

SEQUENCE DIVERGENCE OF *rbcL* GENE AND HYLOGENETIC RELATIONSHIPS IN LILIALES

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Abstract

DNA sequences of the chloroplast gene encoding the large subunit of ribulose-1, 5-bisphosphate-carboxylase (*rbcL*) of 20 taxa representing four different orders of Liliiflorae was compared. Two different methods (maximum parsimony using PAUP and neighbour joining (NJ) were employed for phylogenetic reconstruction. Similar tree topologies were obtained by both methods. Both trees indicate that Liliales *sensu* Dahlgren *et al.*, (1985) is not a monophyletic group. Phylogenetic analysis revealed two clades within order Liliales. Clade "A" includes *Tricyrtidaceae* and *Liliaceae sensu stricto* and corresponds to Liliales *sensu stricto*. Clade "B" includes *Colchicaceae* and *Uvulariaceae*. Monophyly of the taxa within groups A and B is supported in 92 and 100% respectively, of the bootstrap replications for parsimony method and 97 and 100% for neighbour joining method. The *Iridaceae* form a clade with Asparagales that is supported in 100% of the bootstrap replications for both parsimony and NJ methods. *Smilax*, which Dahlgren *et al.*, placed in Dioscoreales, is clustered with those of clade "A" of Liliales.

Introduction

The delimitation of the order Liliales *sensu lato* has been debated for many years (Table 1). Dahlgren *et al.*, (1985) made a comprehensive account of the order Liliales, principally following Huber's (1969) treatment. In Liliales *sensu*, Dahlgren *et al.*, (1985) included 10 families (Iridaceae, Geosiridaceae, Uvulariaceae, Colchicaceae, Liliaceae, Calochortaceae, Alstroemeriaceae). In their system, order Orchidales of Huber (1969) was reduced to three families (Apostasiaceae, Cyripediaceae, Orchidaceae) in Liliales. The shared characters of this order that they recognized are non-phytomelaniferous seed coat, nuclear endosperm formation, perigonal nectaries (rather than septal nectaries), and spotted petals. The families were differentiated into two main groups. Group-I (Iridaceae, Geosiridaceae, Uvulariaceae, Colchicaceae, Liliaceae, Calochortaceae) was distinguished from group-II (Apostasiaceae, Cyripediaceae, Orchidaceae, Alstroemeriaceae) by having stems without vessels (with an exception of Uvulariaceae), extrose anthers and absence of oxalate raphides.

Among Monocotyledons, Takhtajan (1980) hypothesized that Liliales, Alismatales, and Triuridales may have originated from a common ancestor with endospermous seeds and two-celled pollen grains (as in Liliales) and an apocarpus gynoeceum (as in Alismatales). Within Liliales, Melanthiaceae were postulated to be the nearest to the hypothetical ancestor (Lotsy, 1911; Takhtajan, 1959, 1966; Eames, 1961; Radulescu 1973). Hutchinson (1959) considers Liliaceae as the ancestral stock from which several petaloid monocotyledonous families such as Amaryllidaceae, Iridaceae, Palmeae and Orchidaceae have evolved.

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Table 1. Previous classifications of the genera analyzed.

Genus	Dahlgren <i>et al.</i> , (1985)	Bentham et Hooker (1983)	Krause (1930)
<i>Lilium</i>	Liliaceae/Litiales	Tulipeae/Liliaceae/Coronarieae	Lilioideae/Liliaceae/Lilinae
<i>Cardiocrinum</i>	Liliaceae/Litiales	Tulipeae/Liliaceae/Coronarieae	Lilioideae/Liliaceae/Lilinae
<i>Erythronium</i>	Liliaceae/Litiales	Tulipeae/Liliaceae/Coronarieae	Lilioideae/Liliaceae/Lilinae
<i>Medeola</i>	Liliaceae/Litiales	Medeoleae/Liliaceae/Coronarieae	Parideae/Asparagoideae/Liliaceae/Lilinae
<i>Scoliopis</i>	Uvularieae/Uvulariaceae/Litiales	Medeoleae/Liliaceae/Coronarieae	Parideae/Asparagoideae/Liliaceae/Lilinae
<i>Uvularia</i>	Uvularieae/Uvulariaceae/Litiales	Uvularieae/Liliaceae/Coronarieae	Uvularieae/Melanthioideae/Liliaceae/Lilinae
<i>Disporum</i>	Uvularieae/Uvulariaceae/Litiales	Uvularieae/Liliaceae/Coronarieae	Polygonateae/Asparagoideae/Liliaceae/Lilinae
<i>Prosartes</i>	Uvularieae/Uvulariaceae/Litiales	Uvularieae/Liliaceae/Coronarieae	Polygonateae/Asparagoideae/Liliaceae/Lilinae
<i>Streptopus</i>	Uvularieae/Uvulariaceae/Litiales	Polygonateae/Liliaceae/Coronarieae	Polygonateae/Asparagoideae/Liliaceae/Lilinae
<i>Clintonia</i>	Uvularieae/Uvulariaceae/Litiales	Medeoleae/Liliaceae/Coronarieae	Polygonateae/Asparagoideae/Liliaceae/Lilinae
<i>Tricyrtis</i>	Tricyrtideae/Uvulariaceae/Litiales	Uvularieae/Liliaceae/Coronarieae	Tricyrtiae/Melanthioideae/Liliaceae/Lilinae
<i>Gloriosa</i>	Iphigenieae/Colchicaceae/Litiales	Uvularieae/Liliaceae/Coronarieae	Uvularieae/Melanthioideae/Liliaceae/Lilinae
<i>Iris</i>	Iridaeae/Iridoideae/Iridaceae/Litiales	Iridaceae/Epigynae	Iridaceae/Iridinae
<i>Smilax</i>	Smilacoideae/Iridoideae/Iridaceae/Litiales	Liliaceae/Epigynae	Smilacoideae/Liliaceae/Lilinae
<i>Dioscorea</i>	Dioscoreoideae/Dioscoreaceae/Dioscoreales	Dioscoreaceae/Epigynae	Dioscoreaceae/Lilinae
<i>Trillium</i>	Trillaceae/Dioscoreales	Medeoleae/Liliaceae/Coronarieae	Parideae/Asparagoideae/Liliaceae/Lilinae
<i>Convallaria</i>	Convallariaceae/Convallariaceae/Asparagales	Convallariaceae/Liliaceae/Coronarieae	Convallariaceae/Asparagoideae/Liliaceae/Lilinae
<i>Scilla</i>	Hyacinthaceae/Asparagales	Scilleae/Liliaceae/Coronarieae	Scilloideae/Liliaceae/Lilinae
<i>Veratrum</i>	Melanthiaceae/Melanthiales	Veratreae/Liliaceae/Coronarieae	Veratreae/Melanthioideae/Liliaceae/Lilinae

