Systematic Botany (2002), 27(4): pp. 674–692

Phylogenetic Analyses of Trilliaceae based on Morphological and Molecular Data

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Communicating Editor: Jeff H. Rettig

ABSTRACT. These comprehensive analyses of systematic relationships within Trilliaceae focused on the relationships within *Paris* sensu lato (i.e., *Paris*, *Daiswa*, and *Kinugasa*); between species of *Trillium*; and between *Paris* sensu lato and *Trillium*. Seventy species were selected for cladistic analyses and scored for 110 morphological characters; *matK* and ITS molecular characters were obtained from GenBank for a subset of 26 taxa. Based on the preliminary results, *Trillium rivale* was used as a functional outgroup. For the subset of 26 species, analysis of the combined ITS and *matK* sequence data produced six shortest trees; the morphological data, 13 shortest trees; and the combined morphological and molecular data sets, three trees. Analyses of the full morphological data set of 70 species produced 76 shortest trees. *Trillium rivale* was distinct from both *Paris* and *Trillium* and should be placed in its own genus, for which the name ***Pseudotrillium*** is proposed. *Trillium govanianum* was more closely related to *Paris* than to *Trillium* but should be retained as a monotypic genus, *Trillidium*. *Trillium* and *Paris* were monophyletic based on molecular as well as morphological evidence. The cladistic analyses strongly support the separation of *Paris* sensu lato into *Daiswa*, *Kinugasa*, and *Paris*. The monophyly of *Trillium* after removal of ***Pseudotrillium*** and *Trillidium* was supported in all but the large morphological analysis; subgenus *Phyllantherum* was monophyletic in all cases, but subgenus *Trillium* was not monophyletic.

Because of their simple and distinctive morphology (a single flower subtended by a single whorl of leaves on an otherwise naked aerial stem), Trilliaceae have been easy to circumscribe but difficult to place (Table 1). Since initial recognition of the family as a unit by Lindley in 1846 (Reveal 1998), its members have been placed in seven orders and as parts of five families (Table 2; Zomlefer 1996; Farmer 2000). Recent studies such as those using *rbcL* gene sequences (Chase, pers. comm.; Chase et al. 1993, 1995a; Kato et al. 1995b), chloroplast DNA (cpDNA) restriction site analysis (Davis 1995; Kato et al. 1995a), and combined morphological-restriction site analyses (Stevenson and Loconte 1995; Chase et al. 1995b) show that Trilliaceae are monophyletic and clearly separate from other liliaceous genera. This has reinforced the concept that Trilliaceae are not part of a traditional, broad Liliaceae and should in fact be recognized as a separate family. Several of these same studies as well as others (Davis 1995; Chase et al. 1995b; Davis et al. 1998; Fuse and Tamura 1999, 2000; Chase et al. 2000; Rudall et al. 2000; Zomlefer et al. 2001) place Trilliaceae within a larger clade containing Melanthiaceae.

Based on molecular evidence, the APG (1998) includes Trilliaceae in Melanthiaceae as tribe Parideae under the principle of monophyly. The morphology, however, is extremely divergent between these two groups (Zomlefer 1996, 1997; Zomlefer et al. 2001; Farmer 2000); there are no morphological synapomorphies that unite these two groups as separate from other Liliaceous plants (Dahlgren and Clifford 1982; Goldblatt 1995). The characters that they do share such as basifixed anthers, the lack of xylem vessels, and the presence of steroid saponins seem to be plesiomorphic in nature with the rest of Liliaceae s.l. The divergence in ITS sequences (Baldwin et al. 1995) between Melanthiaceae and Trilliaceae adds support for treating Trilliaceae as a separate family. Variability in the 5.8S region is quite high; 21.3% of the sites were variable whereas in the ''Trilliaceae only'' data set 6.7% were variable. (For the whole data set, with *Veratrum maackii* Regel as the outgroup, 55.4% of the sites were variable; with *Trillium rivale* S. Wats. as outgroup only 30% of the sites were variable.) For these reasons, we treat Trilliaceae as a separate family.

The systematics of Trilliaceae are not resolved, despite a long history of study and the distinctiveness of the group. One major question regards generic delineation in Trilliaceae: is the traditional generic split between *Trillium* L. and *Paris* L. valid or should all species be placed in a single genus? The latter hypothesis was generated when initial analysis of *rbcL* data revealed little variation or separation (Chase, pers. comm.). To complement molecular studies, a thorough examination of morphology is desirable to determine if the traditional separation of *Paris* (sensu lato) and *Trillium* is valid.

The traditional view of generic limits in Trilliaceae is separation into the two Linnaean genera based on floral merosity: *Trillium* is trimerous, whereas *Paris* is 4- to 11-merous. Within *Trillium*, the major question involves whether the two subgenera (based on the presence or absence of a pedicel [Freeman 1969, 1975]) are monophyletic (summary in Zomlefer 1996). Within *Paris*, the debate has been whether to recognize a single broad genus (Hara 1969; Li 1984, 1998) or split it

TABLE 1. Generic types of Trilliaceae. Numbers after the genus name refer to numbers of species: National Flora (e.g., Flora of China or Flora of North America)/Tamura/this treatment. All species have a single flower subtended by a whorl of leaves on an otherwise naked stem.

Genus name and type	Description
Trillium L. (43/43/41) *T. cernuum* L.	Trimerous phyllotaxy; flowers sessile (subg. *Phyllantherum*) or pedicellate (subg. *Trillium*).
Paris L. (25/14/14) *P. quadrifolia* L.	4- to 12-merous phyllotaxy; flowers pedicellate;
Daiswa Raf. (NA/10/10) *D. polyphylla* (Smith) Raf. *P. polyphylla* Smith in Rees	4- to 12-merous phyllotaxy; flowers pedicellate;
Trillidium Kunth (NA/NA/1) *T. govanianum* Kunth	Trimerous phyllotaxy; tepaloid inflorescence; petiolate leaves; pedicellate flowers.
Kinugasa Tatew. & Sutô (NA/1/1) *K. japonica* (Franch. & Sav.) Tatew. & Sutô *Trillidium japonicum* Franch. & Sav.	7- to 10-merous phyllotaxy; showy white sepals; pedicellate flowers.
Pseudotrillium S. Farmer (NA/NA/1) *P. rivale* (S. Wats.) S. Farmer *Trillium rivale* S. Wats.	Trimerous phyllotaxy; broad spotted petals; petiolate leaves; pedicellate flowers.

into three genera (Takhtajan 1983). The most recent treatment of the family recognizes *Trillium*, *Paris*, *Daiswa* Raf., and *Kinugasa* Tatew. & Sutô (Tamura 1998).

Despite the fact that molecular data place Trilliaceae near or even within a clade comprised of Melanthiaceae (Davis 1995; Chase et al. 1995b; Davis et al. 1998; Fuse and Tamura 1999, 2000; Chase et al. 2000; Rudall et al. 2000; Zomlefer et al. 2001), taxa of Melanthiaceae are not satisfactory outgroups for Trilliaceae (Farmer 2000). The high amount of both molecular and mor-

TABLE 2. Historical placement and composition of genera associated with Trilliaceae. Liliaceae in assumed to be in Liliales. 1. *Medeola* as *Gyroomia.* 2. *Medeola.* 3. *Scoliopus* 4. *Demidovia* Hoffm. 5. Listed in synonomy for *Trillium,* but mentioned in text. 6. *Clintonia* Raf. 7. *Paris* s.s. 8. *Trillium* and *Trillidium.*

		Included genera				
Reference	Date	*Trillium*	*Paris*	Other	Family	Order
de Jusseau	1789				Liliaceae	
Dumortier	1829	X	X	1	Paridaceae	Paridales
Endlicher	1836–1840	X	X	2, 4	Smilaceae (Parideae)	
Lindley	1846	X	X	5	Trilliaceae	Dioscorales
Kunth	1850	5	X		Smilacineae	
Watson	1879	5	X	2, 3	Liliaceae (Trilleae)	
Bentham & Hooker	1883	X	X	2, 3, 6	Liliaceae (Medeoleae)	
Engler	1888	X	X	2	Liliaceae (Parideae)	
Dalla Torre & Harms	1908	X	X	2, 3	Liliaceae (Parideae)	
Hutchinson	1926	X	X	2, 3	Trilliaceae	Liliales
Rendle	1930	X	X		Liliaceae	
Cronquist	1968				Liliaceae	
Takhtajan	1959				Trilliaceae	Dioscorales
Melchior	1964	X	X	2	Liliaceae (Parideae)	
Huber	1969				Trilliaceae	Stemonales
Takhtajan	1969				Liliaceae	
Willis	1973	X	X	2, 3	Trilliaceae	
Dahlgren	1975				Trilliaceae	Stemonales
Huber	1977				Trilliaceae	Roxburghiales
Takhtajan	1980	X	X		Trilliaceae	Smilicales
Dahlgren et al.	1985	X	X	3	Trilliaceae	Dioscorales
Takhtajan	1987	X	7		Trilliaceae	Dioscorales
Watson & Dallwitz	1991b	X	X	2, 3	Trilliaceae	Dioscorales
Brummitt & Powell	1992	X	7	3		
Thorne	1992	X	X		Trilliaceae	Liliales
Nolte	1994	8	X		Trilliaceae	
Stevenson & Loconte	1995				Trilliaceae	Stemonales
Watson & Dallwitz	1996	X	7		Trilliaceae	Dioscorales
Takhtajan	1997				Trilliaceae	Trilliales
APG	1998				Melanthiaceae	Liliales

phological divergence between Melanthiaceae and Trilliaceae makes assessment of character homologies difficult. Other species that have been linked to Trilliaceae (e.g., *Medeola virginiana* L. or a composite of both species of *Scoliopus* Torr.) have also not proved to be satisfactory outgroups (Farmer 2000). Our preliminary analyses showed, however, that when a putative ingroup species, *Trillium rivale*, was used as a functional outgroup, clear resolution of other members of the family was obtained.

The current study was undertaken to make a critical and systematic analysis of morphological data for Trilliaceae. The initial goal was to test whether or not floral merosity was an adequate character to separate *Trillium* from *Paris* s.l., and whether other morphological characters supported this separation. The availability of molecular data from GenBank for a small subset of species offered the opportunity to integrate molecular with morphological data. The identification of a functional outgroup was essential to clarify intrafamilial relationships and generic boundaries. The results provided comprehensive cladistic analyses of Trilliaceae and have application for the systematics and classification of the family.

Methods

Sample Selection. Species were chosen as the unit for analysis (Kron and Judd 1997). 70 species, including all of those currently recognized as valid, were selected for the morphological analyses (Appendix 1). Samejima and Samejima (1987), Li (1984, 1998), and Takhtajan (1983) served as primary sources although species more recently recognized were also included. The *Flora of China* (Liang and Soukup 2000) was used as the source for currently recognized *Paris* s.l. taxa.

Character Selection. 110 morphological characters were scored (Appendix 2). Some characters were selected because they are widely used field characters (leaf shape, petal color, anther dehiscence), and others because they have been important taxonomically within their particular genus or subgeneric group (placentation, seed arils) or important in separating genera (pollen data or endosperm type). Character states were scored or measured based on a combination of literature reports and observations of herbarium specimens and live plants. Complete details about characters and their scoring are given in Farmer (2000).

DNA sequence information for two gene regions was available from GenBank for a subset of taxa used in the morphological analysis (Table 3). The two molecular data sets were the Internal Transcribed Spacer (ITS) region (ITS1, 5.8s, ITS2 sequences) of nuclear ribosomal DNA, and the chloroplast DNA sequences for the maturase (*matK*) gene. All sequences were visually aligned, and gaps were treated as missing. The ITS sequences, obtained from GenBank (Kazempour Osaloo and Kawano 1999) consisted of 648 base pairs (of which 199 were variable); there were 27 indels (Table 4). The *matK* sequences were also obtained from GenBank (Kazempour Osaloo et al. 1999) and comprised 1578 base pairs, 84 of which were variable; there were seven indels (Table 5). All aligned data matrices, tree files, and supporting data (e.g., sources for morphological characters) are available from the author (sfarmer@goldsword.com) or from http://www.goldsword.com/sfarmer/Article/. The data matrices have also been deposited in TreeBase.

Outgroup Selection. *Trillium rivale* was chosen as the outgroup based primarily on preliminary analyses which indicated basal placement in the molecular analyses, unusual placement in the morphological analysis, and the fact that potential outgroups outside the family were problematical. A variety of genera were considered as potential outgroups, including *Dioscorea* L., *Amianthium* A. Gray, *Veratrum* L., *Xerophyllum* Michx., *Medeola*, and *Scoliopus*, but none of these proved satisfactory. With *T. rivale* as the outgroup, there were fewer shortest trees, and the consensus trees from these were better resolved than with other potential outgroups. This is covered in more detail in the Results section.

Congruency Analysis. When multiple data sets from different sources are under consideration, several options are available: separate analyses followed by combining trees (taxonomic congruence), combined analyses (character congruence), or conditional combined analyses (combination if the data sets are congruent). The primary argument for taxonomic congruence is that characters with different underlying evolutionary assumptions or unequal rates of evolution should not be combined (Bull et al. 1993). The

TABLE 3. Taxa included in the *matK* and ITS sequencing analysis. Legend: species, location, collector, GenBank accession numbers. GenBank accession numbers are listed with *matK* first then ITS. *matK* and ITS sequences published in Kazempour Osaloo and Kawano (1999), and *matK* sequences in Kazempour Osaloo et al. (1999).

