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A NUCLEAR rDNA PHYLOGENY OF *SMILAX* (SMILACACEAE)

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ABSTRACT

Smilacaceae are a taxonomically confused, cosmopolitan family of Liliales characterized by climbing habit, reticulate leaf venation, paired petiolar tendrils, unisexual flowers, and superior ovaries. Deviations from this generalized morphology have led to the division of Smilacaceae into at least seven different genera and five sections within the large genus *Smilax*. In particular, taxa with connate tepals (*Heterosmilax*), more than six stamens (*Pleiosmilax*, *Oligosmilax*), or herbaceous habit (*Nemexia*) have been variously classified. Using DNA sequences of 96 taxa from the nuclear rDNA ITS gene region, parsimony analyses provide moderate resolution, but generally poor bootstrap support for phylogenetic relationships in the family. *Rhipogonum* and *Lapageria* are closely related to *Smilax*, but may be better classified in separate families. Within *Smilax* few of the sections are monophyletic, but three major lineages are evident. The first is a primarily Old World clade that contains species of *Heterosmilax*, the erect, woody species of *Smilax* from Asia, and the herbaceous *Smilax* species. Within this clade there is evidence that the *S. herbacea* complex represents a single eastward dispersal from Asia to North America. A primarily New World clade (or paraphyletic grade in some trees) is also present in the ITS cladogram. From within this group there is evidence of a single westward dispersal from North America to Asia. The third lineage is represented by *S. aspera*, which is the sole member of *Smilax* section *Smilax*, and is sister to the entire genus. Its position in the cladogram is intriguing and may help to shed light on the family's greater evolutionary and biogeographic history.

Key words: DNA, evolution, *Heterosmilax*, Liliales, molecular, Philesiaceae, *Rhipogonum*, Smilacaceae, *Smilax*, systematics.

INTRODUCTION

Smilax L. (Smilacaceae) is well known to both temperate and tropical field botanists because it is one of the most abundant and easily recognized climbing plants in many ecosystems of the Old and New Worlds. Within the Neotropics, Smilacaceae are ranked among the top 25 most diverse plant families with climbing species, and closely tied with Amaryllidaceae s.l. as the second most diverse group of climbing monocots after Araceae (Gentry 1991). Morphologically, however, *Smilax* is unusual among monocots, and is frequently confused as a dicot by students of plant taxonomy because of its "woody" stems and pronounced reticulate leaf venation. In general, members of the genus are characterized as climbing, woody vines with prickles, alternate leaves exhibiting reticulate venation, and paired stipular tendrils. The plants are dioecious, and produce inconspicuous flowers with either six stamens (male) or three stigmas (female); the inflorescence is typically an umbel or panicle of umbels, and the fruit is a berry.

Estimates for the size of the genus are given as ca. 350 species found worldwide (Takhtajan 1997). Species determination, however, is extremely difficult because plants are dioecious, flower infrequently, and show considerable phenotypic variation within populations and even among leaves of the same individual plant. As a result, the taxonomy of *Smilax* is highly problematic, and there has been no monograph published for the entire genus. Koyama (1960) initiated an attempt at such, in which he proposed that the genus be divided into six sections (see Discussion), but this was

never completed. Recently published treatments of *Smilax* for specific geographic areas, however, have helped to sort out some significant nomenclatural problems, and suggest that the size estimate of the genus may be inflated. For example, Andreato's (1995) revision of the Brazilian species of *Smilax* reduced the total number from ca. 50 to 26 species. The treatment of *Smilax* for the *Flora of China* (Chen and Koyama 2000) considered a total of 79 species (39 of which are endemic to that country), and the *Flora of North America North of Mexico* (Holmes 2002) treated 20 species, eight of which are considered by some taxonomists to be synonyms of *S. herbacea*. Australia and Africa possess only two species each, whereas Europe has only one. There are probably at least 15 species in Mexico (Morton 1962), several of which extend into the United States, Central America, and the West Indies, bringing the total number worldwide to no more than 150. This estimate excludes a handful of species endemic to the West Indies, Central, and South America (but not present in Mexico or Brazil), Southeast Asia (absent in China and Australia), and the Pacific Islands. Nevertheless, even if one estimates another 50 species for these three areas, the size of the genus is probably not much larger than ca. 200 species.

Controversy not only surrounds the delimitation of *Smilax* species, but also of the entire Smilacaceae (formerly part of Liliaceae s.l.) and its constituent genera as well. Under the classification system of Takhtajan (1997), for example, three genera: *Smilax*, *Heterosmilax* Kunth, and *Pseudosmilax* Hayata are treated as members of Smilacaceae, which is one

of four families in the order Smilacales. The other families are Philesiaceae (*Philesia* Comm. ex Judd, *Lapageria* Ruiz & Pav.); Rhipogonaceae (*Rhipogonum* Forst.); and Luzuriagaceae (*Luzuriaga* Ruiz & Pav., *Behnia* Didr., *Drymophila* R. Br., *Eustrephus* R. Br., and *Geitonoplesium* A. Cunn.). Cladistic analyses of molecular (Chase et al. 2000) and combined molecular with morphological data (Rudall et al. 2000) have shown that Luzuriagaceae and other climbing members of Liliales with reticulate leaf venation (e.g., *Petermannia* Klotzsch) are only distantly related to Smilacaceae. On the other hand, these data show that *Lapageria*, *Philesia*, and *Rhipogonum* may be sufficiently close enough to *Smilax* to warrant their recognition within a more broadly defined Smilacaceae, rather than treated as separate families (Rudall et al. 2000). The exact relationships among all these taxa are still unclear, but studies with increased data and taxon sampling (see other papers in this volume) are beginning to support relationships in which these three lineages are not monophyletic.

