S. malacodendron* and *S. ovata*) are more closely related to each other than to all other Old World species. The *Hartia* samples included here form a monophyletic lineage embedded within *Stewartia*, supporting the recognition of one broadly-defined genus rather than two genera. The data also support the conclusion that the evergreen habit is a derived character state in this tribe, not the primitive state that might be expected given the evergreen habit of all other members of the family (except *Franklinia*). Molecular phylogenetic analyses are inconsistent with the two latest classification systems published. Additional detailed studies of both extant and fossil taxa are required for the understanding of the biogeography of this group.

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