Veratrum maaxkii Regel, Japan: Nyukawa-mura, H. Kato, AB017417, AB018826

Daiswa fargesii (Franch.) Takht., Japan; Cult. in Royal Bot. Gard. of Setsunan Univ., J. & H. Murata, AB018827, AB018800. *D. polyphylla* (Smith) Raf., Thailand: Chiang Mai; Doi Inthanon, M.N. Tamura, AB018828, AB018801. *D. thibetica* (Franch.) Takht., UK: Cult. in Royal Bot. Gard. of Edinburgh, Unknown, AB018829, AB018802. *D. violacea* (Lév.) Takht., Japan: Cult. in Bot. Gard of Setsunan Univ., J. & H. Murata, AB018830, AB018803

Kinugasa japonica (Franch. & Sav.) Tatew. & Sutô, Japan: Toyama; Tateyama-machi, Mt. Tateyama, H. Kato, AB018831, AB018804

Paris incompleta M. Bieb., U.K.: Cult. in Royal Bot. Gard. of Edinburgh, Unknown, AB018832, AB018805. *P. tetraphylla* A. Gray, Japan: Hokkaido; Hakodate City, Mt. Hakodate-yama, H. Kato, AB018833, AB018806. *P. verticillata* M. Bieb., Japan: Hokkaido; Hakodate City, Mt. Hakodate-yama, H. Kato, AB018834, AB018807

Trillium camschatcense Ker Gawl., Japan: Hokkaido, Samanicho, H. Kato, AB01739, AB018808. *T. chloropetalum* (Torr.) Howell, USA; CA, Santa Cruz Co., M. Ohara et al., AB017382, AB018809. *T. decipiens* Freeman, USA: FL, Jackson, Co., M. Ohara et al. AB017385, AB018810. *T. discolor* Wray ex Hooker, USA: SC, McCormick Co., M. Ohara et al., AB017387, AB018811. *T. erectum* L., USA: PA, Westmoreland Co., S. Kawano et al., AB017388, AB018812. *T. govanianum* Wall. ex Royle, Bhutan: Himalayas, Sin-gonpa, S. Umezawa, AB017391, AB018813. *T. grandiflorum* (Michx.) Salisb., USA: PA, Westmoreland Co., S. Kawano et al., AB017392, AB018814. *T. lancifolium* Raf., USA: SC, McCormick Co., M. Ohara et al., AB017394, AB018813. *T. maculatum* Raf., USA: GA; Early Co., Dry Creek, M. Ohara et al., AB017397, AB018816. *T. ovatum* Pursh, USA: CA, Del Norte Co., S. Kawano et al., AB017399, AB018817. *T. petiolatum* Pursh, USA: WA, Chelan Co., M. Ohara et al., AB017400, AB018818. *T. pusillum* Michx., USA: NC, Sokes Co. (Sic.), S. Kawano et al., AB017401, AB018819. *T. recurvatum* Beck, USA: AR, Newton Co., M. Ohara et al., AB017402, AB018820. *T. reliquum* Freeman, USA: GA, Columbia Co., M. Ohara et al., AB017403, AB018821. *T. rivale* S. Wats., USA: OR, Takilma, Siskiyou Nat. For., K. Hayashi et al., AB017404, AB018822. *T. rugelii* Rendle, Japan: Cult. in Bot. Gard. of Hokkaido Univ., Unknown, AB017405, AB018823. *T. sessile* L., USA: PA, Westmoreland Co., S. Kawano et al., AB017406, AB018824

TABLE 4. Indels for the ITS gene sequence data set for Trilliaceae. Location is the location of the first base pair from the aligned sequences.

Number	Location	Indel sequence	Taxa possessing the base-pair sequence
1	bp45	'aat' 'a-t'	*T. camschatcense*
2	bp48	'gah' 'g-h'	all except *T. rivale* and *T. undulatum*
3	bp49	'ahc' 'a-c'	all except *T. undulatum*
4	bp50	'ahc' 'a-c'	all except *Paris* s.l.
5	bp82	'aat' 'a-t'	*T. pusillum*
6	bp89	'cct' 'c-t'	*T. pusillum*
7	bp96	'ccmt' 'c--t'	*Daiswa*
8	bp144	'yrg' 'y-g'	*T. govanianum, P. incompleta, P. tetraphylla, T. camschatcence*
9	bp145	'rgk' 'r-k'	*Paris* s.s., *Trillium* except for *T. rivale* and *T. govanianum*
10	bp197	'atr' 'a-r'	all except *T. camschatcense*
11	bp202	'rgc' 'r-c'	sessile-flowered *Trillium*
12	bp205	'cya' 'c-a'	all except *D. violacea*
13	bp209	'ygt' 'y-t'	all except *P. incompleta*
14	bp215	'twg' 't-g'	*D. fargesii, D. polyphylla, D. violacea,* and *T. ovatum*
15	bp218	'shy' 's-y'	all except *D. polyphylla* and *D. violacea*
16	bp219	'hyg' 'h-g'	all except *D. fargesii*
17	bp232	'btggt' 'b---t'	*T. rivale*
18	bp449	'gktsraa' 'g-----a'	all except *T. rivale*
19	bp492	'cct' 'c-t'	*T. camschatcense*
20	bp502	'gcgg' 'g--g'	all except *T. rivale*
21	bp518	'tgtk' 't--k'	*P. tetraphylla*
22	bp525	'tatg' 't--g'	*P. incompleta*
23	bp601	'dna' 'd-a'	all except *D. fargesii* and sessile-flowered *Trillium*
24	bp602	'naactc' 'n----c'	*T. rivale*
25	bp611	'wtg' 'w-g'	sessile-flowered *Trillium*
26	bp612	'tgkgacacccam' 't----------m'	all except *T. rivale*
27	bp634	'ygct' 'y--t'	*T. pusillum*

primary advantage of character congruence is that characters from different data sets can strengthen weak phylogenetic signal to the point that it can overcome noise (Sullivan 1996).

Measures of taxonomic congruence and character congruence were examined even though the data sets were combined since the total evidence method (Kluge 1989; Huelsenbeck et al. 1996; Soltis et al. 1998, 1999) was more applicable in this case. Measures of taxonomic congruence included assessment of support for major clades and for rival trees. Major clades were assessed by comparing bootstrap values for each of the clades from each of the analyses (Mason-Gamer and Kellogg 1996; Davis et al. 1998). For the other measure of taxonomic congruence, strict consensus trees were used as constraint trees on which the fits of rival data sets were assessed (related to the Miyamoto incongruence index Swof-

TABLE 5. Indels for the *matK* gene sequence data set for Trilliaceae. Location is the location of the first base pair from the aligned sequences.

Number	Location	Indel sequence	Taxa Possessing the Base-Pair Sequence
1	bp15	'attacagg' 'a------g'	*T. rivale, Paris* s.s., pedicellate *Trillium* except for *T. govanianum*
2	bp51	'acaaaayt' 'a------t'	all except for *T. pusillum*
3	bp119	'atataaat' 'a------t'	*T. decipiens* and *T. reliquum*
4	bp390	'acaaatac' 'a------c'	*T. erectum* and *T. rugelii*
5	bp607	'aatttkat' 'a------t'	*T. camschatcense, T. erectum* and *T. rugelii*
6	bp626	'aatctatt' 'a------t'	all except for *T. petiolatum* and *T. reliquum*
7	bp810	'aatagatg' 'a------g'	*T. maculatum*

ford 1991; Kluge 1989; Davis et al. 1998). Measures of character congruence examined were the Mickevich and Farris original measures computing percent variation due to data, percent variation between characters, and percent variation between data sets (Mickevich and Farris 1981); and the Farris measure of incongruence as implemented in PAUP* via the Homogeneity of Partitions test (Farris et al. 1995a, 1995b; Swofford 1998).

Phylogenetic Analysis. The data sets were analyzed with maximum parsimony using heuristic search methods with TBR and MULPARS and a simple addition sequence. Random addition with steepest descent was used to check for islands of trees (Maddison 1991). Once a minimal tree length was found, branch and bound analysis was used to insure finding all shortest trees. Bootstrap support (Felsenstein 1985) was estimated based on 1,000 replicates with the same search strategy (simple addition) as simple parsimony.

The morphological data set included 70 species and 110 characters (Appendices 1, 2); the ITS, *matK,* and small morphological data sets included 26 of the above taxa. These four separate data sets (ITS, *matK,* small morphological, and full morphological) were analyzed either separately or in combination: ITS data, *matK* data, morphological data, ITS + morphology, *matK* + morphology, ITS + *matK,* ITS + morphology + *matK,* and 70 species morphological data set. Of these eight analyses, only four (ITS-*matK,* small morphology, ITS-*matK*-morphology, and full morphology) are presented here because of their overall similarity; the remainder are given in Farmer (2000) and are available from the author or the previously mentioned web site.

RESULTS

Preliminary Analyses. Several genera were considered as potential outgroups, including *Dioscorea*, *Amianthium*, *Veratrum*, *Xerophyllum*, *Medeola*, and *Scoliopus*, but none of those proved satisfactory. *Dioscorea* was considered because Dahlgren et al. (1985) placed Trilliaceae and Dioscoreaceae together in the Dioscoreales, but molecular data do not support these two groups as being closely related (Chase et al. 1993, 2000). *Scoliopus* and *Medeola* have both been placed in Trilliaceae, but neither is particularly close based on either molecular or morphological data (Gates 1917; Berg 1959, 1962; Chase et al. 1993). *Amianthium* was used initially in the morphological analyses because of the results of the molecular studies (Chase et al. 1993; APG 1998; Fuse and Tamura 1999, 2000; Zomlefer et al. 2001) even though its morphology was quite different from Trilliaceae. *Veratrum maackii* was initially used as the outgroup for the molecular studies, but its ITS sequence in particular was so dissimilar that alignment was problematic; only the 5.8S region could be aligned with any confidence (Baldwin et al. 1995).

Analyses of molecular data supported *Trillium rivale* as basal within Trilliaceae. As in the initial morphological analyses, several well-defined species groups were evident. Dr. Soichi Kawano (pers. comm.) reported that with *Paris polyphylla* as the outgroup in a *matK* analysis of *Trillium* that included several species of *Paris* s.l., *T. rivale* was most basal followed by the rest of the *Paris* s.l. taxa with *Trillium* as most derived (Kazempour Osaloo and Kawano 1999). When we made an analysis with the *matK* sequences using *Veratrum maackii* as outgroup, *Trillium rivale* was basal to all other Trilliaceae taxa in the strict consensus of 1,326 trees (Fig. 1a). Analysis of the ITS sequence data with *V. maackii* as outgroup produced only six trees, and *T. rivale* was again placed in a basal position relative to the rest of the ingroup taxa (Fig 1b). Similar results can also be seen in Kato et al. (1995a) and Kazempour Osaloo and Kawano (1999). ITS sequences could not be reliably aligned when *V. maackii* was the outgroup. The ingroup taxa were relatively easy to align to one another but difficult to align to the outgroup because of the dissimilarity in the sequences. Unambiguous alignment was possible only in the 5.8S region of the sequence.

The problem of potential outgroups being too dissimilar was particularly severe in the morphological analyses. For example, when *Amianthium* was used as the outgroup its dissimilar morphology (a bulbous plant with a bracteate, racemose inflorescence with tepaloid flowers) compared to *Trillium* necessitated the addition of several ''not applicable'' states to the data