The objectives of this study were to produce a preliminary molecular phylogenetic analysis of Smilacaceae, so that a number of fundamental questions related to the systematics of the family could be addressed. These include: (1) what is the natural generic circumscription of Smilacaceae? (i.e., are *Smilax*, *Heterosmilax*, and *Pleiosmilax* monophyletic genera?); (2) are Koyama's (1960) sections monophyletic or should the family be subdivided otherwise?; and (3) are there clear patterns of biogeography within the family? We consider the study to be preliminary because it is based on a limited taxon sample for only a single nuclear gene region (ITS), but we are actively working to add additional taxa and data in order to achieve a long-term goal of revising this large genus.

MATERIALS AND METHODS

Taxon Sampling and Gene Sequencing

Complete ITS sequences (including ITS1, 5.8S, and ITS2) were obtained from 95 species of *Smilax*, *Heterosmilax*, and *Rhipogonum* to represent the ingroup. A sequence of *Lapageria* was included as the functional outgroup taxon based on the close relationship between Philesiaceae and Smilacaceae recovered in broad, family-level analyses of Liliales and other monocots (Chase et al. 2000; Rudall et al. 2000). Complete voucher information is provided in Table 1. The entire data matrix is available from the first author upon request or can be downloaded at <http://www.nybg.org/bsci/res/cullb/dna.html> (May 2005).

All of the sequences were produced by automated methods, briefly described as follows. Most of the total DNA samples were extracted using the FastPrep[®] (Qbiogene Inc., Carlsbad, California, USA) and glassmilk method from approximately 0.5 cm² dried leaf tissue, as described by Struwe et al. (1998). In some cases, leaves were sampled from herbarium specimens (see Table 1). Target loci were amplified in 50 µL volumes using standard polymerase chain reaction (PCR) protocols that included the addition of bovine serum albumin (BSA). Through repeated trial and error with differing combinations of additives or lack thereof, it was discovered that in spite of the fact that the primers are a perfect match, ITS is amplified from Smilacaceae only with the ad-

dition of 10% DMSO. Primers designated "ny43": TATGCTTAAAYTCAGCGGGT and "ny47": AACAAAGGTTTCCGTAGGTGA were commonly used to both amplify and sequence the ITS region. An annealing temperature of 55°C for 30 cycles was discovered to produce the greatest quality amplification product. In all cases, resulting PCR products were purified using QIAquick[®] spin columns (QIAGEN Inc., Valencia, California, USA) according to the manufacturer's protocols. Cycle sequencing reactions were performed using a combination of purified PCR template, primer, and BigDye Reaction mix (Applied Biosystems, Inc., Foster City, California, USA) for 20 cycles. These reactions resulted in complete forward and reverse strands of the genes for nearly all sequences. Centri-Sep[®] sephadex columns (Princeton Separations, Inc., Adelphia, New Jersey, USA) were used according to the manufacturer's instructions to remove excess dye terminators and primer from the cycle sequencing products. These were subsequently dehydrated, resuspended in a mixture of formamide and loading dye, and loaded onto a 5% denaturing polyacrylamide gel. Samples were analyzed on an Applied Biosystems ABI 377XL automated DNA sequencer, and resulting electropherograms were edited using Sequencher vers. 3.0 software (Gene Codes Corporation, Ann Arbor, Michigan, USA). The complete matrix was initially aligned using CLUSTAL_X (Thompson et al. 1997) with default parameters, but then adjusted manually using MacClade vers. 4.0 (Maddison and Maddison 2000).

Phylogenetic Analyses

The ITS data was analyzed using the parsimony criterion in PAUP* vers. 4.0b10 (Swofford 2002) with gaps treated as missing data, characters weighted equally, and with DELTRAN optimization of characters onto resulting trees. Equally parsimonious trees were found by executing a heuristic search of 1000 random addition replicates using tree bisection reconnection (TBR) branch swapping, but keeping only five trees per replicate in order to discover possible "islands" of maximum parsimony (Maddison 1991). All trees obtained in the first round of searching were then used as starting trees for a second heuristic search using the same parameters, but this time saving all shortest trees (MULTREES option in effect) until a MAXTREE limit of 10,000 trees was hit. Support values for the relationships discovered by analysis of each matrix were calculated by performing bootstrap (bts) analyses of 1000 heuristic search replicates using the TBR branching swapping algorithm, and saving two trees per replicate.

