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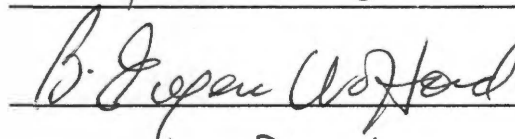
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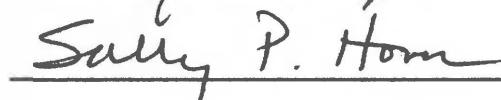

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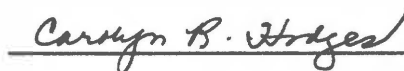
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Carolyn R. Hodges,
Vice Provost and Dean of the Graduate School

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A Systematic Study of *Trillium* subgenus *Delostylis*

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Susan Baker Farmer

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This work is first dedicated to the memory of John D. Freeman who first asked the question "Is the variety different from the species" but most of all to my family without whose support this never would have happened.

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ABSTRACT

This study focused on the systematics of *Trillium* subg. *Delostylis*, an enigmatic group endemic to the southeastern United States. Phylogenetic analysis of DNA sequence data was used to examine the circumscription and phylogenetic placement of *Delostylis*. The results of the phylogenetic analysis of data from the ITS, *matK*, and *psbA-trnH* sequence data suggested that the circumscription of *Delostylis* be narrowed to consist of *T. persistens*, *T. catesbaei*, and *T. pusillum sensu lato*. A revised *Delostylis* was characterized by the presence of a style with three slender stigmatic branches in those plants whose flowers are either white-fading-to-pink or pink. Results of the phylogenetic analyses showed *Delostylis* to be one of four innermost clades in *Trillium* (the other three being *T. ovatum* [bootstrap 100%; posterior probability 1.0], *T.* subg. *Phyllanthium* [100%; 1.0], and sister taxa *T. grandiflorum* and *T. nivale* [86%; 1.0]). Phylogenetic analyses of DNA sequence data as well as statistical analysis of quantitative morphological characters in conjunction with geographic distribution, physiography, and habitat preference were used to address the questions of species-level taxonomy. Molecular evidence suggested that the narrowly endemic *Trillium persistens* was monophyletic, but *T. catesbaei* was not. The remaining members of *Delostylis* form the Pusillum Complex which was found to be composed of ten species (four of them new) in three groups rather than a single species with one or more varieties. The Pusillum Cluster included *T. pusillum*, *T. texanum*, *T. georgianum* (*sp. nov.*), and *T. telmacola* (*sp. nov.*); the Ozarkanum Cluster included *T. ozarkanum* and *T. alabamicum* (*sp. nov.*); and the Virginianum Cluster included *T. virginianum*, *T. monticola* (*stat. nov.*), *T. carolinianum* (*sp. nov.*), and *T. palustris* (*sp. nov.*). Five of the 10 species that are included within the Pusillum Complex are known from five counties or less (*T. telmacola*, *T. carolinianum*, *T. georgianum*, *T. monticola* and *T. pusillum*) and should certainly be considered for protection.

TABLE OF CONTENTS

1.0	INTRODUCTION	1
1.1	Introduction to Trilliaceae and <i>Trillium</i>	1
1.2	<i>Delostylis</i> and its Distribution	3
1.3	Taxonomic History of <i>Trillium pusillum</i>	5
1.4	Goals of this Project	12
2.0	MATERIALS AND METHODS	19
2.1	Sampling Strategy	19
2.1.1	Taxon sampling	19
2.1.2	Molecular markers	19
2.1.3	Outgroup selection	27
2.1.4	Morphology	27
2.2	DNA Extraction, PCR, and Sequencing Protocols	31
2.3	Sequence Manipulation and Alignment	33
2.4	Phylogenetic Analyses	33
2.5	DNA Sequence Data as Taxonomic Characters	34
2.6	Morphological Analysis	35
3.0	Results	37
3.1	Parsimony Analyses	39
3.1.1	ITS sequence data	39
3.1.2	<i>matK</i> sequence data	39
3.1.3	<i>psbA-trnH</i> sequence data	42
3.1.4	Combined sequence data	42
3.2	Bayesian Analyses	42
3.2.1	ITS sequence data	42
3.2.2	<i>matK</i> sequence data	47
3.2.3	<i>psbA-trnH</i> sequence data	47
3.2.4	Combined sequence data	52
3.3	Molecular Markers as Taxonomic Data	52
3.3.1	<i>matK</i> sequence data	52
3.3.2	<i>psbA-trnH</i> sequence data	55
3.4	Morphological Analysis	61
3.4.1	The Pusillum Complex	61
3.4.2	The Pusillum Cluster	63
3.4.3	The Ozarkanum Cluster	67
3.4.4	The Virginianum Cluster	74
3.4.5	The individual species	74
4.0	Discussion	85
4.1	<i>Delostylis</i> : Composition and Placement	85
4.2	The Pusillum Complex	90
4.2.1	The Pusillum Cluster	95
4.2.2	The Ozarkanum Cluster	96
4.2.3	The Virginianum Cluster	97
4.3	The Synthetic View	98
4.3.1	Conservation Issues	100