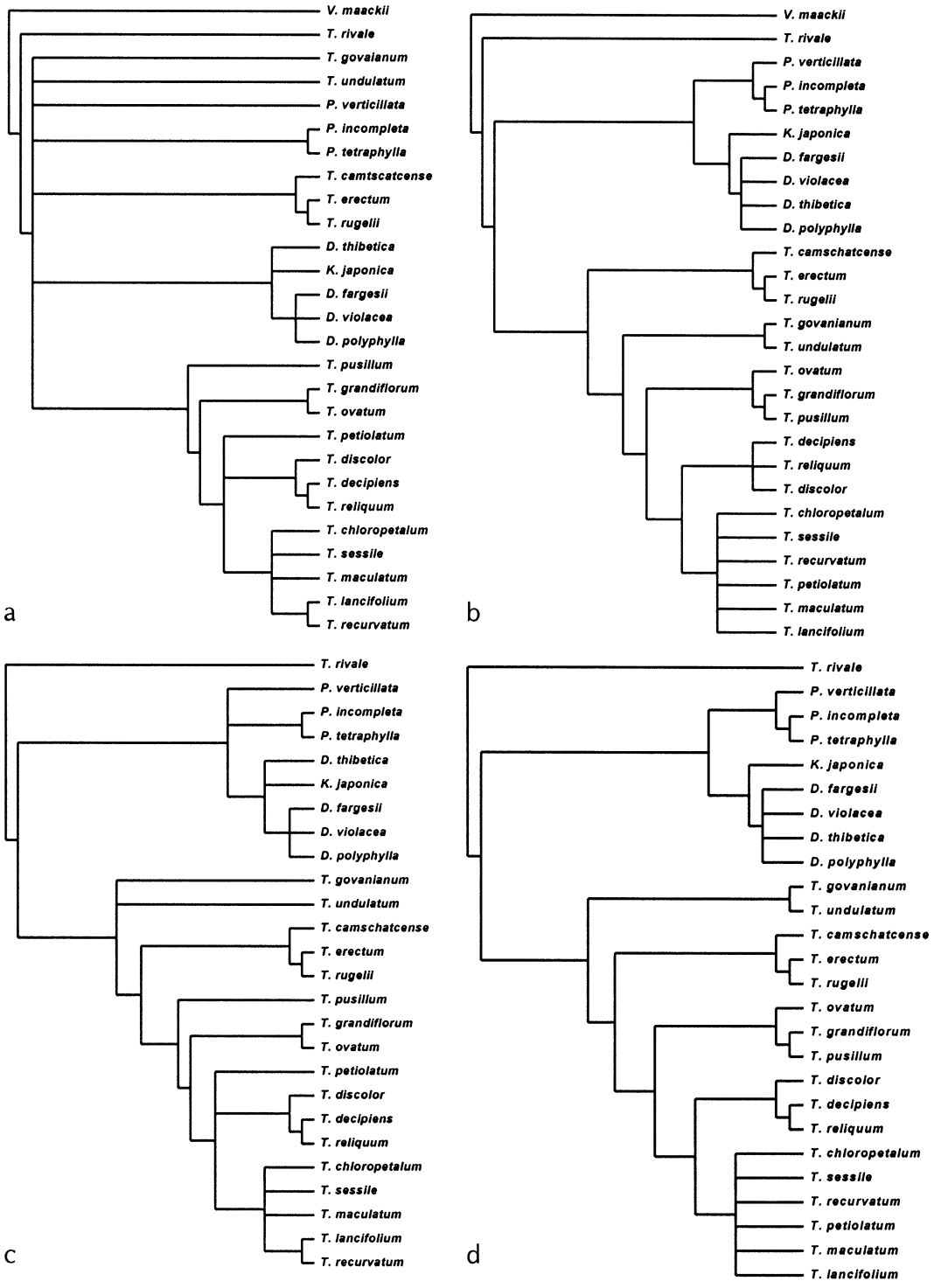

FIG. 1A–D. Strict consensus trees from preliminary analysis of Trilliaceae with different data sets and different outgroups. A, C. Tree produced using *matK* data. B, D. Tree produced using ITS data. A. *Veratrum maackii* as the outgroup. Strict consensus of 1326 trees; length 285. B. Strict consensus of 6 trees; length 748. C. *Trillium rivale* as the outgroup. Strict consensus of 6 trees; length 107. D. Tree produced using ITS data. Strict consensus of 6 trees; length 331.

TABLE 6. Summary of character congruence measures for the combined data sets for Trilliaceae.

	ITS-*matK*	ITS-morphology	*matK*-morphology	All data
Homogeneity of Partitions test	P = 0.20	P = 0.01	P = 0.01	P = 0.01
Variation within data	70.99%	72.54%	76.82%	73.3%
Variation between characters	25.27%	24.68%	21.22%	23.48%
Variation between data sets	3.74%	2.69%	1.95%	3.21%

set. Initial analyses of Trilliaceae with the morphological data set using *Amianthium* as outgroup did not go to completion but probably produced more than 100,000 shortest trees. With PAUP 3.1.1, the maximum of 32,760 trees was reached quickly; with PAUP*, over 80,000 trees were saved before overloading the system, with still over 70,000 trees to swap. In the resulting consensus tree, a grade was present with many of the internal clades as expected (e.g., *T*. subg. *Phyllantherum*, *Paris* s.s.). However, *T. rivale* was basal to the clade containing *Paris* s.l. rather than clustering with any of the *Trillium*.

Congruency Analysis. Homogeneity of partitions analyses using 100 random re-partitions as provided in PAUP* were performed on each of the combined data sets. The results indicated congruency between the molecular data sets but incongruency between the partitions of the molecular+morphological data set. However, the original Mickevich-Farris measures of percent variation indicated little variation between data sets. The character congruency measures are summarized in Table 6. The bootstrap support values for the major clades are presented in Table 7; conflicts between analyses included position of clades, the relationships among pedicellate *Trillium*, and the placement of *Kinugasa*. The rival tree analyses values (Table 8) indicate the number of extra steps required to map a particular set of data onto a particular tree; in several cases, a rival tree or the putative tree (the TEST Tree) was actually the shortest tree for a given data set.

Phylogenetic Analysis. MOLECULAR DATA. Parsimony analysis of the combined ITS and *matK* sequence data produced six shortest trees of length 455 with a CI (consistency index) of 0.73 (Table 9); the strict consensus is shown in Fig. 2. The data set included 2219 characters, 283 variable, 145 informative. A basal dichotomy separated *Paris* s.l. and *Trillium* as sister clades with bootstrap support of 65% and 81%, respectively. Within the ''Paris clade,'' *Paris* and *Daiswa+Kinugasa* were placed as sister clades with bootstrap support of 95% and 80%, respectively. *Kinugasa* was sister to *Daiswa* and the latter clade had a bootstrap value of 100%. In the ''Trillium clade,'' a basal split separated *T. govanianum* and *T. undulatum* with bootstrap support of 73% from the remaining *Trillium* (bootstrap support of 81%). *Trillium* subg. *Phyllantherum* was placed in a well-defined, monophyletic clade with 99% bootstrap support. *Trillium* subg. *Trillium* formed a grade, within which there was a subclade consisting of the members of the ''Erectum clade'' with 99% support. The ''Grandiflorum clade,'' with marginal bootstrap support (51%), formed a polytomy in the consensus tree at the base of subg. *Phyllantherum*.

SMALL MORPHOLOGICAL DATA SET. Parsimony analysis of the morphological data for the same set of taxa for which molecular data were available produced 12 shortest trees (length 660; CI 0.75); the strict consensus is shown in Fig 3. This data set included 97 characters, 76 variable, and 63 informative (Table 9).

A basal split in the tree separated *Paris* s.l. + *T. govanianum* (74%) from *Trillium* (Fig. 3). The ''Paris clade'' was supported in the bootstrap analysis at 69%; if floral merosity was included, this value jumped to 93%. *Trillium govanianum* was basal to *Paris* s.l.; within the ''Paris clade,'' *Kinugasa japonica* was basal to sister clades containing *Paris* and *Daiswa*. *Trillium* subg. *Trillium* was a paraphyletic group, but *T*. subg. *Phyllantherum* was monophyletic (53%). *Trillium* subg. *Trillium* formed a polytomy with a clade composed of *T. grandiflorum* and *T. ovatum* (bootstrap support = 69%) and a clade composed of *T. erectum* and *T. rugelii* (bootstrap support = 53%).

TABLE 7. Bootstrap support for major clades in each of the analyses of Trilliaceae. Note: 1. excludes *T. pusillum.* 2. 100% if *K. japonica* is excluded. 3. 70% excluding *K. japonica* 4. excluding *T. camtschatcense* 5. excluding *P. verticillata.*

	ITS	*matK*	Morphology	ITS-*matK*	ITS-morphology	*matK*-morphology	All data
Phyllantherum clade (9 taxa)	*99%*	*76%*	*56%*	*100%*	*99%*	*93%*	*100%*
Grandiflorum clade (3)	*polytomy*	*98%*[1]	*69%*[1]	*51%*[1]	*polytomy*	*99%*[1]	*83%*[1]
Erectum clade (3)	*98%*	*67%*	*54%*[3]	*99%*	*94%*	*polytomy*	*98%*
Daiswa clade (4 + *Kinugasa*)	57%[2]	85%[2]	polytomy	80%[2]	63%[2]	51%[3]	72%[2]
Paris clade (3)	96%	55%	89%[5]	95%	97%	63%	92%
govanianum + *undulatum*	72%	polytomy	separate	73%	separate	separate	polytomy

TABLE 8. A comparison of lengths of constraint consensus trees optimized against rival data sets for Trilliaceae. Note: numbers in bold pertain to a tree tested against its own data set. * Indicates trees that are the same length as the tree tested against its own data. ** Indicates trees that are shorter than the tree tested against its own data.

	Data set						
Tree	ITS	*matK*	Morphology	ITS-*matK*	ITS-morphology	*matK*-morphology	ITS-*matK*-morphology
ITS	**349**	+9	+23	+8	+24	+26	+30
matK	+32	**110**	+19	+31	+52	+13	+49
Morphology	+50	+32	**660**	+81	+51	+26	+80
ITS-*matK*	−1**	+2	+18	**460**	+18	+14	+17
ITS-morphology	0*	+11	−1**	+10	**1008**	+4	+8
matK-morphology	+14	+5	+1	+18	+16	**776**	+18
ITS-*matK*-morphology	−1**	0*	+3	−2**	+3	−3**	**1121**
TEST tree	0*	+11	−4**	+10	−3**	+1	+5

COMBINED ANALYSIS. The combination of morphological and both molecular data sets produced three trees; the strict consensus was almost fully resolved (Fig. 4). The three trees, which differed only for the relative placement of members of *Trillium* subg. *Phyllantherum*, were 1,171 steps long with a CI of 0.71 (Table 9). This data set included 2,219 base pairs and 97 morphological characters (359 variable characters; 208 informative).

A basal split separated *Paris* s.l. from *Trillium*. Within the ''Paris clade,'' *Paris* and *Daiswa*+*Kinugasa* were placed in sister clades (97% and 75%). *Kinugasa* was separated as the sister group to *Daiswa*, with the latter having 100% bootstrap support. *Trillium undulatum* and *T. govanianum* were basal to the rest of *Trillium*. Within the ''Trillium clade,'' *T*. subg. *Phyllantherum* was a monophyletic clade supported at 100%; the ''Pedicellate group'' was a paraphyletic grade consisting of an ''Erectum clade'' with 97% support and a ''Grandiflorum clade,'' supported at 86%.

FULL MORPHOLOGICAL DATA SET. With the full data set of 70 species, 76 shortest trees with a length of 1,537 and a CI of 0.69 (Table 9) were produced in two islands of 39 and 37 trees, respectively (Fig. 5). In one island, *Daiswa* was completely resolved (Fig. 6a); in the other island, *T*. subg. *Phyllantherum* was more fully resolved (Fig. 6b). In both islands, *T*. subg. *Trillium* was resolved as is evident in the strict consensus tree. This data set included 97 characters (80 variable; 71 informative).

Paris s.l. (with the basal *Trillium govanianum* and *T. taiwanense*) and most *Trillium* were placed as sister taxa in the strict consensus tree produced by the analysis of this data set (Fig. 5); however, a small group of pedicellate *Trillium* was basal to these two sister groups. *Paris* s.s. was most derived in a paraphyletic *Daiswa*. *Kinugasa japonica* was basal to *Paris* s.s. and served to separate those genera (Fig. 6a). In the ''Trillium clade,'' *T. undulatum* was basal with the rest of the *Trillium* forming cohesive sister clades: the ''Pedicellate clade (minus *T. undulatum*),'' and the ''Sessile-flowered clade.''

DISCUSSION

When we began this project, the goal was to determine if the traditional generic circumscriptions were correct. Traditionally, the genera were separated only on the basis of floral merosity; based on early *rbcL* sequence data (Chase, pers. comm.), there was no separation between any of the genera in Trilliaceae (Kato et al. 1995b; Kazempour Osaloo and Kawano 1999). In the morphological analysis there was clear separation between *Trillium* and *Paris* s.l. (Fig. 5), but it was when DNA sequence data were obtained from GenBank and analyzed that we saw clear separation within *Paris* s.l.

TABLE 9. Statistics for the data sets and for the most parsimonious trees.