(Cont'd. Table 1)

Genus	Hutchinson (1959)	Cronquist (1981)	Takhtaja (1987)
<i>Lilium</i>	Tulipeae/Liliaceae/Liliales	Liliaceae/Liliales	Liliaceae/Liliaceae/Liliales
<i>Cardiocrinum</i>	Tulipeae/Liliaceae/Liliales	Liliaceae/Liliales	Liliaceae/Liliaceae/Liliales
<i>Erythronium</i>	Tulipeae/Liliaceae/Liliales	Liliaceae/Liliales	Liliaceae/Liliaceae/Liliales
<i>Medeola</i>	Tricyrtideae/Liliales	Liliaceae/Liliales	Medeolaceae/Liliales
<i>Scotiopus</i>	Tricyrtideae/Liliales	Liliaceae/Liliales	Scoliopeae/Melanthioideae/Melanthiaceae/Liliales
<i>Uvularia</i>	Uvularieae/Liliaceae/Liliales	Liliaceae/Liliales	Uvularieae/Melanthioideae/Melanthiaceae/Liliales
<i>Disporum</i>	Polygonateae/Liliaceae/Liliales	Liliaceae/Liliales	Polygonateae/Convallarioideae/Convallariaceae/Asparagales
<i>Prosartes</i>	Polygonateae/Liliaceae/Liliales	Liliaceae/Liliales	Polygonateae/Convallarioideae/Convallariaceae/Asparagales
<i>Streptopus</i>	Polygonateae/Liliaceae/Liliales	Liliaceae/Liliales	Polygonateae/Convallarioideae/Convallariaceae/Asparagales
<i>Clintonia</i>	Polygonateae/Liliaceae/Liliales	Liliaceae/Liliales	Polygonateae/Convallarioideae/Convallariaceae/Asparagales
<i>Tricyrtis</i>	Tricyrtideae/Liliaceae/Liliales	Liliaceae/Liliales	Tricyrtideae/Melanthioideae/Melanthiaceae/Liliales
<i>Gloriosa</i>	Uvularieae/Liliaceae/Liliales	Liliaceae/Liliales	Glorioseae/Colchicoideae/Liliales
<i>Iris</i>	Iridaceae/Iridales	Iridaceae/Liliales	Iridoideae/Iridaceae/Liliales
<i>Smilax</i>	Smilacaceae/Liliales	Smilacaceae/Liliales	Smilacaceae/Smilacales
<i>Dioscorea</i>	Dioscoreaceae/Dioscoreales	Dioscoreaceae/Dioscoreales	Dioscoreaceae/Dioscoreales
<i>Trillium</i>	Tricyrtideae/Liliaceae	Liliaceae/Liliales	Trilliaceae/Dioscoreales
<i>Convallaria</i>	Convallarieae/Liliaceae/Liliales	Liliaceae/Liliales	Convallarieae/Convallarioideae/Convallariaceae/Asparagales
<i>Scilla</i>	Scillaceae/Liliaceae/Liliales	Liliaceae/Liliales	Hyacintheae/Hyacinthaceae/Amarylidales
<i>Veratrum</i>	Veratreeae/Liliaceae/Liliales	Liliaceae/Liliales	Melanthieae/Melanthioideae/Liliales

Delimitation of Liliales has frequently varied during the last decade (Takhtajan, 1980, 1987; Cronquist, 1981; Dahlgren & Clifford, 1982; Thorne, 1983, 1992; Dahlgren *et al.*, 1985; Conran, 1987; Dahlgren, 1989; Oganezova, 1990; Goldberg, 1989). No consensus is apparent regarding the placement of i) Orchidales and Iridales in Liliales, and ii) Smilacaceae in Liliales, Dioscoreales or Asparagales. Moreover, the familial assignment of many genera is in a state of chaos. Disputes arise from the fact that taxonomists have used different criteria for defining orders and lower taxonomic categories. Nevertheless, many authors (Oganezova, 1990; Dahlgren *et al.*, 1985, Dahlgren, 1989; Conran, 1987) have confirmed the overall arrangements of the Asparagales-Liliales by Dahlgren & Clifford (1982). Phenetic and cladistic approaches both favour the fragmentation of the Liliales *sensu lato* into small more homogenous orders.

In an attempt to reconstruct the phylogeny of the controversial taxa of Liliiflorae, we have been analyzing the DNA sequences of chloroplast gene (*rbcL*) encoding the large subunit of ribulose-1, 5-bisphosphate-carboxylase (Shinwari *et al.*, 1994a,b). In this paper, we address the following questions by comparing *rbcL* sequences of 20 taxa representing four different orders of Liliiflorae *sensu* Dahlgren *et al.*, (1985).