RESULTS

The strict consensus of the 10,000 equally parsimonious trees is presented as Fig. 1 with bootstrap values >50% indicated. The ITS matrix contains 955 characters of which 308 (32%) are variable and 176 (18%) are parsimony informative. Analysis of these data resulted in more than 10,000 trees of maximum parsimony with a length of 634 steps, CI of 0.637 (0.534 excluding uninformative sites), and RI of 0.803. The tree is characterized by several monophyletic groups. The first is a primarily Old World clade that contains species of *Heterosmilax*, the erect, woody species of *Smilax*

Table 1. Species of Smilacaceae, Rhipogonaceae, and Philesiaceae analyzed for this study (arranged alphabetically). New ITS sequences for this study will be made available in GenBank at a future time.

Taxon	Locality	Voucher
<i>Heterosmilax chinensis</i> F. T. Wang	Hunan, China	<i>Fu 010845</i> (ZJU)
<i>H. japonica</i> Kunth	Zhejiang, China	<i>Fu 010807</i> (ZJU)
<i>H. yunnanensis</i> Gagnep.	Yunnan, China	<i>Fu 20019</i> (ZJU)
<i>Lapageria rosea</i> Ruiz & Pav.	Chile, ex cult.	<i>Cameron 1108</i> (NY)
<i>Rhipogonum discolor</i> F. Muell.	Queensland, Australia	<i>AQ 657912</i> (BRI)
<i>R. elseyanum</i> F. Muell.	Australia ex hort., Sydney	<i>Cameron 1155</i> (NY)
<i>R. scandens</i> Forst.	(1) New Zealand	<i>Cameron 2226</i> (NY)
<i>R. scandens</i>	(2) New Zealand	<i>Cameron 1149</i> (NY)
<i>Smilax aberrans</i> Gagnep.	Guizhou, China	<i>Fu 20037</i> (ZJU)
<i>S. arisanensis</i> Hayata	Guizhou, China	<i>Fu 20036</i> (ZJU)
<i>S. aspera</i> L.	(1) France	Kew—living 1997–4668
<i>S. aspera</i>	(2) Italy	<i>Delprete s. n.</i>
<i>S. aspericaulis</i> Wall	Yunnan, China	<i>Fu 010835</i> (ZJU)
<i>S. australis</i> R. Br.	New South Wales, Australia	<i>Cameron 1139</i> (NY)
<i>S. austrozhejiangensis</i> Q. Lin	Zhejiang, China	<i>Fu 20040</i> (ZJU)
<i>S. biltmoreana</i> (Small) J. B. Norton ex Pennell	S. Carolina, USA	<i>Wurdack s. n.</i> (NY)
<i>S. bockii</i> Warb. ex Diels	Sichuan, China	<i>Fu 20022</i> (ZJU)
<i>S. bona-nox</i> L.	N. Carolina, USA	<i>Cameron 1053</i> (NY)
<i>S. bracteata</i> C. Presl.	Yunnan, China	<i>Fu 20065</i> (ZJU)
<i>S. china</i> L.	(1) Sichuan, China	<i>Fu Fc02</i> (ZJU)
<i>S. china</i>	(2) Hubei, China	<i>Fu 20006</i> (ZJU)
<i>S. china</i>	(3) Japan	<i>Fu Fc03</i> (ZJU)
<i>S. china</i>	(4) Korea	<i>Fu Fc01</i> (ZJU)
<i>S. chingii</i> F. T. Wang & T. Tang	Sichuan, China	<i>Fu 20020</i> (ZJU)
<i>S. cocculoides</i> Warb. ex Diels	Yunnan, China	<i>Fu 20057</i> (ZJU)
<i>S. corbularia</i> Kunth	(1) Guangxi, China	<i>Fu 20050</i> (ZJU)
<i>S. corbularia</i>	(2) Yunnan, China	<i>Fu 20056</i> (ZJU)
<i>S. darrissi</i> H. Lévl.	Sichuan, China	<i>Fu Fw112</i> (ZJU)
<i>S. davidiana</i> A. DC.	Zhejiang, China	<i>Fu Fw108</i> (ZJU)
<i>S. discotis</i> Warb.	Hubei, China	<i>Fu Fw111</i> (ZJU)
<i>S. ferox</i> Wall. ex Kunth	Yunnan, China	<i>Fu 20059</i> (ZJU)
<i>S. glauca</i> Walter	New Jersey, USA	<i>Fu 9982</i> (ZJU)
<i>S. glauco-china</i> Warb. ex Diels	Zhejiang, China	<i>Fu Fw114</i> (ZJU)
<i>S. glycyphylla</i> Sm.	New South Wales, Australia	<i>Fu 98101</i> (ZJU)
<i>S. guiyangensis</i> C. X. Fu & C. D. Shen	Guizhou, China	<i>Fu Fw31</i> (ZJU)
<i>S. herbacea</i> L.	(1) New York, USA	<i>Cameron 1066</i> (NY)
<i>S. herbacea</i>	(2) N. Carolina, USA	<i>Cameron 9967</i> (NY)
<i>S. herbacea</i>	(3) New York, USA	<i>Fu 99810</i> (ZJU)
<i>S. herbacea</i>	(4) N. Carolina, USA ex hort.	Kew—living 1973–16729
<i>S. herbacea</i>	(5) N. Carolina, USA ex hort.	Kew—living 1973–16729
<i>S. hispida</i> Muhl. ex Torr.	Virginia, USA ex hort.	Kew—living 1969–9240
<i>S. hugeri</i> J. B. Norton ex Pennell	Alabama, USA	<i>Wood 8675</i> (GH)
<i>S. jamesii</i> G. A. Wallace	California, USA	<i>ARK 3725</i> (NY)
<i>S. kraussiana</i> Meisn.	Gabon, Africa	<i>JMR 3151</i> (NY)
<i>S. lanceifolia</i> Roxb.	Guizhou, China	<i>Fu Fw86</i> (ZJU)
<i>S. lasioneura</i> Hook.	Florida, USA	
<i>S. megalantha</i> C. H. Wright	W. China, ex hort.	Kew—living 1908–13001
<i>S. melastomifolia</i> Sm.	Hawaii, USA	<i>Motley 1986</i> (NY)
<i>S. microphylla</i> C. H. Wright	Guizhou, China	<i>Fu 20007</i> (ZJU)
<i>S. nipponica</i> Miq.	(1) Liaoning, China	<i>Fu 91277</i> (ZJU)
<i>S. nipponica</i>	(2) Hunan, China	<i>Li 00718</i> (ZJU)
<i>S. nipponica</i>	(3) Nagano, Japan	<i>Fu 99062</i> (ZJU)
<i>S. nipponica</i>	(4) Zhejiang, China	<i>Fu 88032</i> (ZJU)
<i>S. nipponica</i>	(5) Jiangxi, China	<i>Fu 950174</i> (ZJU)
<i>S. ocreata</i> A. DC.	Yunnan, China	<i>Fu 20066</i> (ZJU)
<i>S. perfoliata</i> Lour.	(1) Hainan, China	<i>Fu Fw43</i> (ZJU)
<i>S. perfoliata</i>	(2) Yunnan, China	<i>Fu Fw116</i> (ZJU)