		Number of characters			Trees					
Data set	Total	Constant	Uninformative	Informative	Number	Length	CI	HI	RI	RC
ITS	641	444	92	105	6	331	0.70	0.30	0.72	0.50
matK	1578	1494	44	40	6	107	0.86	0.14	0.90	0.77
morphology	97	621	13	63	13	646	0.77	0.61	0.58	0.45
ITS-*matK*	2219	1936	138	145	6	455	0.73	0.28	0.76	0.56
ITS-morphology	738	463	107	168	6	1005	0.74	0.51	0.63	0.46
matK-morphology	1675	1515	57	103	54	768	0.77	0.55	0.67	0.50
all data	2316	1957	151	208	3	1120	0.74	0.48	0.65	0.49
full morphology	97	17	9	71	1,296	1785	0.70	0.63	0.44	0.82

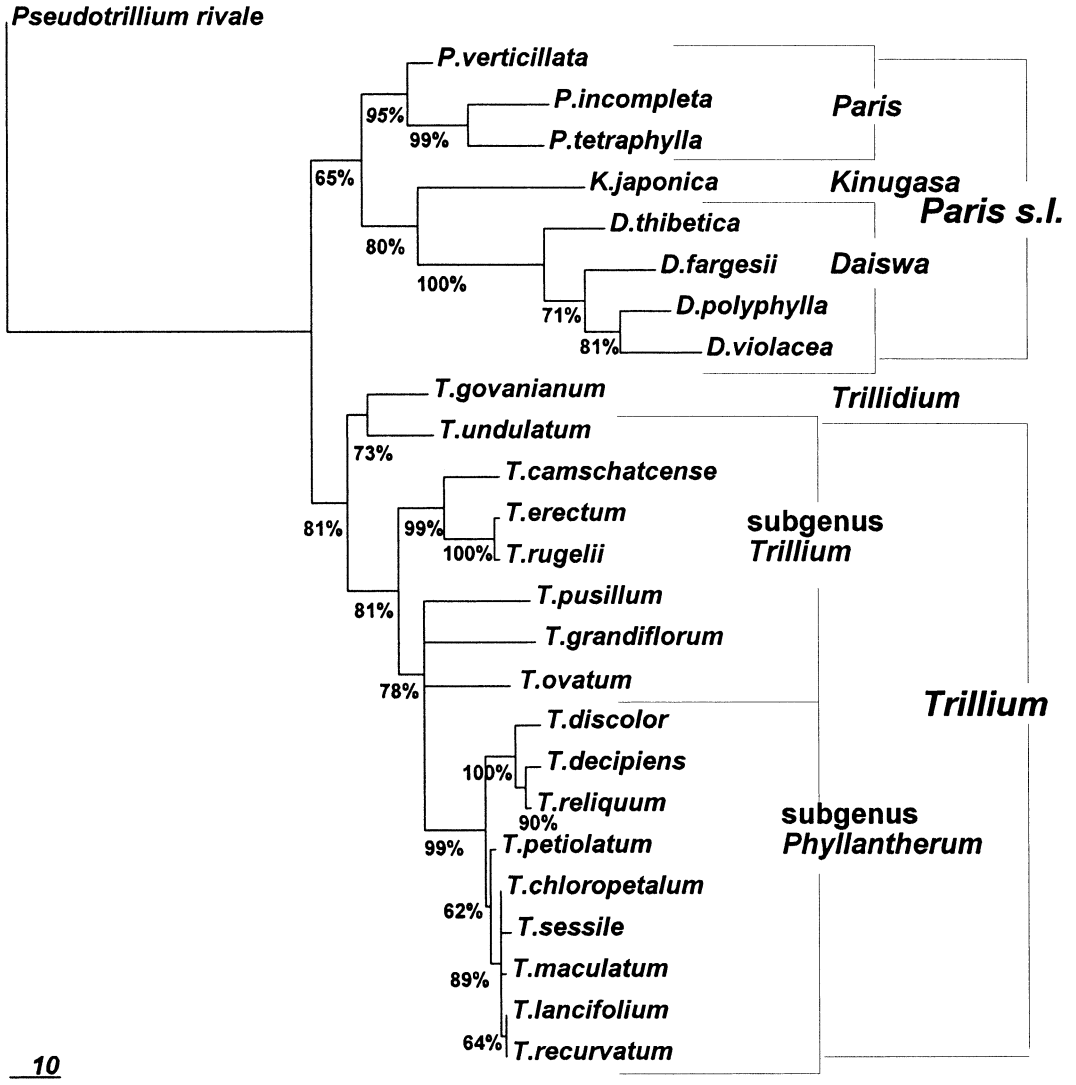

FIG. 2. Strict consensus of six trees from the ITS-*matK* analysis of 26 species of Trilliaceae. Length 455 steps; CI = 0.73. Percentages below the branches are bootstrap values.

Based on morphological and molecular separation and the molecular alignment of *Kinugasa* with *Daiswa* rather than *Paris* we decided that taxonomic decisions had to be made. With nomenclatural stability in mind, we decided to use existing and recently revived generic names for the entities within Trilliaceae.

Detailed phylogenetic analyses resolved generic delineation within Trilliaceae. *Trillium rivale* was distinct from both *Paris* and *Trillium* and should be placed in its own genus. *Trillium govanianum* was morphologically more similar to *Paris* than to *Trillium*, but molecular evidence indicated it as a separate monotypic genus, *Trillidium*. *Trillium* and *Paris* s.l. are distinct based on molecular as well as morphological evidence. The cladistic analyses provided support for the separation of *Paris* s.l. into *Daiswa*, *Kinugasa*, and *Paris*. The monophyly of *Trillium* as now redefined was supported in all but the large morphological analyses. We believe that with further analysis, *Trillium taiwanense* S.S. Ying will be shown to be either *Paris* or *Daiswa* (it has been listed in synonymy for *Paris fargesii* var. *brevipetalata* [Huang & Yang] Huang & K.C.Yang [Huang, et al 1989], but Liang and Soukup [2000] treat it as separate).

Trillium rivale. The main conclusion from outgroup considerations is that *Trillium rivale* is distinct from both *Paris* s.l. and from *Trillium*. Despite its traditional classification in *Trillium*, *T. rivale* was shown to be sister and basal to the remaining taxa in the family Trilliaceae. Although *Trillium rivale* is superficially

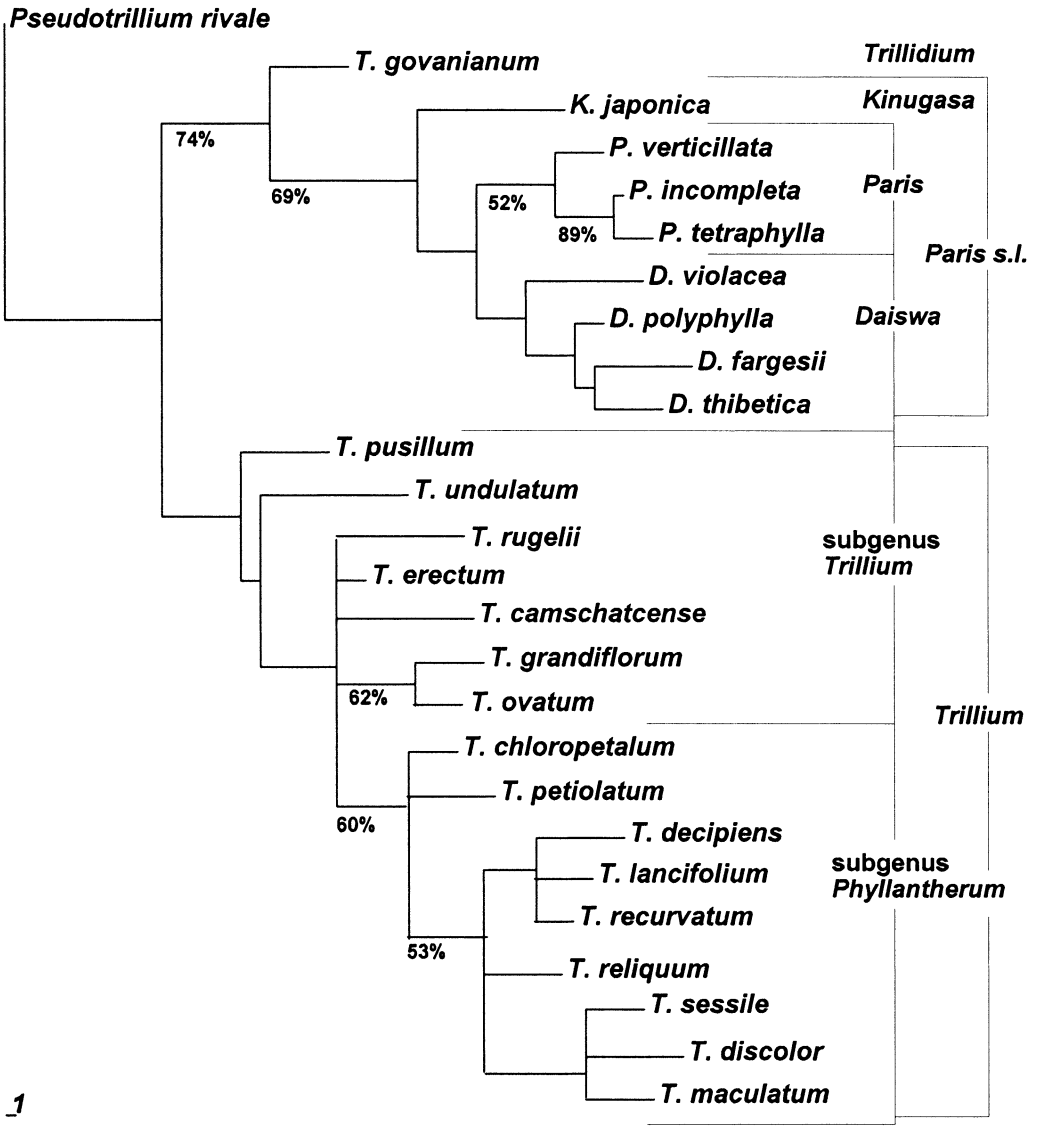

FIG. 3. Strict consensus of 13 trees from the small morphological analysis of 26 species of Trilliaceae. Length 646 steps; CI = 0.77. Percentages below the branches are bootstrap values.

similar to other *Trillium* species, it also differs in a number of features: thick, leathery, cordate leaves; spotted petals; the cotyledon shape is more leaf-like than strap-like; and the pedicel elongates from anthesis until the ripe fruit is in contact with the ground. This status was supported by numerous morphological apomorphies, 138 single base-pair changes, and four base-pair indels. Based on these results, *T. rivale* should be placed in its own genus, for which the name *Pseudotrillium* is proposed below.

Trillium govanianum *and* T. undulatum. A puzzling outcome was that some (but not all) analyses resolved *Trillium govanianum* and *T. undulatum* as closely related. *Trillium govanianum* and *T. undulatum* were either shown as basal to *Trillium* (Figs. 2, 4), or *T. govanianum* was basal to *Paris* s.l. and *T. undulatum* was basal or near-basal to *Trillium* (Figs. 3, 5).

In a recent pair of papers, Fukuda (2001a, 2001b) explores the origins of the tetraploid *Trillium govanianum* and its relationship to *T. undulatum*. Based on comparative analysis of the floral and vegetative morphology and the chromosome morphology of *T. govanianum*, *T. tschonoskii*, *T. undulatum*, and *Daiswa polyphylla*, he concludes that the allotetraploid *T. govanianum* is an intergeneric hybrid between *Trillium* and *Daiswa* with part of the chromosome complement of *T. govanianum*

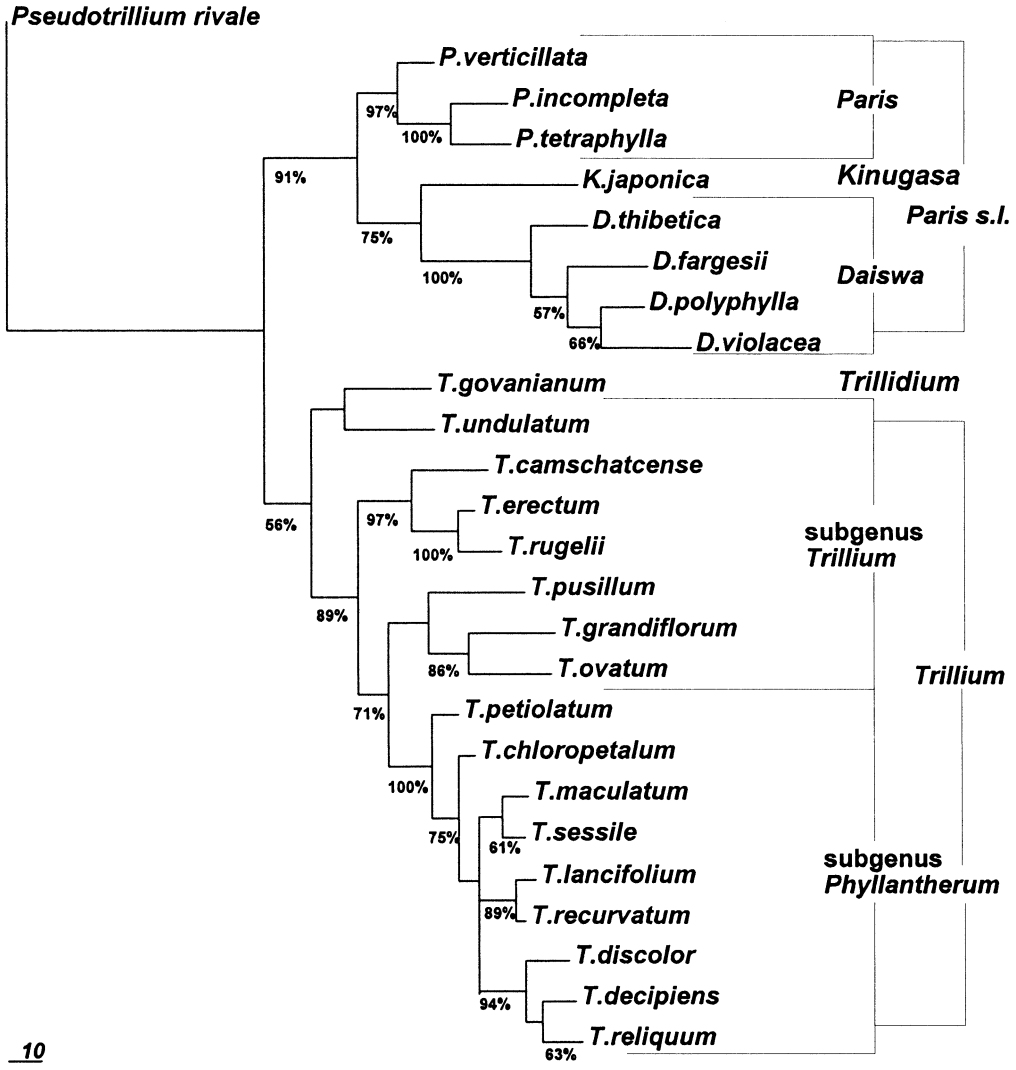

FIG. 4. Strict consensus of three trees from the combined analysis of 26 species of Trilliaceae. Length 1120 steps; CI = 0.74. Percentages below the branches are bootstrap values.

closer to *T. undulatum* than to *T. tschonoskii*. To date, the presence of more than one genome in the molecular sequence data that would substantiate this hypothesis has not been detected. There is little difference in the placement of *Trillidium* between the *matK* and the ITS analysis. Further molecular analyses might shed light on the relationships of these taxa.