- 1) Is Liliales *sensu* Dahlgren *et al.*, (1985) a monophyletic group?
- 2) Can *rbcL* data help to resolve the question of the placement of Iridaceae in Liliales raised by Dahlgren *et al.*, (1985)?
- 3) Is merging of Tricyrtidaceae with Uvulariaceae supported by *rbcL* sequence data?
- 4) Do *rbcL* data give any insight to solve the phylogenetic relationship of Smilacaceae with different orders

Material and Methods

Plant materials: *rbcL* sequences of 21 taxa, representing four different orders of Liliiflorae *sensu* Dahlgren *et al.*, (1985), was determined. Voucher specimens of the plant materials (Table 2) are deposited in the Herbarium of Kyoto University (KYO). Among 19 genera analyzed 12 (*Lilium*, *Cardiocrinum*, *Erythronium*, *Medeola*, *Uvularia*, *Disporum*, *Prosartes*, *Streptopus*, *Clintonia*, *Tricyrtis*, *Gloriosa*, and *Iris*) belong to Liliales, four (*Smilax*, *Scoliopus*, *Trillium* and *Dioscorea*) to Dioscoreales, two (*Scilla* and *Convallaria*) to Asparagales, and one (*Veratrum*) to Melanthiales *sensu* Dahlgren *et al.*, (1985).

DNA extraction: Total genomic DNA was extracted from fresh or frozen leaves according to the method of Tai & Tanksley (1990), except that liquid nitrogen was used to assist in the grinding of plant tissue.

Polymerase chain reaction: PCR amplification of the almost entire part of the *rbcL* gene (1411pb) was conducted using two primers which anneal to the start position (*rbcLN*:5'-ATGTCACCA CAAACAGAAACT-3') and close to the end of the *rbcL* coding region (DBRBAS2:5'-GCTTGAATTCGAATTTGATC-3). To obtain the sequence of the 5' end of *rbcL* gene, PCR was conducted using additional primer corresponding to the *atp* β gene (*atp* β 232 5'-CCGTCCGTAGCATCATAGC-3') which locates at the upstream of *rbcL* gene (Table 3). The amplification reaction mixture (100 μ l) contained 50-100ng of genomic DNA, 40 pmol of each primer, 0.2mM each of

Table 2. Sources of plant materials.

Species	Localities	Collector(s)
<i>Clintonia borealis</i> Rafin	USA: Wisconsin: Mrathen Co. Forest	S. Kawano <i>et al.</i>
<i>Convallaria majalis</i> L.	Russia: Caucasia	H.H. Iltis
<i>Disporum sessile</i> Don	Japan: Kyoto Pref: Ohmiya cho, Mt. Takano	Z.K. Shinwari
<i>Prosartes lanuginose</i> Michx.	USA: Pennsylvania: Somerset Co.: Powder Mill	S. Kawano <i>et al.</i>
<i>Streptopus lanceolatus</i> (Aiton) Reveal	USA: Wisconsin: Marathen Co. Forest	S. Kawano <i>et al.</i>
<i>Tricyrtis affinis</i> Makino	Japan: Kyoto Pref.: Ohmiya-cho	Z.K. Shinwari
<i>Uvularia sessilifolia</i> L.	USA: Pennsylvania: Somerset Co.: Powder Mill	S. Kawano <i>et al.</i>
<i>U. floridana</i> Chapman	USA: Florida: Gadsen Co.: Flat Creak	S. Kawano <i>et al.</i>
<i>Scoliopus bigelovii</i> Torr.	USA: California, Humboldt Co.	S. Kawano <i>et al.</i>
<i>Trillium kamtschaticum</i> Pallas	Japan: Hokkaido, Chitose city	H. Kato
<i>Medeola virginiana</i> L.	USA: Pennsylvania, Somerset Co.	S. Kawano <i>et al.</i>
subsp. <i>Oxysepalum</i> Hulten	Japan: Nara Pref., Kamikitayama-mura	H. Takahashi
<i>Veratrum album</i> L.		
<i>Gloriosa superba</i> L.	Thailand: Loei, Ban Na Noi	H. Takahashi
<i>Lilium lancifolium</i> Thunb.	Japan: Nagasaki Pref., Izuhara-cho, Shiine	K. Hayashi
<i>Cardiocrinum cordatum</i> (Thun.) Makino	Japan: Osaka Pref., Chihayaakasaka-mura	K. Hayashi
<i>Erythronium japonicum</i> Decne.	Japan: Toyama Pref., Yatsuo-machi	H. Kato
<i>Dioscorea bulbifera</i> L.	Madagascar: Antananarivo	R. Terauchi
<i>Smilax china</i> L.	Japan: Shiga Pref., Gamo-cho	H. Kato
var. <i>spontanea</i> (Makino) Nakai		
<i>Iris ensata</i> Thunb.	Japan: Shiga Pref., Gamo-cho	H. Kato
<i>Scilla scilloides</i> (Lindl.) Druce	China:	S. Noda
<i>Acorus calamus</i> L.	Japan: Kyoto Pref., Kyoto University Bot. Garden	J. Katsuchi

Table 3. PCR and sequencing primers used in this study.