Table 1. Continued.

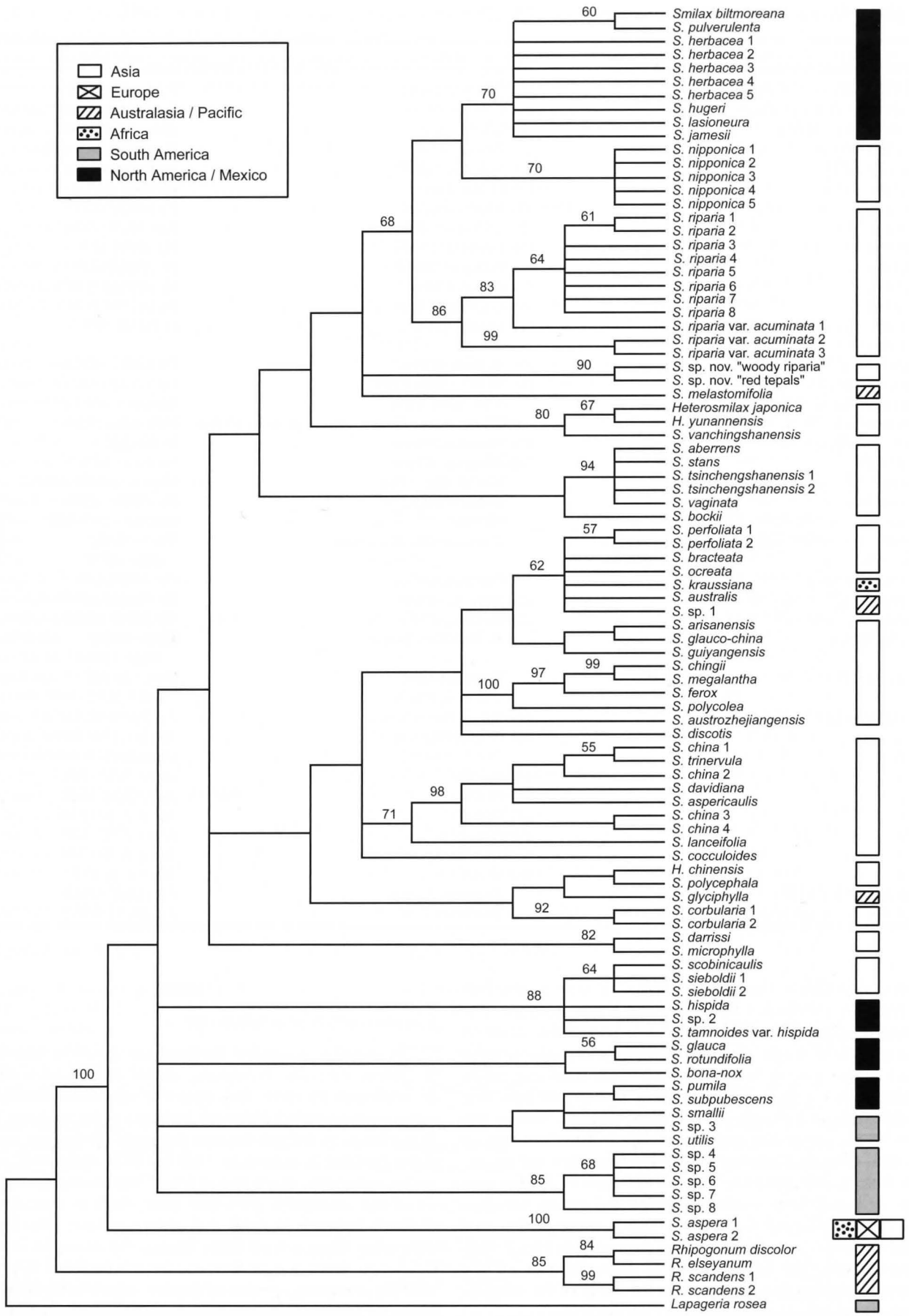
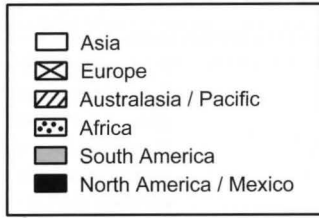
Taxon	Locality	Voucher
<i>S. polycephala</i> F. T. Wang & T. Tang	Yunnan, China	<i>Fu Fw69</i> (ZJU)
<i>S. polycolea</i> Warb. ex Diels	China	<i>Fu 20010</i> (ZJU)
<i>S. pulverulenta</i> Michx.	USA	<i>Horr 4460</i> (GH)
<i>S. pumila</i> Walter	Florida, USA	<i>Cameron 1065</i> (NY)
<i>S. riparia</i> A. DC.	(1) Nagano, Japan	<i>Fu 99902</i> (ZJU)
<i>S. riparia</i>	(2) Osaka, Japan	<i>Fu 9904</i> (ZJU)
<i>S. riparia</i>	(3) Nagano, Japan	<i>Fu 99914</i> (ZJU)
<i>S. riparia</i>	(4) Samchuck, Korea	<i>Kim 0529</i> (ZJU)
<i>S. riparia</i>	(5) Guangxi, China	<i>Fu 91091</i> (ZJU)
<i>S. riparia</i>	(6) Guangxi, China	<i>Fu 20067</i> (ZJU)
<i>S. riparia</i>	(7) Anhui, China	<i>Fu 010880</i> (ZJU)
<i>S. riparia</i>	(8) Liaoning, China	<i>Fu 912691</i> (ZJU)
<i>S. riparia</i> var. <i>acuminata</i> (C. H. Wright) F. T. Wang & T. Tang	(1) Hunan, China	<i>Li 00726</i> (ZJU)
<i>S. riparia</i> var. <i>acuminata</i>	(2) Sichuan, China	<i>Fu 92052</i> (ZJU)
<i>S. riparia</i> var. <i>acuminata</i>	(3) Hubei, China? HN	<i>Fu 92105</i> (ZJU)
<i>S. rotundifolia</i> L.	New York, USA	<i>Cameron 1068</i> (NY)
<i>S. scobinicaulis</i> C. H. Wright	China	<i>Fu Fw113</i> (ZJU)
<i>S. sieboldii</i> Miq.	(1) Zhejiang, China	<i>Li 18</i> (ZJU)