Morphological and biogeographic data suggest that *T. govanianum* and *T. undulatum* do not form a monophyletic group. *Trillium govanianum* is found in the Sikkim and Nepalese Himalayan Mountains at elevations above 3,200 m; *T. undulatum*, in the eastern United States at elevations below 2,000 m. *Trillium undulatum* and *T. govanianum* were paired in the molecular and combined analyses, but in the morphological analysis, were separated. Mapping morphological characters on the molecular tree shows they share the unusual characters of extrorse anther dehiscence and distinctly petiolate leaves. However, *T. govanianum* shares several diagnostic characters with *Paris* s.l., including pollen shape (ellipsoidal) and apertures (monosulcate), endosperm type (nuclear), and narrow filiform petals; *T. undulatum* shares these character states with *Trillium* rather than with *T. govanianum* or *Paris* s.l.

Long-branch attraction (Felsenstein 1978; Huelsenbeck 1997) is a possible explanation for the placement of *T. undulatum* and *T. govanianum*. The artificial constraint tree (the TEST tree in table 8) that was generated placed *T. govanianum* basal to *Paris* s.l. and *T. undulatum* basal to *Trillium*, and the tree was either shorter than the strict consensus trees that were generated by PAUP* (Table 8) or less than 2% longer. Even though

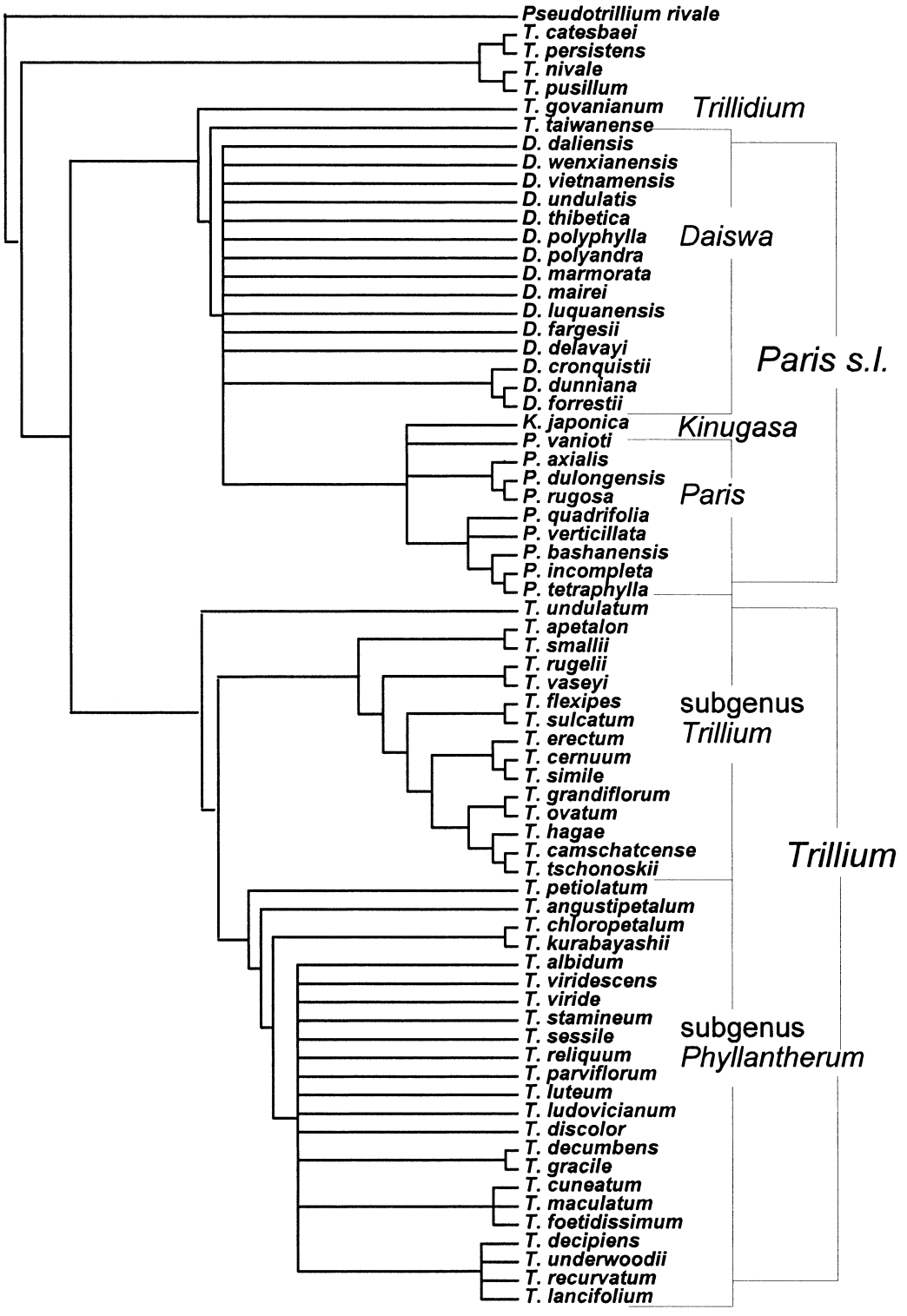

FIG. 5. Strict consensus of 76 trees from the full morphological analysis of 70 species of Trilliaceae. Length 1,537 steps; CI = 0.69. See Fig. 6 for more fully resolved clades produced from the two islands of most parsimonious trees.

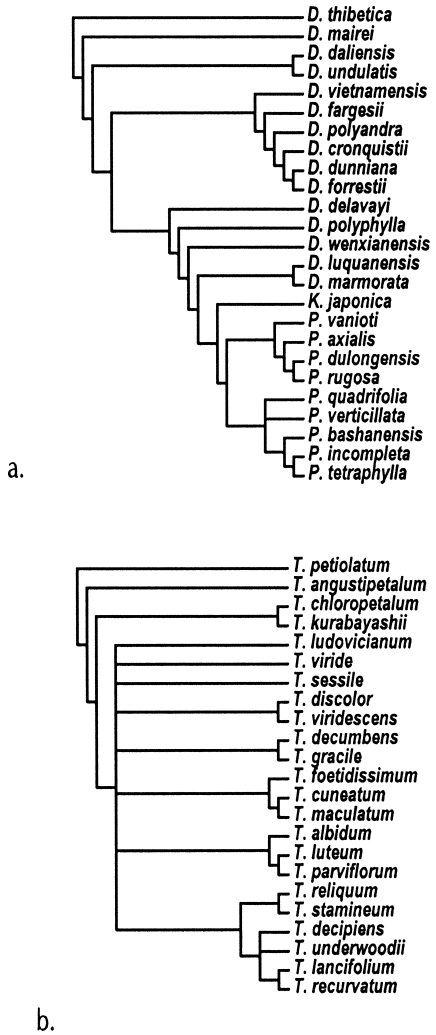

FIG. 6. More fully resolved clades produced from the two islands of most parsimonious trees. a. *Daiswa* clade from island 1. b. *Trillium* subg. *Phyllantherum* clade from island 2. See Fig. 5 for the strict consensus tree containing both islands of most parsimonious trees.

long branch attraction may not be satisfactorily accounted for, it can most often be remedied by the addition of more taxa (Graybeal 1998; Halanych 1998).

TRILLIDIUM GOVANIANUM. *Trillium govanianum* was distinct from *Trillium* and *Paris* on molecular as well as morphological grounds and should be recognized as distinct at the genus level as *Trillidium govanianum*. It occurs in the Himalayan Mountains, a region most known for *Paris* s.l. rather than *Trillium*. It has trimerous phyllotaxy and broad leaves like *Trillium*, but it shares several unusual synapomorphies with *Paris* s.l., including endosperm type, pollen shape and apertures, and narrow filiform petals. *Trillidium govanianum* has a pigmented, tepaloid periath rather than the plesiomorphic condition of distinct sepals and petals; in *Paris* s.l. this usually consists of filiform yellowish-green petals and broad sepals. It also lacks an aril on the seeds, a synapomorphy that is shared with *Paris* s.s., but not with *Trillium* (partial aril) or *Daiswa* (enveloping sarcotesta). For these reasons, Hara et al. (1978) revived the genus *Trillidium*; it has also been recognized by Nolte (1994) and Case and Case (1997). Our analyses supported recognition of *Trillidium* as a distinct genus.

TRILLIUM UNDULATUM. *Trillium undulatum* has distinct phylogenetic placement and morphology. It is clearly a *Trillium* based on both morphology and biogeography. Like other *Trillium* species, it has broad leaves and trimerous phyllotaxy. It usually has a red, v-shaped pattern at the base of each petal that is unique in Trilliaceae; the coloration of most petals within Trilliaceae is usually unmarked. It has the relatively rare features within *Trillium* of extrorse anther dehiscence and petiolate leaves. *Trillium undulatum* also has the plesiomorphic spherical, omniaperturate pollen that is typical of *Trillium* species. The distribution of *T. undulatum* in the Appalachian Mountains places it within the general range of *Trillium* and at its center of greatest diversity.

Paris *s.l.* *Paris* s.l. is consistently resolved as monophyletic in all of the analyses. In addition to the traditionally used trait of merosity, *Paris* s.l. and *Trillium* can be distinguished by several other characters. Most notable are synapomorphies for *Paris* s.l. such as narrower leaves, filiform petals, elliptical, monosulcate pollen, and nuclear endosperm. The synapomorphies that defined the *Paris* clade (including *Trillidium*) were two absolute base pair changes (one *matK* and one ITS), petal width, pollen shape and aperture, and endosperm type. Excluding *Trillidium*, there were an additional three base-pair changes, the general absence of anthocyanin pigments, lateral anther dehiscence, and presence of a style.

The molecular cladistic analyses supported the separation of *Paris* s.l. into two sister clades, *Paris* s.s. and *Kinugasa*+*Daiswa*. The support for the monophyly of *Kinugasa*+*Daiswa* was provided primarily by the molecular and the combined analysis (Figs. 2, 4) but not from morphology. There were three indels and two base-pair changes that separated *Paris* and *Daiswa*+*Kinugasa* in the ITS sequence and two base-pair changes in the *matK* sequences.

The placement of *Kinugasa japonica* with *Daiswa* rather than with *Paris* s.s. in the molecular and combined analyses was unexpected. In traditional, morphological classifications, *Kinugasa* has always been aligned with *Paris* subg. *Paris* but both ITS and *matK* analyses suggested a relationship between *Kinugasa* and *Daiswa*, with both exhibiting many of the same indels and base-pair changes. Morphologically, *Kinugasa* shares several features with *Paris* s.s. such as slender stigmatic branches,

an indehiscent berry, and seeds without an enveloping sarcotesta; a thick rhizome and angular ovary are characters that it shares with *Daiswa*. Because of the unusual morphology of the species (i.e., the showy, white sepals and octoploid chromosome count) and the difficulty in aligning it with either *Paris* or *Daiswa*, the separate genus *Kinugasa* should be retained for this species.

Daiswa was usually placed as a monophyletic sister group to *Paris* s.s. Its monophyly was supported by indels, base-pair changes, and morphological characters such as placentation, stigma size, fruit type, fruit dehiscence, and seed arils. Although stem height was not used as a character, most species of *Daiswa* are more than 40 cm tall (with 11 of 14 species to 80 cm or more; *D. dunniana* [Lév.] Takht. grows to 3 m), whereas most species of *Paris* are under 50 cm. tall (Liang and Soukup 2000). Only fruit type and seed arils are listed by Takhtajan (1983) as diagnostic for *Daiswa*. Because of the recognition of *Kinugasa* as a genus, and because *Paris* and *Daiswa* were separated on a molecular level, we recommend recognition of *Daiswa* as distinct from *Paris*.

Trillium. The results of the molecular analyses strongly supported the monophyly of the ''*Trillium* clade,'' which was defined by several base pair changes as well as morphological characters. Within this clade, the subgroups were not as well defined as those in *Paris* s.l. Subgenus *Phyllantherum* was a well-defined, monophyletic group but the pedicellate taxa in the *Trillium* clade usually formed a paraphyletic group.

Trillium subg. *Phyllantherum* was shown to be quite cohesive with character support besides the sessile-flowered habit. Other apomorphic characters such as petal transverse posture, filament color, ovary color, and petal vertical posture also helped to define this group.

Of Freeman's (1969, 1975) subgroups, the only one that was supported in the results of the phylogenetic analyses was the ''*T. recurvatum* group,'' composed of *T. recurvatum* and *T. lancifolium*. The other groups (the ''*T. sessile* group'' and the ''*T. maculatum* group'') were not cohesive in these analyses.