Primer	Sequence	Location*		Design based on and reference
		Strand	Location*	
<i>rbcL</i> N'	5'-ATGTCACCA CAAACAGAAACT-3'	1 sense		Wheat <i>rbcL</i> , Terachi <i>et al.</i> , 1987
S1	5'-AGGACGATGACTACCCATCG-3'	243 sense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
S2	5'-AAAACCTTCCAAGGCC-3'	435 sense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
S3	5'-TTTATGCGTTGGAGAGACCCG-3'	631 sense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
S4	5'-AATGCATGCAGTTATTG-3'	887 sense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
S5	5'-GGTATTCAATGTTTG GCA-3'	1141 sense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
DBRBAS2	5'-GCTTGAATTCGAATTTGATC-3'	1411 antisense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
DBRBAS1	5'-TTACGAGCTTGTACACACGC-3'	1295 antisense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
TRRVI	5'-TAGAGACCCAA TCITGAGTG-3'	1111 antisense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
RV7	5'-ATATGCCAAACATGAATACCC-3'	1160 antisense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
RV6	5'-TGAGCCAAAGCTAGTTAATTTC-3'	845 antisense		Consensus sequence of 60 species of Liliiflorae's <i>rbcL</i> . (Terauchi unpublished)
RV3	5'-GCTAAGTAGTCA TGCAT-3'	812 antisense		Consensus sequence of 60 species of Liliiflorae's <i>rbcL</i> . (Terauchi unpublished)
RV5	5'-CCGTAGTTCITTTGGGATAA-3'	557 antisense		Consensus sequence of 60 species of Liliiflorae's <i>rbcL</i> . (Terauchi unpublished)
RV1	5'-TTGTAACGATCAAGACT-3'	242 antisense		Consensus sequence of 60 species of Liliiflorae's <i>rbcL</i> . (Terauchi unpublished)
RV4	5'-TCAGTCCACACAGTTGTCCA-3'	215 antisense		Consensus sequence of 60 species of Liliiflorae's <i>rbcL</i> . (Terauchi unpublished)
PX6	5'-GC ATCGTCCCTTGTAAACGA-3'	252 antisense		Consensus sequence of wheat (Terachi <i>et al.</i> , 1987) and <i>Dioscorea rbcL</i> . (Terauchi unpublished)
atp β 232	5'-CCGTCCG IAGC AICATAGC-3'	atp β 232 antisense		Consensus sequence of wheat atp β (Howe <i>et al.</i> , 1985), rice (Moon <i>et al.</i> , 1987) and <i>Nicotiana bigelovii</i> (Avni <i>et al.</i> , unpublished, X 61316)

*Location of 5' end base of the primer is indicated with regard to the site number in *rbcL* gene.

dNTPs; 50mM KCl, 10mM Tris HCl pH8.8, 1.5mM MgCl₂, 0.1% Triton X-100 and 2.0 unit of *Taq* DNA polymerase (Wako Chemicals). Amplification was conducted in a DNA thermal cycler (Perkin Elmer Cetus) for 35 cycles. Each cycle consisted of a denaturing step of 1 min at 94°C, an annealing step of 2 min at 54°C and an extension step of 3 min at 72°C. The final extension step was extended to 10 min. The amplified DNA fragment was isolated from the remaining primers and dNTPs by electrophoresis through 1% agarose gel. Excised DNA was purified using a DNA purification kit (Gene Clean II, Bio101) and was resuspended in 20 µl of TE (10mM Tris-HCl pH8.0, 1mM EDTA). The final yield was about 4 µg of DNA, enough for eight sequencing reactions.

DNA sequencing: Purified double-stranded DNA was used as a template for direct sequencing by an auto-sequencer (ABI 373A) and Taq DyeDeoxy™ terminator cycle sequencing kit (ABI) according to the manufacturer's instructions. Six primers were used for the sense-strand and 10 primers were used for antisense-strand (Table 3).

Tree reconstruction: Relationships among 20 *rbcL* sequences were analyzed using the *Acorus calamus* sequence as outgroup. *Acorus* was hypothesized to be a sister group of other monocotyledons (Duvall *et al.*, 1993). Two different tree reconstruction methods were employed viz., the maximum parsimony method applied to the character change data sets of nucleotide sequences (Fitch, 1977), and the neighbour-joining method (Saitou & Nei, 1987) applied to the distance matrix data sets.

For the maximum parsimony methods, the computer program PAUP version 3.1.1 (Swofford, 1993) was used. The heuristic bootstrap option was used to find the maximum parsimony tree, and a bootstrapping of 100 replications was conducted to put the confidence values to the tree topology. A most parsimony tree was obtained (length=573, CI=0.64 and RI=0.667). To make a distance matrix (Table 4) among the taxa, Kimura's (1981) two parameter estimates of the number of substitutions per site between the sequences were estimated by DNADIST program of PHYLIP ver 3.4 (Felsenstein, 1991). The resulting distance matrix was then analyzed by NEIGHBOUR program of PHYLIP to obtain the tree by the neighbour-joining method (Saitou & Nei, 1987). The SEQBOOT program of PHYLIP was used to obtain the bootstrapping probabilities for each branch of the tree.

Results

Phylogenetic analyses: Similar tree topologies were given by the maximum parsimony method (Fig. 1) and the neighbour-joining method (Fig. 2). Both the trees indicate that the Liliales *sensu* Dahlgren *et al.*, (1985) is not a monophyletic group and has at least two different groups. Group "A" consisting of *Tricyrtis*, *Prosartes*, *Scoliopus*, *Streptopus*, *Clintonia*, *Medeola*, *Erythronium*, *Lilium*, *Cardiocrinum* and *Smilax* corresponds to Liliales *sensu stricto*: these are Liliaceae *sensu stricto* and Tricyrtidaceae. Group "B" includes Colchicaceae (*Gloriosa*) and Uvulariaceae (*Disporum* and *Uvularia*). Monophyly of the taxa within groups A and B is supported in 94-97, 100% respectively of the bootstrap replications for parsimony method and neighbour joining methods (Fig. 1 and 2). The Iridaceae apparently do not come together with order Liliales *sensu* Dahlgren *et al.*, (1985). Instead it forms a clade with Asparagales that is supported in 100% of the bootstrap replications for PAUP and NJ methods. *Smilax*, which Dahlgren *et al.*, (1985) placed in Dioscoreales, is clustered with those of clade "A" of Liliales.