<i>S. sieboldii</i>	(2) Zhejiang, China	<i>Fu Fw53</i> (ZJU)
<i>S. smallii</i> Morong	N. Carolina, USA	<i>Cameron 1054</i> (NY)
<i>S. stans</i> Maxim.	Hubei, China	<i>Fu 20008</i> (ZJU)
<i>S. subpubescens</i> A. DC.	Mexico	unknown (UNAM)
<i>S. tannoides</i> var. <i>hispida</i> (Muhl. ex Torr.) Fernald	Tennessee, USA ex hort.	Kew—living 1980–4557
<i>S. trinervula</i> Miq.	Guizhou, China	<i>Fu 20038</i> (ZJU)
<i>S. tsingchengshanensis</i>	(1) Yunnan, China	<i>Fu Fw115</i> (ZJU)
<i>S. tsingchengshanensis</i>	(2) Sichuan, China	<i>Fu 20013</i> (ZJU)
<i>S. utilis</i> Wright	W. Indies ex hort.	Kew—living 1969–18324
<i>S. vaginata</i> Decne.	China ex hort. Guizhou, China	Kew—living 1995–3022
<i>S. vanchingshanensis</i>	(1) Papua New Guinea	<i>Fu 20011</i> (ZJU)
<i>S. sp.</i>	(2) Central Mexico	<i>Motley 2399</i> (NY)
<i>S. sp.</i>		<i>Cameron 2139</i> (NY)
<i>S. sp.</i>	(3) Brazil	<i>Alvez 2152</i> (RB)
<i>S. sp.</i>	(4) Brazil	<i>Alvez 2201</i> (RB)
<i>S. sp.</i>	(5) Brazil	<i>Alvez 2160</i> (RB)
<i>S. sp.</i>	(6) Brazil	<i>Alvez 2272</i> (RB)
<i>S. sp.</i>	(7) Brazil	<i>Alvez 2336</i> (RB)
<i>S. sp.</i>	(8) Brazil	<i>Alvez s. n.</i> (RB)
<i>S. sp. nov.</i> “red tepals”	Zhejiang, China	<i>Fu 20041</i> (ZJU)
<i>S. sp. nov.</i> “woody riparia”	Yunnan, China	<i>Fu 20053</i> (ZJU)