Trillium subg. *Trillium*, which traditionally includes all of the pedicellate taxa, is most cohesive in the large morphological analysis. In that analysis, two monophyletic groups were resolved: the ''erectum group,'' and the ''delostylis group.'' Unfortunately, the ''delostylis group'' was represented only by *T. pusillum* in the molecular analyses, so its coherence as a group in the smaller analyses was difficult to assess; in the large morphological analysis, this group consisted of *T. pusillum*, *T. nivale*, *T. catesbaei*, and *T. persistens*. The name ''delostylis group'' is based on Rafinesque's genus name reflecting the presence in all of these taxa of a fused style (Rafinesque 1819). Clearly, *T. pusillum* itself was distinct from the other *Trillium*, placed basal to *Trillium* in the small morphological analysis and as part of a clade basal to all other taxa in the large morphological analysis. Its placement in the ITS and *matK* analyses was different as indicated by the polytomy in the combined ITS-*matK* tree. Because of the general congruence between the molecular and morphological data sets and because of the lack of supporting molecular evidence, it is inappropriate to elevate the ''delostylis group'' to generic status without further research.

Traditionally, *T. grandiflorum* and *T. ovatum* have been placed within the ''delostylis group'' because of their anthocyanin chemistry and petal texture, but they do not share the rare character states of a fused style and sub-petiolate to petiolate leaves. These species were positioned as most derived in the ''erectum group.'' The ''erectum group'' was cohesive in the molecular as well as combined analyses; the ''*grandiflorum*+*ovatum* group'' were paired in all but the ITS-*matK* analysis.

Taxonomic Treatment

Pseudotrillium S.B. Farmer, gen. nov. –TYPE: *Pseudotrillium rivale* (S. Watson) S.B. Farmer, comb. nov.

Genus propria ob petalis gutatis. *Trillium* L. affinis sed pedicellis elongatus continuus dum fructus maturus et adpressus humus; *Trillidium* Kunth affinis sed petalis latis, non tepalis angustus; *Paris* L. affinis sed petalis latis, rhizomatis incrassatus, semina cum eliasoma; *Kinugasa* Tatew. & Sutô affinis sed sepalis viridis et petalis latis; *Daiswa* Raf. affinis sed petalis latis, fructus indehiscens et semina cum eliasoma non arrilatus.

The genus is monotypic, and differs from *Trillium* because of the spotted petals and a pedicel that continues to elongate; different from *Trillidium* because of the broad spotted petals rather than narrow purple tepals; different from *Paris* s.s. because of the broad, spotted petals, the thickened rhizome, and the presence of an eliasome on the seed; different from *Kinugasa* because that genus has broad, colored sepals and filiform petals unlike the other genera in the family; and different from *Daiswa* because that genus has narrow petals, a dehiscent capsule, and a complete aril covering the seed.

Pseudotrillium rivale (S. Watson) S.B. Farmer, *comb. nov.*—TYPE: USA. California: Big Flat, 30 miles west of Crescent City in Del Norte County, California. *W.H. Shockley s.n.* Range: Siskiyou Mountains of Oregon and California (LECTOTYPE here chosen: GH!). Basionym: *Trillium rivale* S. Watson. Proc. Amer. Acad. Arts 20: 378 1885.

In Serrano Watson's original description (1885) of *T. rivale*, he cites two specimens, one by Shockley, and the other by Thomas Howell. Both specimens are on the same herbarium sheet. The Shockley specimen is in an envelope on the sheet labeled *T. ovatum*, corrected to *T. rivale* and already annotated as being the type with a

stamp by an unknown individual. This specimen appears to be more typical of the species than the Howell specimens.

Based on the recognition of *Daiswa* as separate from *Paris*, several species need to be transferred to *Daiswa*, but these will be addressed at a later date.

Key to the genera of Trilliaceae

1. Inflorescence composed of tepals (if outer perianth segments are green, shape and size of inner and outer segments similar); phyllotaxy trimerous . . . . . *Trillidium*
1. Inflorescence composed of sepals and petals (shape and size of inner and outer segments dissimilar); phyllotaxy trimerous to numerous 2
   2. Sepals showy, white; petals filiform (to 1[-2] mm wide) or absent . . . . . *Kinugasa*
   2. Sepals green or purplish; petals filiform to broad (0.1–6 cm wide), or absent 3
      3. Phyllotaxy mostly 4- to 11-merous; leaves (0.8-) 2–5 (-7) cm wide (rarely to 60 cm with fewer leaves and height to 1m or more); petals filiform 1–2 (-3) mm wide (rarely 5–6 mm) 4
         4. Placentation axile; seeds with partial green aril or aril absent . . . . . *Paris*
         4. Placentation parietal; seeds with enclosing red or orange sarcotesta . . . . . *Daiswa*
      3. Phyllotaxy mostly trimerous with leaves (0.8-) 5–15 (-25) cm wide; petals (2-) 7–15 (-60) cm wide (if narrower, petals either white or pink, or plants sessile-flowered) 5
         5. Petals generally spotted, ovate, frequently appearing clawed; leaves cordate to rounded, coriaceous . . . *Pseudotrillium*
         5. Petals not spotted, from ovate to obovate; leaves ovate to obovate, ''herbaceous'' or not coriaceous . . . . . *Trillium*

Acknowledgements. The authors thank the Botany Department of the University of Tennessee for support of this work, Gene Wofford and Randy Small for helpful comments; Cynthia Morton and an anonymous reviewer for critical review of the manuscript; Heng Li, Soichi Kawano, Vic Soukup, and Carl Denton for valuable assistance to this project; and GH for providing the images of the type specimens of *Trillium rivale*.

Literature Cited

APG (Angiosperm Phylogeny Group). 1998. An ordinal classification for the families of flowering plants. Annals of the Missouri Botanical Garden 85: 531–553.

Baldwin, B. G., M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell, and M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden 82: 247–277.

Bentham, G. and J. D. Hooker. 1883. *Genera plantarum*. London: Reeve & Co.

Berg, R. Y. 1959. Morphology and taxonomic position of *Scoliopus*, Liliaceae. Skrifter Utgitt av det Norske Videnskaps-Akademi i Oslo I. Matematisk-Naturvidenskapelig Klasse. Ny Serie. No. 4.

———. 1962. Morphology and taxonomic position of *Medeola*, Liliaceae. Skrifter Utgitt av det Norske Videnskaps-Akademi i Oslo I. Matematisk-Naturvidenskapelig Klasse. Ny Serie. No. 3.

Brummitt, R. K. and C. E. Powell. 1992. *Vascular plant families and genera.* Kew, U.K.: Royal Botanic Gardens.

Bull, J. J., J. P. Huelsenbeck, C. W. Cunningham, D. L. Swofford, and P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. Systematic Biology 42: 384–397.

Case, F. W. and R. B. Case. 1997. *Trilliums*. Portland: Timber Press.

Chase, M. W., M. R. Duvall, H. G. Hills, J. G. Conran, A. V. Cox, L. E. Eguiarte, J. Hartwell, M. F. Fay, L. R. Caddick, K. M. Cameron, and S. Hoot. 1995a. Molecular phylogenies of Lilianae. Pp. 109–137 in *Monocotyledons: systematics and evolution,* eds. P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries. Kew: Royal Botanic Gardens.

———, D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y. L. Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedren, B. S. Gaut, R. K. Jansen, K. J. Kim, C. F. Wimper, J. F. Smith, G. R. Furnier, S. H. Strauss, Q. Y. Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, Jr., S. W. Graham, S. C. H. Barrett, S. Dayanandan, and V. A. Albert. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. Annals of the Missouri Botanical Garden 80: 528–580.

———, ———, P. S. Soltis, P. J. Rudall, M. F. Fay, W. H. Hahn, S. Sullivan, J. Joseph, M. Molvray, P. J. Kores, T. J. Givnish, K. J. Sytsma, and J. C. Pires. 2000. Higher-level systematics of the monocotyledons: an assessment of current knowledge and a new classification. Pp. 3–16 in *Monocots: systematics and evolution,* eds K. L. Wilson and D. A. Morrison. Collingwood: CSIRO Publishing.

———, D. W. Stevenson, P. Wilkin, and P. J. Rudall. 1995b. Monocot systematics: a combined analysis, Pp. 685–730 in *Monocotyledons: Systematics and evolution,* eds. P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries. Kew: Royal Botanic Gardens.

Cronquist, A. 1968. *The evolution and classification of flowering plants.* London, U.K.: Nelson.

Dahlgren, R. M. T. 1975. A system of classification to be used to demonstrate the distribution of characters. Botaniska Notiser 128: 119–147.

——— and H. T. Clifford. 1982. *The monocotyledons: a comparative study.* London: Academic Press.

———, ———, and P. F. Yeo. 1985. *The families of the monocotyledons: structure, evolution, and taxonomy.* Berlin: Springer-Verlag.

Dalla Torre, K. W. von and H. Harms. 1908. *Genera siphonogamarum ad systems Englerianum conscripta.* Lipsiae: G. Engelmann.

Davis, J. I. 1995. A phylogenetic structure for the monocotyledons, as inferred from chloroplast DNA restriction site variation, and a comparison of measures of clade support. Systematic Botany 20: 503–527.

———, M. P. Simmons, D. W. Stevenson, and J. F. Wendel. 1998. Data decisiveness, data quality, and incongruence in phylogenetic analyses: an example from the monocotyledons using the mitochondrial *atpA* sequences. Systematic Biology 47: 282–310.

De Jussieu, A. L. 1789. *Genera plantarum secundum ordines naturales disposita.* Paris: Viduam Herissant.

Dumortier, ParB.-C. 1829. *Analyse des families des plantes avec l'indication des principaus cenres que s'y battachent.* Tournay: J. Casterman.

Endlicher, S. 1836–1840. *Genera plantarum*. Vindobonae: Apud Fr. Beck Universitatis Bibliopolam.

ENGLER, A. 1888. *Die natürlichen pflanzenfamilien.* II Leipzig: W. Engleman.

FARMER, S. B. 2000. Toward an understanding of the global phylogeny of Trilliaceae. M.S. Thesis. University of Tennessee, Knoxville, TN.

FARRIS, J. S., M. KÄLLERSJÖ, A. C. KLUGE, and C. BULT. 1995a. Constructing a significance test for incongruence. Systematic Biology 44: 570–572.

———, ———, ———, and ———. 1995b. Testing significance of incongruence. Cladistics 10: 315–319.

FELSENSTEIN, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Systematic Zoology 27: 401–410.

———. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

FREEMAN, J. D. 1969. A revisionary study of sessile-flowered *Trillium* L. (Liliaceae). Ph.D. Dissertation. Vanderbilt University, Nashville TN.

———. 1975. Revision of *Trillium* subgenus *Phyllantherum* (Liliaceae). Brittonia 27: 1–62.

FUKUDA, I. 2001a. The origin and evolution in *Trillium*. 1. The origin of the Himalayan *Trillium govanianum*. Cytologia 66: 106–111.

———. 2001b. The origin and evolution in *Trillium*. 2. Chromosome variation of *Trillium undulatum* in North America. Cytologia 66: 319–327.

FUSE, S. and M. N. TAMURA. 1999. Early evolutionary process of the monocotyledons inferred from *matK* DNA sequences with special reference to the phylogeny of Melanthiaceae s. l. (abstract). International Botanical Congress XVII (unpublished).

——— and ———. 2000. A phylogenetic approach of the plastid *matK* gene with emphasis on Melanthiaceae sensu lato. Plant Biology 2: 415–427.

GATES, R. R. 1917. A systematic study of the North American genus *Trillium,* its variability, and its relation to *Paris* and *Medeola*. Annals of the Missouri Botanical Garden 4: 43–93.

GOLDBLATT, P. 1995. The status of R. Dahlgren's orders Liliales and Melanthiales. Pp. 181–200 in *Monocotyledons: systematics and evolution,* eds. P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries. Kew: Royal Botanic Gardens.

GRAYBEAL, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Systematic Biology 47: 9–17.

HALANYCH, K. M. 1998. Lagomorphs misplaced by more characters and fewer taxa. Systematic Biology 47: 138–146.

HARA, H. 1969. Variations in *Paris polyphylla* Smith with reference to other Asiatic species. Journal of the Faculty of Science, University of Tokyo, Section 3, Botany 10(10): 141–180, pl. 1–6.

———, W. T. STEARN, and L. H. J. WILLIAMS. 1978. *An enumeration of the flowering plants of Nepal.* London: British Museum (Natural History).

HUANG, T. C., S. F. HUANG, and K. C. YANG. 1989. Notes on the flora of Taiwan. Taiwania 34: 45–52.

HUBER, H. 1969. Die samenmerkmale und verwandtschaftsverhältnisse der Liliiflorae. Mitteilung Botanische Staatssammlung München. 8: 219–538.

———. 1977. The treatment of the monocotyledons in an evolutionary system of classification. Plant Systematics and Ecology, Supplement 1: 285–298.

HUELSENBECK, J. P. 1997. Is the Felsenstein zone a fly trap? Systematic Biology 46: 69–74.

———, J. J. BULL, and C. W. CUNNINGHAM. 1996. Combining data in phylogenetic analysis. Trends in Ecology and Evolution 11: 152–158.

HUTCHINSON, J. 1926. *The families of flowering plants.* Vol. 2. *Monocotyledons.* London: Clarendon Press.