Table 4. The number of base substitutions per site given as 100xd which was calculated according to Kimura (1981).

	<i>Tric.</i>	<i>Pros.</i>	<i>Stre.</i>	<i>Scoli.</i>	<i>Clin.</i>	<i>Med.</i>	<i>Eryt.</i>	<i>Lil.</i>	<i>Car.</i>	<i>Smilax</i>	<i>Dis.</i>	<i>Uvul.</i>	<i>Glorio.</i>	<i>Verat.</i>	<i>Scilla</i>	<i>Con.</i>	<i>Iris</i>
<i>Tricyrtis</i>	--	2.08	1.93	2.16	2.68	3.6	3.68	2.44	2.54	4.04	7.57	6.98	8.08	6.68	7.75	5.5	6.27
<i>Proxartes</i>	28	--	1.03	0.81	2.99	3.22	3.45	2.84	3.04	4.04	7.41	6.58	7.44	6.15	7.66	5.67	6.09
<i>Streptopus</i>	26	14	--	1.11	2.68	2.91	3.29	2.6	2.87	3.68	7.08	6.33	7.34	6.32	7.01	5.4	5.9
<i>Scoliopis</i>	29	11	15	--	2.76	3.14	3.68	2.84	2.96	4.03	7.33	6.74	7.16	6.05	6.93	5.59	5.91
<i>Clintonia</i>	36	40	36	37	--	1.48	2.76	1.56	1.96	4.12	7.56	6.98	8.05	6.77	7.41	5.66	5.99
<i>Medeola</i>	48	43	39	42	20	--	2.76	2.28	2.87	4.39	8.55	7.45	8.51	7.04	7.24	6.1	6.63
<i>Erythronium</i>	46	41	42	41	33	39	--	2.44	2.87	5.0	8.14	7.14	8.26	7.51	7.83	6.19	6.35
<i>Lilium</i>	31	36	33	36	20	29	29	--	0.97	4.38	7.41	6.55	7.79	6.86	7.25	5.84	6.26
<i>Cardiocrinu</i>	31	37	35	36	24	35	35	12	--	4.57	7.73	6.82	8.28	6.66	7.82	5.81	6.37
<i>Smilax</i>	51	57	54	55	53	55	64	55	60	--	6.82	7.09	7.93	7.55	7.3	6.1	6.36
<i>Disporum</i>	98	96	92	95	98	110	96	91	91	94	--	2.01	3.54	8.44	8.83	7.35	7.46
<i>Uvularia</i>	90	85	82	87	91	97	86	81	82	90	26	--	3.19	7.77	8.4	6.97	7.44
<i>Gloriosa</i>	93	86	85	83	93	98	93	90	93	97	42	37	--	8.72	9.0	7.43	8.1
<i>Veratrum</i>	77	71	73	70	78	81	84	79	75	81	96	90	99	--	7.07	6.77	6.97
<i>Scilla</i>	84	85	81	82	92	88	84	77	75	81	110	102	96	75	--	3.54	4.39
<i>Convallaria</i>	66	68	65	67	68	73	74	70	68	73	87	85	86	78	32	--	3.41
<i>Iris</i>	78	78	73	75	76	75	76	71	72	76	102	92	90	75	45	21	--

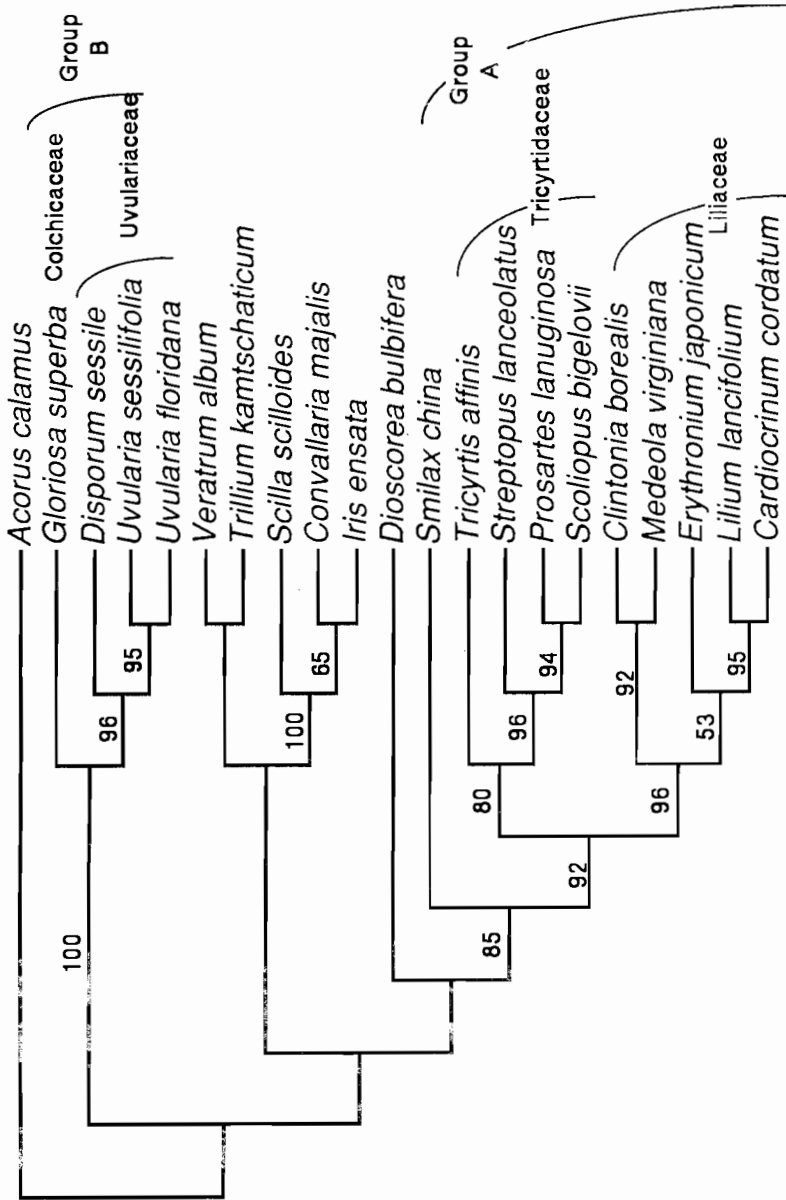


Fig. 1. The most parsimonious tree (Length = 573, CI = 0.640, RI = 0.667). The values on branches indicate bootstrap probabilities (%) out of 100 Bootstrap replications (only more than 50% are shown).