from Asia, Africa, Oceania, Australia, as well as the herbaceous *Smilax* species from Asia and North America. The Hawaiian species, *S. melastomifolia*, is part of this clade. A primarily New World clade (or paraphyletic grade in some trees) is also present, but unresolved in the strict consensus. *Smilax sieboldii* from East Asia is a member of this New World clade and closely related to *S. hispida*. Within this New World group, the majority of North American species are more closely related to each other than they are to species from South America. The third lineage of *Smilax* contains *S. aspera* from southern Eurasia and Africa, which is sister to the entire genus. *Rhipogonum* is monophyletic and sister to *Smilax*. A summary tree is presented in Fig. 2. Resolution within the larger clades is generally lacking and bootstrap support is poor or absent for many of the relationships, especially along the “spine” of the tree.

DISCUSSION

Circumscription of Smilacaceae

The question of whether Smilacaceae should be broadened to include *Philesia*, *Lapageria*, and/or *Rhipogonum* cannot be addressed by these data unless a substantial number of outgroup taxa from Liliales are included in the analysis. That is not likely to happen because sequence divergence among these families is extremely high for ITS, and would make for an ambiguous alignment at best. Even among the branches of the cladogram presented here, there is considerable distance between ingroup and outgroup taxa. The branch separating *Rhipogonum* from *Smilax*, for example, is more than eight times longer than the branch between *S. aspera* and the remaining species of *Smilax*, which have fairly low levels of sequence variation among themselves (phylogram