KATO, H., S. KAWANO, R. TERAUCHI, M. OHARA, and F. H. UTECH. 1995a. Evolutionary biology of *Trillium* and related genera (Trilliaceae) I. Restriction site mapping and variation of chloroplast DNA and its systematic implications. Plant Species Biology 10: 17–29.

———, R. TERAUCHI, F. H. UTECH, and S. KAWANO. 1995b. Molecular systematics of the Trilliaceae sensu lato as inferred from *rbcL* sequence data. Molecular Phylogeny and Evolution 4: 184–193.

KAZEMPOUR OSALOO, S. and S. KAWANO. 1999. Molecular systematics of Trilliaceae II. Phylogenetic analyses of *Trillium* and its allies using sequences of *rbcL* and *matK* genes of cpDNA and internal transcribed spacers of 18s–26s nrDNA. Plant Species Biology 14: 75–94.

———, F. H. UTECH, M. OHARA, and S. KAWANO. 1999. Molecular systematics of Trilliaceae I. Phylogenetic analysis of *Trillium* using *matK* gene sequences. Journal of Plant Research 112: 35–49.

KLUGE, A. G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Systematic Zoology 38: 7–25.

KRON, K. A. and W. S. JUDD. 1997. Systematics of the *Lyonia* group (Andromedeae, Ericaceae) and the use of species as terminals in higher-level cladistic analyses. Systematic Botany 22: 479–492.

KUNTH, C. S. 1850. *Enumeratio Plantarum*, vol. 5. Stutgardiae: Sumtibus J. G. Cottae.

LI, H. 1984. The phylogeny of the genus *Paris*. Acta Botanica Yunnanica 6: 351–362.

———. 1998. *The genus Paris* (*Trilliaceae*). Beijing: Science Press.

LIANG, S. and V. G. SOUKUP. 2000. *Paris*. Pp. 88–95 in *Flora of China,* Vol. 24, *Flagellariaceaem-Marantaceae,* eds. Z. Wu and P. H. Raven. Beijing: Science Press and St. Louis: Missouri Botanical Garden Press.

LINDLEY, J. 1846. *The vegetable kingdom,* 3rd edition. London: Bradley and Evans.

MADDISON, D. R. 1991. The discovery and importance of multiple islands of most parsimonious trees. Systematic Zoology 40: 315–328.

MASON-GAMER, R. J. and E. A. KELLOGG. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Systematic Biology 45: 524–545.

MELCHIOR, H. 1964. Liliaceae. Pp 512–524 in *Syllabus der Pflanzenfamilien,* vol. 2. Berlin: Gebrüder Borntraeger.

MICKEVICH, J. E. and J. S. FARRIS. 1981. The implications of congruence in *Menidia*. Systematic Zoology 30: 351–370.

NOLTE, H. J. 1994. *Flora of Bhutan including a record of plants from Sikkim and Darjeeling*. Vol 3, part 1, eds. A. J. C. Grierson and D. G. Long. Edinburgh: Royal Botanic Garden.

RAFINESQUE, C. S. 1819. Prodrome des nouveaux genres des plantes observés en 1817 et 1818 dans l'interieur des Ètats-Unis d'Amerique. Journal de physique, de chimie, d'historie naturelle et des arts 89: 102

RENDLE, A. B. 1930. *The classification of flowering plants* Vol. 1. *Gymnosperms* and *monotocyledons.* Ed. 2. Cambridge: Cambridge University Press.

REVEAL, J. L. 1998. Comments on Art. 14, footnote 2: first place of publication for seed plant family names. Taxon 47: 851–856.

RUDALL, P. J., K. L. STOBART, W-P. HONG, J. G. CONRAN, C. A. FURNESS, G. C. KITE and M. W. CHASE. 2000. Consider the lilies: systematics of Liliales. Pp. 347–357 in *Monocots: systematics and evolution,* eds. K. L. Wilson and D. A. Morrison. Collingwood: CSIRO Publishing.

SAMEJIMA, K. and J. SAMEJIMA. 1987. *Trillium genus illustrated.* Sapporo: University of Hokkaido Press.

SOLTIS, D. E., M. E. MORT, P. S. SOLTIS, C. HIBSCH-JETTER, E. A. ZIMMER, and D. MORGAN. 1999. Phylogenetic relationships of the enigmatic angiosperm family Podostemaceae inferred from 18s rDNA and *rbcL* sequence data. Molecular Phylogenetics and Evolution 1: 261–272.

———, P. S. SOLTIS, M. E. MORT, M. W. CHASE, V. SAVOLAINEN,

S. B. HOOT, and C. M. MORTON. 1998. Inferring complex phylogenies using parsimony: an empirical approach using three large DNA data sets for angiosperms. Systematic Biology 47: 32–46.

STEVENSON, D. W. and H. LOCONTE. 1995. Cladistic analysis of monocot families. Pp. 543–578 in *Monocotyledons: systematics and evolution,* eds. P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries. Kew: Royal Botanic Gardens.

SULLIVAN, J. 1996. Combining data with different distributions of among-site variations. Systematic Biology 45: 375–380.

SWOFFORD, D. L. 1991. When are phylogenetic estimates from molecular and morphological data sets incongruent? Pp. 295–333 in *Phylogenetic analysis of DNA sequences,* eds. M. M. Miyamoto and J. Cracraft. New York: Oxford Press.

———. 1998. *Paup* Phylogenetic analysis using parsimony (* and other methods).* Version 4. Sunderland: Sinauer Associates.

TAKHTAJAN, A. 1959. *Die evolution der angiospermen.* Jena: G. Fischer.

———. 1969. *Flowering plants: origin and dispersal.* Washington D.C.: Smithsonian Institution Press.

———. 1980. Outline of the classification of flowering plants. Botanical Review 46: 225–359.

———. 1983. A revision of *Daiswa* (Trilliaceae). Brittonia 35: 255–270.

———. 1987. *Systema magnoliophytorum.* Lenningrad: Soviet Sciences Press.

———. 1997. *Diversity and classification of flowering plants.* New York: Columbia University Press.

TAMURA, M. N. 1998. Trilliaceae. Pp. 444–452 in *The families and genera of vascular plants,* Vol III, *Monocotyledons,* ed. K. Kubitzki. Berlin: Springer-Verlag.

THORNE, R. F. 1992. Classification and geography of the flowering plants. Botanical Review 58: 225–348.

WATSON, L. and M. J. DALLWITZ. 1991a. The families of angiosperms: automated descriptions with interactive identification and information retrieval. Australian Systematic Botany 4: 681–695.

——— and ———. 1991b. Trilliaceae in The families of flowering plants: descriptions and illustrations. http://muse.bio.cornell.edu/delta/angio/extra/index.htm. (note that this URL now points to the data referenced in the 1996 citation.)

——— and ———. 1996. Trilliaceae in The families of flowering plants: descriptions and illustrations. http://muse.bio.cornell.edu/delta/angio/extra/index.htm.

WATSON, S. 1879. Contributions to American botany. I. Revision of the North American Liliaceae. Proceedings of the American Academy of Arts and Sciences 14: 213–288.

———. 1885. Contributions to American Botany. 2. Descriptions of some new species of plants, chiefly from our western territories. Proceedings of the American Academy of Arts and Sciences 20: 352–378.

WILLIS, J. C. 1973. *A dictionary of flowering plants and ferns.* 8th ed. Cambridge, U.K.: Cambridge University Press.

ZOMLEFER, W. B. 1996. The Trilliaceae in the southeastern United States. Harvard Papers in Botany 1: 91–120.

———. 1997. The genera of Melanthiaceae in the southeastern United States. Harvard Papers in Botany 2: 133–177.

———, N. H. WILLIAMS, W. M. WHITTEN, and W. S. JUDD. 2001. Generic circumscription and relationships in the tribe Melanthieae (Liliales: Melanthiaceae), with emphasis on *Zigadenus*: evidence from ITS and *trnL-F* sequence data. American Journal of Botany 88: 1657–1669.

APPENDIX 1

Taxa of Trilliaceae included in the analysis. Numbers in parenthesis indicate the proportion (%) of morphological characters scored as unknown.

Trillium albidum J.D. Freeman (1.8%), *T. angustipetalum* (Torr.) J.D. Freeman (8.2%), *T. apetalon* Makino (0.9%). *T. camtschatcense* Ker Gawl. (0.9%), *T. catesbaei* Elliott, *T. cernuum* L. (2.7%), *T. chloropetalum* (Torr.) Howell (4.5%), *T. cuneatum* Raf., *T. decipiens* J.D. Freeman (2.7%), *T. decumbens* Harb. (0.9%), *T. discolor* T.Wray ex Hook. (0.9%), *T. erectum* L. (2.7%), *T. flexipes* Raf., *T. foetidissimum* J.D. Freeman (1.8%), *T. govanianum* Wall. in Royle (4.5%), *T. gracile* J.D. Freeman (1.8%), *T. grandiflorum* (Michx.) Salisb., *T. hagae* Miyabe & Tatew. (2.7%), *T. kurabayashi* J.D. Freeman (5.5%), *T. lancifolium* Raf. (3.6%), *T. ludovicianum* Harb. (1.8%), *T. luteum* (Muhl.) Harb., *T. maculatum* Raf., *T. nivale* Riddell (1.8%), *T. ovatum* Pursh (0.9%), *T. parviflorum* V.G. Soukup (8.2%), *T. persistens* W.H. Duncan (5.5%), *T. petiolatum* Pursh (0.9%), *T. pusillum* Michx. (0.9%), *T. recurvatum* L.C. Beck, *T. reliquum* J.D. Freeman (6.4%), *T. rivale* S. Watson (0.9%), *T. rugelii* Rendle (1.8%). *T. sessile* L. (2.7%), *T. simile* Gleason (6.4%), *T. smallii* Maxim. (0.9%), *T. stamineum* Harb. (2.7%), *T. sulcatum* T.S. Patrick (1.8%), *T. taiwanense* S.S. Ying (23.6%), *T. tschonoskii* Maxim. (1.8%), *T. underwoodii* Small (3.6%), *T. undulatum* Kin ex Elliott, *T. vaseyi* Harb. (0.9%), *T. viride* L.C. Beck (0.9%), *T. viridescens* Nutt. (2.7%), *Daiswa cronquistii* Takht. (3.6%), *Paris daliensis* H. Li & V.G. Soukup (4.5%), *D. delavayi* (Franch.) Takht. (6.4%), *D. dunniana* (H. Lév.) Takht. (13.6%), *D. fargesii* (Franch.) Takht. (10.9%), *D. forrestii* Takht. (7.3%), *P. luquanensis* H. Li (5.5%), *P. mairei* H. Lév. (12.7%), *P. marmorata* Stearn (14.5%), *P. polyandra* S.F. Wang (19.1%), *D. polyphylla* (Sm.) Raf. (10.0%), *D. thibetica* (Franch.) Takht. (11.8%), *Paris undulatis* H. Li & V.G. Soukup (6.4%), *P. vietnamensis* (Takht.) H. Li (11.8%), *P. wenxianensis* Z.X. Peng & R.N. Zhao (15.5%), *Kinugasa japonica* (Franch. & Sav.) Tatew. & Sutô (3.6%), *P. axialis* H. Li (4.5%), *P. bashanensis* F.T. Wang & Ts. Tang (3.6%), *P. dulongensis* H. Li & Kurita (5.5%), *P. incompleta* M. Bieb. (12.7%), *P. quadrifolia* L. (7.3%), *P. rugosa* H. Li & Kurita (5.5%), *P. tetraphylla* A. Gray (10.9%), *P. vaniotii* H. Lév. (13.6%), *P. verticillata* M. Bieb. (8.2%).

APPENDIX 2

List of Characters used in the morphological analysis of Trilliaceae. Numbers in parentheses indicate proportion (%) of samples for which data were missing. Characters preceded by * were omitted in the phylogenetic analysis.