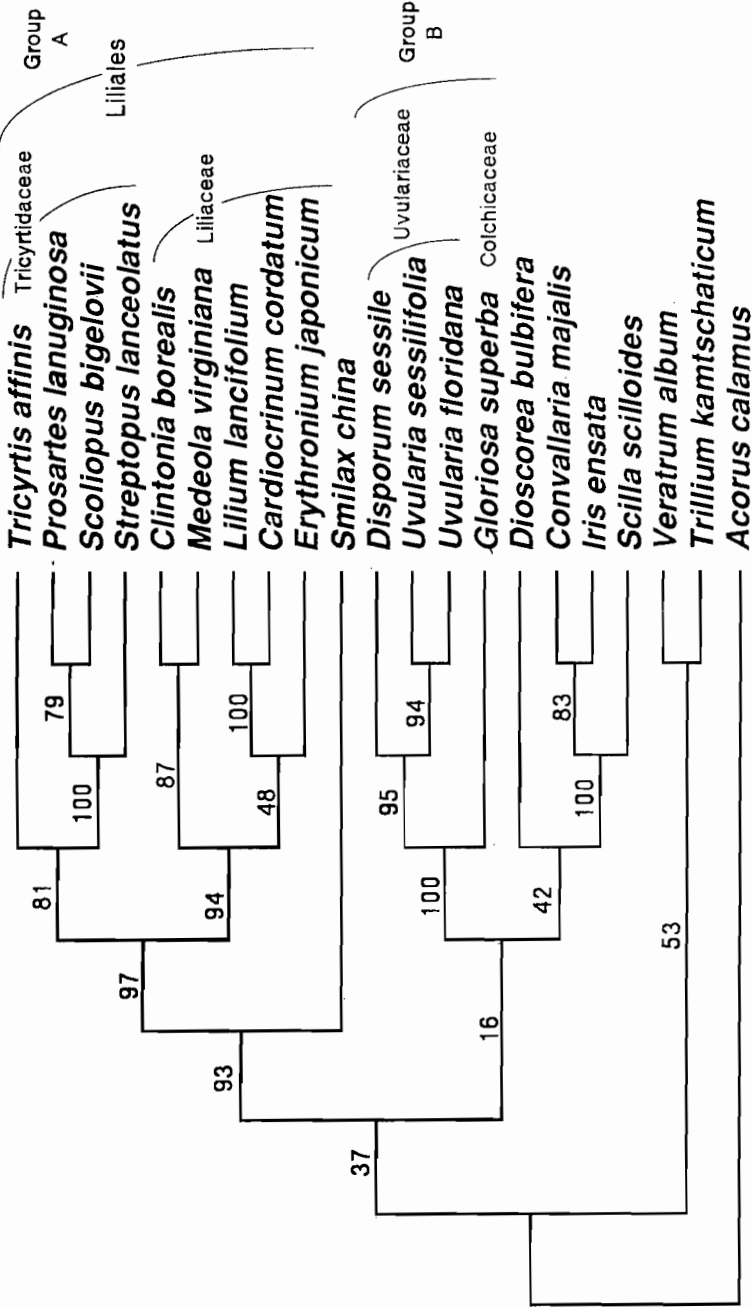


Fig. 2. Consensus tree obtained by the neighbour joining method. Value on branches indicate the bootstrapping probabilities.

TOP SENESCENCE IN SOME MEMBERS OF AMARYLLIDACEAE

Number of base substitutions among 22 genera: The sequences of 18 *rbcL* genes were determined. *Clintonia borealis*, *Prosartes lanuginose* (= *Disporum lanuginosum*), *Disporum sessile*, *Streptopus lanceolatus* (= *S. roseus*), *Tricyrtis affinis*, *Uvularia sessilifolia*, *U. floridana*, *Erythronium japonicum*, *Medeola virginiana*, *Scilla scilliodes*, *Scoliopus bigelovii*, *Trillium kamtschaticum*, *Veratrum album*, *Dioscorea bulbifera*, *Iris ensata*, *Smilax china* and *Convallaria majalis*, are registered at DDBJ (DNA Data Bank of Japan) under the accession number D 17372, D 17374, D 17376, D 17381, D 17382, D 17383, D 28328, D 28156, D 28158, D 28161, D 28162, D 28165, D 28168, D 28327, D 28332, D 28333 and D 28334, respectively. *Lilium lancifolium*, *Cardiocrinum cordatum*, *Gloriosa superba* and *Acorus calamus* are our unregistered data. From the sequence data set, the number of substitution per site was calculated by Kimura's (1981) two-parameter method (Table 4). The base pair differences among taxa within group "A" (Fig. 1 and 2) ranged from 11-48 (100d=1.03-3.68) and within group "B" from 26-42 (100d=1.7-3.54). Group "A" differed from group "B" in 81-110 base pairs (100d=6.33-8.55). *Iris* differed from Liliales *sensu* Dahlgren *et al.*, (1985) by 71-102 base pairs (100d=5.9-8.79), while its difference from Asparagales was 21-45 base pairs (100d=3.41-4.39).