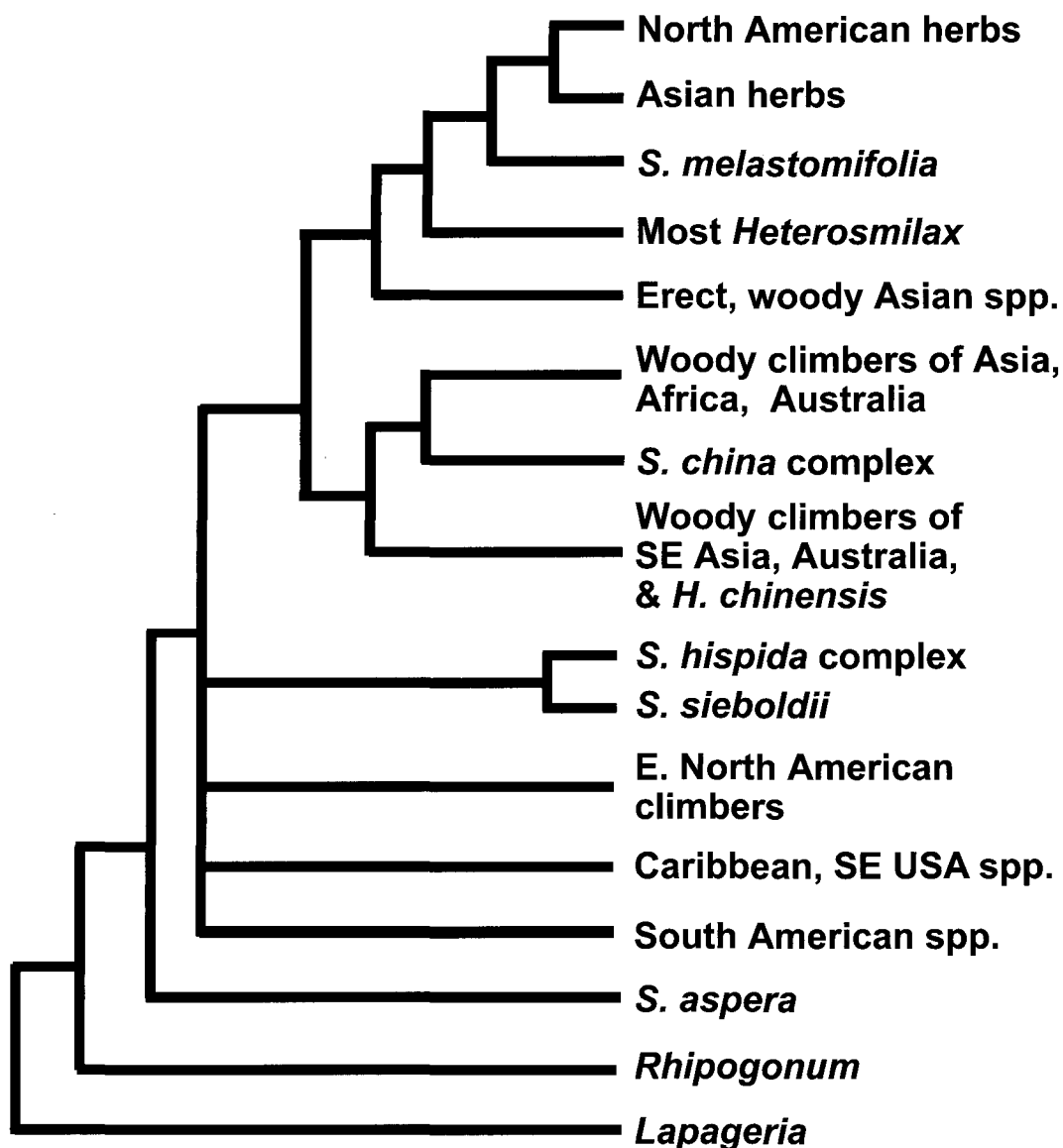


Fig. 2.—A generalized summary cladogram of the ITS strict-consensus tree for species of Smilacaceae. Major groups are identified by habit and geography.

not shown). As stated earlier, however, there is growing evidence that *Rhipogonum* and *Philesia* + *Lapageria* are more closely related to each other than either is to *Smilax*, and that each would be best classified as a distinct family since they do not form a monophyletic group (Fay 2003; and see other papers in this volume).

On the other hand, these ITS data do clearly indicate that *Heterosmilax*, *Nemexia* Raf., *Oligosmilax* Seem., *Pleiosmilax*, and *Pseudosmilax*—genera segregated from *Smilax* in some taxonomic systems—are embedded within *Smilax*. Recognition of any of them would render *Smilax* a paraphyletic genus at best. Seemann (1868) treated the Hawaiian

species *S. sandwicensis* Kunth (= *S. melastomifolia*) and *S. vitiensis* A. DC. from Fiji as members of *Pleiosmilax*. Rather than the six stamens typical in Smilacaceae, these three species possess numerous stamens (typically 12–18) in the male flowers. Although only one of these species is included in our matrix (*S. melastomifolia*), it is firmly embedded within *Smilax*, and clearly not in a position to be recognized as a separate genus. Why these Pacific Islands species should double or triple their stamen number is unclear. There may be a selective advantage for this condition related to restricted pollinators, or perhaps the plants are polyploid and/or of hybrid origin. We intend to explore this latter hypothesis in

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Fig. 1.—The strict consensus of 10,000 equally parsimonious trees resulting from analysis of ITS for Smilacaceae. The tree is oriented with *Lapageria rosea* (Philesiaceae) as the designated outgroup taxon. Bootstrap values >50% are presented above branches and geographic distribution for each species is indicated. Note that *Smilax aspera* is widespread in southern Europe, Africa, the Middle East, and south-western Asia.

future studies by comparing maternally inherited plastid gene sequences with our nuclear data.

The generic name *Nemexia* is applied by some taxonomists to those species of *Smilax* with herbaceous, aerial stems that die back each year. The two Asian species of this group (*S. nipponica* and *S. riparia*), as well as the several North American species (e.g., *S. biltmoreana*, *S. herbacea*, and *S. jamesii*) do form a monophyletic group (68% bts) in our tree. However, this clade is also embedded within *Smilax*, and does not merit generic status.

Likewise, species of *Heterosmilax* (= *Oligosmilax*, *Pseudosmilax*) are also nested within *Smilax* based on ITS data. In fact, *H. chinensis* is far removed from the two other included species of *Heterosmilax* (*H. japonica* and *H. yunnanensis*), making the genus polyphyletic. These morphologically distinct species are characterized by complete connation of the tepals into a tubular perianth. The stamens of the male flowers also fuse to form a single androecial column. Koyama (1984) monographed *Heterosmilax* and commented on the fact that *H. chinensis* was unlike any of the other 11 species in the genus because of its pubescent stems and three elongated, lanceolate, acute-tipped perianth lobes. According to our molecular phylogeny, these outstanding features may actually indicate that perianth fusion has evolved independently at least two times in Smilacaceae. The condition is probably related to a shift in pollinators, as there are reports of small gnats entering the flowers of *Heterosmilax*, whereas mostly larger flies visit *Smilax* flowers. There are also a few species of *Smilax* s.s. that are described as having flowers with partially connate stamens and/or tepals (Chen and Koyama 2000). Curiously, one of these species, *S. vanchingshanensis*, shows a close relationship with the *H. japonica* lineage, whereas the other, *S. corbularia*, is closely related to *H. chinensis*. These patterns of relationship suggest that both clades containing species with tubular flowers may have undergone a similar evolutionary transition from species with distinct tepals to fully connate perianth via intermediate floral forms. This speculation needs to be confirmed with sampling of additional *Heterosmilax* species.

Smilacaceae, it would seem, are to be treated as a monogeneric family containing only the genus *Smilax*. The type species of the genus, *S. aspera*, is sister to all others in the genus/family, and, thus the placement of this species makes it nearly impossible to divide *Smilax* into more than one monophyletic genus of reasonable size.