1. *Hybrid status: 1-yes, 2-no. **2. *Flowering time** (4.7%): 1–February, 2–March, 3–April, 4–May, 5–June, 6–July, 7–August. **3. *Chromosome number**: 1-diploid, 2-triploid, 3-tetraploid, 4-hexaploid, 5-octoploid. **4. *Geographical area**: 1-China, 2-wNA, 3-eNA, 4-Europe, 5-nAsia, 6-sAsia. **5. *Genus**: 1-outgroup, 2-*Trillium*, 3-*Paris*. **6. *Subgenus**: 1-outgroup, 2-*Trillium*, 3-*Phyllantherum*, 4-*Paris*, 5-*Kinugasa*, 6-*Daiswa.* **7. *Section** (18.6%): 1-outgroup, 2-*grandiflorum*, 3-*erectum*, 4-*catesbaei*, 5-*recurvatum*, 6-*sessile*, 7-*maculatum*, 8-*paris*, 9-*axiparis*, a-

kinugasa, b-*daiswa*, c-*dunnaniana*, d-*marmorata*, e-*fargesiana*, f-*thibetica*. **8. *Rafinesque's genus**: 1-outgroup, 2-*Paris*, 3-*Daiswa*, 4-*Phyllantherum*, 5-*Trillium*, 6-*Delostylis*. **9. Plant type**: 1-herb, 2-vine. **10. Foliaceousness**: 1-scapose, 2-leafy, 3-subscapose. **11. Plant sexuality**: 1-bisexual, 2-dioecious, 3-polygamous. **12. Inflorescence type**: 1-solitary, 2-simple umbel, 3-raceme, 4-panicle of racemes, 5-panicle, 6-spike, 7-cyme. **13. Number flowers**: 1-one, 2-more than one. **14. Composition**: 1-tepals, 2-sepals+petals. **15. Pedicel vertical posture**: 1-erect, 2-above the leaves, 3-horizontal, 4-below the leaves, 5-none. **16. Root type**: 1-rhizome, 2-bulb, 3-tuber, 4-fibrous. **17. Rhizome size** (7.0%): 1-very thin (to 5 mm), 2-slender (5–10 mm), 3-thick (1–2.5 cm), very thick (over 2.5 cm). **18. Stem habit**: 1-erect, 2-partially decumbent, 3-decumbent. **19. Plant vestiture**: 1-glabrous, 2-puberulent, 3-pubescent, 4-pilose, 5-papilose, 6-scabrous, 7-striate. **20. Stem color**: 1-green, 2-yellowish-green, 3-reddish-purple, 4-greenish-purple, 5-purplish, 6-brownish-green, 7-reddish, 8-green over purple, 9-purple tinged. **21. Stem color distribution**: 1-throughout, 2-geographic (e.g., segregated by area), 3-suffused. **22. *Leaf number**: 1-two, 2-three, 3-four, 4-five, 5-six, 6-seven, 7-eight, 8-nine, 9-ten, a-eleven and up. **23. Leaf location**: 1-terminal, 2-cauline, 3-basal, 4-reduced cauline. **24. Leaf arrangement**: 1-whorled, 2-alternate, 3-opposite, 4-spirally inserted. **25. Leaf: # whorls**: 1-one, 2-more than one, 3-none. **26. Leaf attachment**: 1-petiolate, 2-subsessile, 3-sessile. **27. Leaf shape: widest point**: 1-ovate (¼), 2-elliptic-ovate, 3-elliptic/rhombic/oblong (½), 4-elliptic-obovate, 5-obovate (¾). **28. Leaf shape: width LxW**: 1-linear, 2-narrow, 3-average, 4-broad, 5-very broad, 6-depressed or transverse. **29. Leaf shape: sides**: 1-elliptic (curved), 2-oblong-elliptic, 3-oblong (parallel) 4-oblong-rhombic, 5-rhombic (straight), 6-rhombic-elliptic. **30. Leaf margin**: 1-entire, 2-undulate, 3-serrulate. **31. Leaf color distribution**: 1-throughout, 2-mottled, 3-geographic, 4-suffused. **32. Leaf color**: 1-green, 2-more or less purple mottled, 3-green with maroon hue, 4-green with small brownish spots, 5-green with white variegation on veins, 6-mottled, 7-mottled with dark green, 8-green with spots of light green, 9-multiple shades of green, a-shades of green beside pale midrib, b-dark green spots on mottled background, c-dark green between veins with pale midrib. **33. Leaf lower surface**: 1-pubescent on veins, 2-scabrous on veins, 3-glossy, 4-dull, 5-purple, 6-purple nerves, 7-glabrous, 8-pubescent. **34. Leaf apex type**: 1-entire, 2-with sinuses, 3-with mid-rib, midvein or vein extension. **35. Leaf apices** (3.5%): 1-acuminate, 2-acute, 3-obtuse, 4-rounded, 5-emarginate, 6-sub-acute, 7-cuspidate, 8-blunt, 9-attenuate. **36. Leaf base type**: 1-entire, 2-with sinuses. **37. Leaf bases** (5.8%): 1-cuneate, 2-sheathing, 3-obtuse, 4-rounded, 5-attenuate, 6-hastate, 7-cordate. **38. Leaf texture**: 1-herbaceous, 2-membranous, 3-papery, 4-rugose, 5-coriaceous. **39. Leaf # main nerves** (8.1%): 1-many, 2-three, 3-five, 4-seven. **40. Bracts**: 1-absent, 2-present. **41. Sepal form**: 1-normal, 2-petaloid. **42. Sepal fusion**: 1-separate, 2-fused. **43. *Sepal number**: 1-three, 2-four, 3-five, 4-six, 5-seven, 6-eight, 7-nine, 8-ten. **44. Sepal shape: widest point**: 1-ovate, 2-elliptic-ovate, 3-elliptic/rhombic/oblong, 4-elliptic-obovate, obovate. **45. Sepal shape: width LxW**: 1-narrow, 2-average, 3-broad. **46. Sepal shape: sides**: 1-elliptic, 2-oblong-elliptic, 3-oblong. **47. Sepal duration**: 1-persistent, 2-deciduous. **48. Sepal texture**: 1-herbaceous, 2-membranous. **49. Sepal color**: 1-reddish-purple, 2-yellow, 3-purple, 4-purplish-green, 5-red, 6-green, 7-reddish-green, 8-yellowish-green, 9-white, a-white with green veins, b-greenish with red or purple veins, c-greenish-purple basally, d-greenish, reddish-purple basally, e-greenish-brown, f-greenish with red veins, g-greenish with reddish-purple margins, h-greenish with purple margins, i-green with white veins. **50. Sepal color distribution**: 1-throughout, 2-suffused, 3-marginal, 4-mottled, 5-geographic. **51. Sepal apices** (11.6%): 1-acuminate, 2-acute, 3-obtuse, 4-rounded, 5-acuminate-rounded, 6-sub-acute, 7-cuspidate, 8-emarginate, 9-caudate, a-blunt, b-attenuate. **52. Sepal apex type**: 1-entire, 2-midrib, midvein, or vein extension. **53. Petal form**: 1-normal, 2-foliaceous, 3-staminoid, 4-none. **54. Petal presence**: 1-present, 2-absent. **55. *Petal number**: 1-zero, 2-three, 3-four, 4-five, 5-six, 6-seven, 7-eight. **56. Petal fusion**: 1-separate, 2-fused. **57. Petal: widest point** (3.5%): 1-ovate, 2-elliptic-ovate, 3-elliptic/rhombic/oblong, 4-elliptic-obovate, 5-obovate. **58. Petal: width LxW**: 1-filiform, 2-linear, 3-narrow, 4-average, 5-broad, 6-very broad. **59. Petal: sides** (1.2%): 1-elliptic, 2-oblong-elliptic, 3-oblong, 4-rhombic-elliptic. **60. Petal duration**: 1-persistent, 2-deciduous. **61. Petal color** (3.5%): 1-white, 2-yellow, 3-cream, 4-pink, 5-red, 6-purple, 7-bronze, 8-green, 9-reddish-purple, a-greenish-purple, b-purplish-black, c-brownish-purple, d-greenish-purple, e-purple with greenish-claw, f-yellow with greenish claw, g-green with purple claw, h-yellow with purple claw, i-yellow-green with purple claw, j-white spotted with rose, k-white with purple basally, l-white with basal red ''V'', m-olive, n-purplish green with purple claw. **62. Petal color distribution**: 1-throughout, 2-spotted, 3-geographic. **63. Petals: pigmented**: 1-yes, 2-no, 3-white fading to pink. **64. Petal: transverse posture** (32.6%): 1-incurved, 2-plane, 3-recurved, 4-undulate, 5-outcurved. **65. Petal: vertical posture** (5.8%): 1-erect, 2-divergent upwards, 3-horizontal, 4-divergent downwards, 5-declined. **66. Petal: longitudinal posture** (1.2%): 1-straight, 2-twisted. **67. Petal apices** (32.6%): 1-acuminate, 2-acute, 3-obtuse, 4-rounded, 5-blunt, 6-subacute, 7-cuspidate, 8-apiculate, 9-emarginate, a-mucronate. **68. Petal apex type**: 1-entire, 2-midrib, midvein, or vein extension. **69. Stamen form**: 1-normal, 2-petaloid-abortive. **70. *Stamen number**: 1-zero, 2–1x

sepals, 3–2x sepals, 4–3x sepals, 5–4x sepals. **71. Stamen fusion**: 1-free, 2-adnate to tepal. **72. Stamen transverse posture** (33.7%): 1-straight, 2-incurved, 3-slightly recurved. **73. Stamen vertical posture** (30.2%): 1-erect, 2-spreading. **74. Anther dehiscence** (8.1%): 1-introrse, 2-latrorse, 3-extrorse. **75. Pollen shape** (1.2%): 1-spherical, 2-ellipsoid, 3-irregular. **76. Pollen aperture** (1.2%): omniaperturate, 2-monosulcate. **77. Pollen ornamentation** (45.3%): 1-granulate, 2-echinate, 3-corrugate, 4-verrucate, 5-spinulate, 6-clavate, 7-foveolate, 8-reticulate, 9-psilate, a-gemmate. **78. Connective prolongation** (5.8%): 1-none, 2-acute, 3-truncate, 4-dilated, 5-round, 6-emarginate, 7-obtuse. **79. Filament color** (29.1%): 1-green, 2-purple, 3-whitish-purple, 4-reddish-purple, 5-whitish-green, 6-reddish-purplish-brown, 7-pinkish-white, 8-greenish-yellow, 9-white, a-pink. **80. Pollen color** (27.9%):1-yellow, 2-orange, 3-olive, 4-brownish, 5-olive-orange, 6-purple, 7-greyish-purple, 8-pink, 9-yellowish pale purple, a-green, b-maroon, c-orange-yellow. **81. Connective color** (31.4%): 1-green, 2-pink, 3-purple, 4-pinkish-purple, 5-reddish-purple, 6-brown, 7-whitish-green, 8-white, 9-reddish-purple-brown. **82. Stamen color distribution** (3.5%): 1-throughout, 2-pollen different, 3-all segments different. **83. Pistil form**: 1-normal, 2-abortive. **84. Ovary position**: 1-inferior, 2-superior. **85. Ovary # locules** (24.4%): 1-one, 2-three, 3-four to ten. **86. Ovary placentation** (25.6%): 1-axile, 2-parietal, 3-combination. **87. Ovary plane shape** (5.8%): 1-conical, 2-angular-ovoid, 3-ovoid, 4-ellipsoid, 5-obovoid, 6-flask-shaped, 7-subglobose, 8-oval-globose, 9-fusiform, a-ovate-conical. **88. Ovary X-section shape** (12.8%): 1-round, 2-ridged, 3-angled, 4-winged. **89. Ovary # ribs** (30.2%): 1-zero, 2-two, 3-three, 4-four, 5-five, 6-six, 7-seven. **90. Ovary apex** (7.0%): 1-truncate, 2-crowned, 3-attenuate, 4-obtuse. **91. Style presence** (1.2%): 1-present, 2-absent. **92. *Stigma number**: 1-three, 2-four, 3-five, 4-six, 5-eight, 6-ten, 7-seven. **93. Stigma shape** (47.7%): 1-linear-subulate, 2-subulate. **94. Stigma vertical posture** (29.1%): 1-divergent, 2-erect. **95. Stigma transverse posture** (23.3%): 1-incurved, 2-straight, 3-outcurved. **96. Stigma duration**: 1-deciduous, 2-persistent. **97. Stigma size** (8.1%): 1-very thin, 2-thin, 3-average, 4-thick. **98. Ovary color** (11.6%): 1-yellowish-white, 2-green, 3-purple, 4-pink, 5-cream yellow, 6-white, 7-reddish-purple, 8-purplish-brown, 9-yellow-green, a-reddish-purplish-brown, b-green over purple, c-purple over green, d-purple over yellow, e-purple over white, f-pink over white, g-green with purple disk, h-green with yellow disk, i-green with white disk, j-yellow with black disk. **99. Ovary color distribution**: 1-throughout, 2-spotted, 3-striped, 4-geographic. **100. Stigma color** (17.4%): 1-green, 2-purple, 3-yellowish-green, 4-creamy white, 5-yellow, 6-yellowish-white, 7-pink, 8-black, 9-brownish-violet, a-reddish-purplish-brown, b-brown, c-purple in, green out, d-purple-brown out, yellow in, e-purple out, yellow in, f-white, basally pink, g-green out, yellow in, h-pinkish orange. **101. Stigma color distribution**: 1-throughout, 2-banded, 3-geographic. **102. Pistil color distribution** (1.2%): 1-throughout, 2-geographic by part, 3-all different. **103. Fruit type** (4.7%): 1-berry, 2-capsule, 3-fleshy capsule. **104. Fruit dehiscence** (4.7%): 1-indehiscent, 2-basally dehiscent, 3-septicidal, 4-irregular, 5-loculicidal, 6-dehiscent, 7-decay. **105. Fruit plane shape** (31.4%): 1-conical, 2-angular-ovoid, 3-ovoid, 4-globose, 5-obovoid, 6-elliptic. **106. Fruit color** (20.9%): 1-green, 2-maroon, 3-greenish-yellow, 4-rusty red, 5-greenish-white, 6-red, 7-black, 8-bluish-black, 9-brown, a-purple, b-green with purple dots, c-purplish green, d-greenish-brown, e-green with brown ribs. **107. Fruit color distribution**: 1-throughout, 2-mottled, 3-spotted, 4-striped, 5-geographic. **108. Seed arils** (2.3%): 1-incomplete, 2-absent, 3-complete. **109. Endosperm development** (2.3%): 1-helobial, 2-nuclear. **110. Cotyledon shape** (1.2%): 1-strap-like, 2-leaf-like.