Discussion

The *rbcL* results obtained indicate that Liliales *sensu* Dahlgren *et al.*, (1985) are not a monophyletic group. Analysis of present data suggests the presence of two main phylogenetic groups in Liliales *sensu* Dahlgren *et al.*, (1985) (Figs. 1, 2). Group "A" includes families Liliaceae and Tricyrtidaceae *sensu* (Dahlgren and Clifford 1982), and group "B" includes Colchicaceae and Uvulariaceae.

Taxa in group "A" can be differentiated morphologically from those in groups B by having loculicidal capsule in contrast to septicidal. Liliaceae *sensu stricto* has *Fritillaria* type embryo sac formation, (this character is an important autapomorphy for this family), all others *Polygonum* or *Allium* type of embryo sac formation. Members of group "B" have alkaloids whereas the remaining Liliales, Asparagales and Melanthiales have steroidal saponins Dahlgren *et al.*, (1985). Uvulariaceae were treated as a tribe of Colchicaceae by Takhtajan (1980), the former having rhizomatous underground part whereas the later have a corn.

Iridaceae were placed in Liliales by Dahlgren *et al.*, (1985), but they were not certain of this placement. Its placement varies in almost all the systems (Table 1). Morphologically Iridaceae were different from Liliales by having epigynous flowers with three stamens only in contrast to hypogynous (except Alstroemeriaceae) and 6 stamens. Moreover, Iridaceae have septal nectaries rather than perigonal nectaries, which is characteristic of Asparagales. In Figs. 1 and 2, they form a clade with Asparagales, which is concordant with morphological data suggesting that Iridaceae better be transferred to Asparagales.

Björnstad (1970) suggested that some of the genera like *Disporum*, *Prosartes*, *Clintonia* should be transferred to Uvulariaceae. Dahlgren *et al.*, (1985) merged Tricyrtidaceae into Uvulariaceae. In Conran's (1987) cladistic analysis of Liliiflorae, *Streptopus* and *Tricyrtis* were isolated from Uvulariaceae. In his analysis, Uvulariaceae were associated with Liliales in the PAUP analysis (Kluge & Farris 1969; Farris 1970)

and with Asparagales in the BOOTM (bootstrap confidence by mixed method parsimony analysis) (Felsenstein, 1985).

The present data suggest that Tricyrtidaceae should be retained as an independent family including *Prosartes*, *Scoliopus*, *Streptopus* and *Tricyrtis*, *Disporum* should be retained in Uvulariaceae. Moreover, *Medeola* and *Scoliopus* should be transferred to Liliales as suggested by Berg (1962), and Dahlgren *et al.*, (1985), and also confirmed by our recent studies (Shinwari, 1998).

Smilacaceae was placed under Liliaceae by Krause (1930) Hutchinson (1959) and Cronquist (1981) gave family rank for Smilacaceae, because of its advanced karyotype (Sen, 1975) and woody climbing habit. Cronquist (1981) reported that Smilacaceae were intermediate between the Liliales and Dioscoreales, with both Smilacaceae and Dioscoriaceae derived from Liliaceous ancestors. Thorne (1983, 1992) placed Smilacaceae under suborder Dioscorinae. Dahlgren *et al.*, (1985) reported that Smilacaceae form a bridge between Asparagales and Dioscoreales. Smilacaceae form a clade with Liliales *sensu stricto* (Figs. 1, 2), which is concordant to Hutchinson (1959), Sen (1975) and Cronquist (1981) by keeping it next to Liliales *sensu stricto* and seems to be the sister family of the latter.

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References

- Bentham, G. and J.D. Hooker. 1883. *Genera Plantarum*, 3: 748-837. London.
- Berg, R.Y. 1962a. Morphology and taxonomic position of *Medeola*, Liliaceae. *Skr. Nov. Videns Akad. Oslo N Ser.*, 3: 1-55.
- Berg, R.Y. 1962b. Contribution to the comparative embryology of Liliaceae: *Scoliopus*, *Trillium*, *Paris* and *Medeola*. *Skr. Nor. Videns Akad. Oslo N Ser.*, 4: 1-64.
- Björnstad, I. 1970. Comparative embryology of Asparagoideae-Polygonatae, Liliaceae. *Nytt. Mag. Bot.*, 17: 169-207.
- Conran, J.G. 1987. A phenetic study of the relationships of *Drymophila* R. Br. Within the reticulate veined *Liliiflorae*. *Austr. J. Bot.*, 35: 283-300.
- Conran, J.G. 1989. Cladistic analysis of some net-veined *Liliiflorae*. *Pl. Sys. Evol.*, 168: 123-141.
- Cronquist, A. 1981. *An integrated system of classification of flowering plants*. Columbia Univ. Press, New York.
- Dahlgren, G. 1989. An updated angiosperm classification. *Bot. J. Linn. Soc.*, 100: 197-203.
- Dahlgren, R.M.T. and H.T. Clifford. 1982. *The Monocotyledons- A Comparative Study*. Academic Press, London.
- Dahlgren, R.M.T., H.T. Clifford and P.F. Yeo. 1985. *The Families of Monocotyledons*. Springer, Berlin.