Relationships within Smilax

Since *Smilax* s.l. is the only monophyletic genus within Smilacaceae, then it seems desirable to classify the 200 or more species into natural (= monophyletic) subgenera or sections. Koyama (1960) proposed that *Smilax* (including the other segregate genera, but excluding *Heterosmilax*) could be divided into six sections. These are as follows: (1) *Smilax* sect. *Pleiosmilax* (species in which staminate flowers have more than six stamens, usually 12–18); (2) *Smilax* sect. *Smilax* (species with six stamens in male flowers, six prominent staminodes in pistillate flowers, and a spicate inflorescence); (3) *Smilax* sect. *Macranthae* Kunth (species with six stamens, no staminodes in female flowers, and umbellate inflorescences arranged in compound panicles); (4) *Smilax* sect.

Coilanthus A. DC. (species in which the anthers of the six stamens are sessile, and the inflorescence consists of single umbels); (5) *Smilax* sect. *China* T. Koyama (woody, perennial species with single umbels and six stamens, but these with anthers on long filaments); and (6) *Smilax* sect. *Coprosmanthus* (Torrey) Kunth (= sect. *Nemexia* (Raf.) A. DC.; species with six stamens, single umbels, and anthers on long filaments, but in which the herbaceous aerial stem dies back each year).

The ITS tree shows that Koyama's (1960) system is inconsistent with the recognition of exclusively monophyletic groups. *Smilax* sect. *Smilax* is monotypic (containing only *S. aspera*) and sister to the entire family; its inflorescence morphology is unique in *Smilax*, and therefore not unexpected as the basal lineage of Smilacaceae. *Smilax* sect. *Coprosmanthus* (the herbaceous species complex) is monophyletic, and is positioned as the most derived clade of Smilacaceae. The remaining members of the family, however, are a mixture of species from the other sections, none of which are monophyletic. There are a few select clades of species that can be defined by shared habit or vegetative features (e.g., the erect woody species) as shown in Fig. 2, but there is no way obvious to us at the present time in which *Smilax* could be better divided into sections based on morphology.

Phylogeny and Geography

Although morphological characters (especially those of inflorescence structure, filament length, and stamen number) appear to be homoplastic and poor indicators of interspecific relationships within *Smilax*; there is an evident pattern of geographic distribution to be found in the ITS tree. *Smilax aspera*, the most widespread species of the genus, is exceptional in that it is distributed over three continents (Africa, Europe, and Asia). Its position as sister to the entire family is intriguing from the standpoint of historical biogeography, but is beyond the scope of this paper. Other than this taxon, most species of *Smilax* are fairly restricted in their distribution.

The ITS phylogeny shows that all Old World species, with the exception of one species pair (*S. scobinicaulis* and *S. sieboldii* from China) share a common ancestor. The clade includes one of the few African species, *S. kraussiana*, as well as both Australian species, *S. australis* and *S. glyciophylla*, which are only distantly related to each other and intermixed among species from temperate and tropical Asia. The clade is not exclusively Old World in membership, however, since species of the *S. herbacea* lineage from North America are also found within it. In this respect, the placement of *S. herbacea* and its relatives within an otherwise Old World group represents the sole example of *Smilax* dispersal from the Old to the New World. Fu et al. (2005) discussed the historical biogeography of this fascinating clade in detail, and attributed its disjunct distribution to Pleistocene vicariance events that separated contiguous transcontinental populations following eastward migration from East Asia to North America. Such a pattern has been documented for a number of flowering plant groups (Bufford and Spongberg 1983).

With the exception of *S. scobinicaulis* and *S. sieboldii*, all remaining species of *Smilax* in the cladogram are native to

the New World. These lineages are not resolved as monophyletic in the strict consensus tree, but they do share a common ancestor (i.e., they may be paraphyletic). The enigmatic placement of Chinese *S. scobinicaulis* and *S. sieboldii* within the phylogeny is worthy of further discussion. This species pair is part of a complex that includes other conspicuously armored species of *Smilax* from Mexico and the mostly midwestern and western United States. Norton (1916) proposed that these taxa, together with *S. californica* A. Gray, *S. hispida*, and the fossil taxon *S. lamarensis* from Wyoming, might represent a migration of *Smilax* from Asia to North America. In opposition to Norton's (1916) hypothesis, our ITS data indicate that the presence of *S. scobinicaulis* and *S. sieboldii* in Asia more likely represents a dispersal from North America, perhaps also across the Bering Straits prior to Pleistocene glaciation, but in this case from east to west. This hypothesis would be strengthened if *S. californica*, the most northwestern American species of *Smilax*, is sampled and found to be the sister to the *S. sieboldii*-*S. scobinicaulis* pair.

Conclusions and Future Initiatives

This study documents the first hypothesis of phylogenetic relationships within Smilacaceae, but we concede that it is based on a limited number of taxa and characters. Already, steps are underway to generate a second complementary data set from a plastid gene (*rpl16*) for this same set of taxa, so that issues of possible hybrid origin and/or chloroplast capture may be addressed. We are also in the process of adding more Central and South American species to the matrix since those areas are not fully represented in this study. Although the results presented here should be considered preliminary, they nevertheless indicate with strong support that *Smilax* may be a genus of lilioid monocots with a more complex evolutionary history than previously thought.

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