- Duvall, M.R., G.H. Learn, L.E. Eguiarte and M.T. Clegg. 1993. Phylogenetic analysis of *rbcl* sequences identifies *Acorus calamus* as the primal extant monocotyledon. *Proc. Natl. Acad. Sci., USA.*, 90: 4641-4644.
- Eames, A.J. 1961. *Morphology of the Angiosperms*. McGraw-Hill. New York.
- Farris, J.S. 1970. Methods for computing Wagner trees. *Syst. Zool.*, 19: 83-92.
- Felsenstein, J. 1985. Phylogenetic and comparative method. *Amer. Naturalist*, 125: 1-15.
- Felsenstein, J. 1991. PHYLIP (Phylogeny Inference Package). Version 3.4.
- Fitch, W.M. 1977. On the problem of discovering the most parsimonious tree. *Am. Nat.*, 111: 223-257.
- Garay, L.A. 1960. On the origin of Orchidaceae. *Leaflet Harv. Univ. Bot. Mus.*, 19: 57-96.
- Gobi, C. 1916. *A review of the systems of plants*. Petrograd. (in Russian).
- Goldberg, A. 1989. Classification, evolution, and phylogeny of the families of monocotyledons. *Smithsonian Contr. Bot.*, 71: 1-74.
- Hallier, H. 1912. L'origine et le système phyletique des Angiosperms exposés à l'aide de leur arbre généalogique. *Arch. Neerl. Sci. Exact. Nat. Ser.*, 3, 1: 146-234.
- Howe, C.J., I.M. Fearnley, J.E. Walter, T.A. Dyer and J.C. Gray. 1985. Nucleotide sequences of the genes of the alpha, beta and epsilon subunits of wheat chloroplast ATP synthase. *Plant Mol. Biol.*, 4: 333-345.
- Huber, H. 1969. Die Samenmerkmale und Verwandtschaftsverhältnisse der Liliifloren. *Mitt. Bot. Staatssamml. München*, 8: 219-539.
- Hutchinson, J. 1959. *The Families of Flowering Plants*. 2: 591-621. Oxford Clarendon Press.
- Kimura, M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. *Proc. Natl. Acad. Sci., USA.*, 78: 454-458.
- Kluge, A.G. and J.S. Farris. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.*, 18: 1-32.
- Krause, K. 1930. *Liliaceae* - In: Krause, A. and Prantl, K. (Eds.): *Die natürlichen Pflanzenfamilien*, 15a, pp. 227-386. Leipzig: Engelmann.
- Lotsy, J.P. 1911. Vortrage über botanische Stammesgeschichte. Cormophyta siphonogamia, Vol III. Fischer, Jena.
- Melchior, H. 1964. In: Krause, A. and Prantl, K. (Eds.): *Syllabus der Pflanzenfamilien Angiospermen*. 12, Vol. 2.
- Moon, E., T. Kao and R. Wu. 1987. Sequence of the chloroplast encoded *atp B-atp E-trn M* gene clusters from rice. *Nucleic Acid Research*, 15: 4358-4369.
- Oganezova, G.G. 1990. Seed and fruit anatomy of some Amaryllidaceae in connection with their systematics and phylogeny. *Bot. Zn.*, 75: 615-630.
- Radulescu, D. 1973. Liliiflorae. Discussions et considerations phylogenetiques à l'aide de quelques recherches morphologiques. *Lucr. Gard. Bot. Bucuresti.*, 1972-1973: 249-283.
- Rao, V.S. 1969. The floral anatomy and relationships of the rare *Apostasias*. *J. Indian Bot. Soc.*, 68: 374-385.
- Rao, V.S. 1974. The relationships of the *Apostasiaceae* on the basis of floral anatomy. *Bot. J. Linn. Soc.*, 68: 319-327.
- Reveal, L.J. 1993. *Streptopus lanceolatus* (Aiton) Reveal, a new name for *Streptopus roseus* Michx. (Convallariaceae). *Phytologia*, 74: 185-189.
- Saitou, N. and M. Nei. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-425.
- Sen, S. 1975. Cytotaxonomy of Liliales. *Fedd. Rep.*, 86: 255-305.
- Shinwari, Z.K., R. Terauchi, F.H. Utech and S. Kawano. 1994a. Recognition of the new world *Disporum* section *Prosartes* as *Prosartes* (Liliaceae) based on the sequence data of *rbcl* gene. *Taxon*, 43: 353-366.
- Shinwari, Z.K., R. Terauchi and S. Kawano. 1994b. Phylogenetic relationship of the genera of tribe Polygonatae-Asparagoideae-Liliaceae *sensu lato*. *Pl. Syst. Evol.*, 192: 263-277.
- Shinwari, Z.K. 1998. Systematics of the genus *Uvularia* and related taxa based upon *rbcl* gene sequence data. *Pak. J. Bot.*, 30: 161-172.

- Swofford, D. 1993. PAUP: Phylogenetic Analysis Using Parsimony, ver. 3.1.1. Computer programe distributed by the Illionois Natural History Survey, Champaign, IL. USA.
- Tai, T.H. and S.D. Tanksley. 1990. A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. *Plant Mol. Biol. Reporter*, 8: 297-303.
- Takhtajan, A. 1959. Die Evolution der Angiospermen. Fischer. Jena.
- Takhtajan, A. 1966. A system and phylogeny of the flowering plants. *Nauka. Moscow and Leningard*. (in press).
- Takhtajan, A. 1969. Flowering plants. Origin and dispersal. Oliver and Boyd, Edinbergh.
- Takhtajan, A. 1980. Outline of the classification of flowering plants. *The Botanical Review*, 46: 225-359.
- Takhtajan, A. 1987. Systema Magnoliophytorum. 287-309. Leninopoli Officina Editoria "NAUKA" Sectio Leninopolitana MCML XXXVII.
- Terachi, T., Y. Ogihara and K. Tsunwaki. 1987. The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops* VI. Complete nucleotide sequences of the *rbcL* genes encoding H. and L-type rubisco large subunits in common wheat and *Aegilops-crassa* 4X. *Jpn. J. Genet.*, 62: 375-388.
- Thorne, R.F. 1983. Proposed new realignments in the Angiosperms. *Nord. J. Bot.*, 3: 85-117.
- Thorne, R.F. 1992. Classification and geography of the flowering plants. *Bot. Rev.*, 58: 225-349.

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