5.0	Taxonomic Treatment	103
5.1	Subgeneric Classification of <i>Trillium</i>	103
5.2	<i>Trillium</i> subgen. <i>Delostylis</i> (Raf.) stat. nov.	104
5.2.1	<i>Trillium</i> subgen. <i>Delostylis</i> sect. <i>Catesbaei</i> nom nov.	106
5.2.2	<i>Trillium</i> subgen. <i>Delostylis</i> sect. <i>Pusillum</i> nom nov.	106
	Literature Cited	121
	Appendix A. Herbarium Specimens of the <i>Pusillum</i> Complex Examined	143
	Appendix B. Aligned DNA Sequence Data Matrices	157
	B.1 ITS data set	159
	B.2 <i>matK</i> data set	165
	B.3 <i>psbA-trnH</i> data set	179
	B.4 Combined data set	189
	Appendix C. Standard Statistics from the Morphological Characters	207
	Appendix D. Discriminant Analyses	215
	D.1 The Clusters of the <i>Pusillum</i> Complex	217
	D.2 The Taxa of the <i>Pusillum</i> Cluster of the <i>Pusillum</i> Complex	220
	D.3 The Taxa of the <i>Ozarkanum</i> Cluster of the <i>Pusillum</i> Complex	223
	D.4 The Taxa of the <i>Virginianum</i> Cluster of the <i>Pusillum</i> Complex	225
	D.5 The Taxa of the <i>Pusillum</i> Complex	228
	Vita	231

LIST OF TABLES

1. Subgeneric groups within <i>Trillium</i> subgen. <i>Trillium</i>	4
2. Character differences between putative members of <i>Delostylis</i>	7
3. Taxonomic and nomenclatural history for <i>Trillium pusillum</i> and varieties.	13
4. Taxonomic treatments for entities included within <i>Trillium pusillum: texanum</i>	14
5. Taxonomic treatments for entities included within <i>Trillium pusillum: ozarkanum</i>	15
6. Taxonomic treatments for entities included within <i>Trillium pusillum: virginianum</i>	16
7. Taxonomic treatments for entities included within <i>Trillium pusillum: monticola</i>	17
8. Taxon sampling for the molecular analyses of <i>Delostylis</i>	21
9. Reported county-level distribution and taxon sampling of <i>Trillium pusillum</i> for the morphological analyses (adapted from Freeman [undated]).	24
10. DNA sequence data available from GenBank used in these analyses.	28
11. PCR and sequencing primers used in the molecular analyses of <i>Delostylis</i>	32
12. Summary statistics for the four data sets used in the phylogenetic analyses of <i>Delostylis</i>	38
13. Scores from MrModeltest analyses with different starting trees.	45
14. <i>psbA-trnH</i> markers that are diagnostic for cluster membership within the Pusillum Complex. ..	59
15. <i>psbA-trnH</i> markers that are taxon specific within the Pusillum Complex.	60
16. Means for the standard morphometric characters for the clusters of the Pusillum Complex.	62
17. Discriminant model classification results for the clusters of the Pusillum Complex.	64
18. Casewise statistics for misclassified cases for the clusters of the Pusillum Complex.	64
19. Centroids of the clusters of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.	64
20. Means for the standard morphometric characters for the taxa of the Pusillum Cluster of the Pusillum Complex.	66
21. Discriminant model classification results for the Pusillum Cluster of the Pusillum Complex.	68
22. Centroids of the taxa of the Pusillum Cluster of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.	68
23. Means for the standard morphometric characters for members of the Ozarkanum Cluster of the Pusillum Complex.	70
24. Discriminant model classification results for the Ozarkanum Cluster of the Pusillum Complex.	72
25. Casewise statistics for misclassified cases in the analysis of the Ozarkanum Cluster of the Pusillum Complex.	72
26. Centroids of the taxa of the Ozarkanum Cluster of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.	73
27. Means for the standard morphometric characters for members of the Virginianum Cluster of the Pusillum Complex.	75
28. Discriminant model classification results for the Virginianum Cluster of the Pusillum Complex.	76
29. Casewise statistics for misclassified cases in the analysis of the Virginianum Cluster of the Pusillum Complex.	76

30.	Centroids of the taxa of the Virginianum Cluster of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.	76
31.	Discriminant model classification results for the taxa of the Pusillum Complex.	79
32.	Casewise statistics for misclassified cases in the analysis of the taxa of the Pusillum Complex. ..	80
33.	Centroids of the taxa of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.	81
34.	Comparisons of clade support between DNA sequences and analysis techniques.	87
A.1	Herbarium specimens of the Pusillum Complex examined for this study.	145
C.1	Descriptive statistics from the SPSS analysis of the taxa of the Pusillum Complex.	209
D.1	Wilks lambda illustrating the significance of the functions calculated for the clusters of the Pusillum Complex.	217
D.2	Eigenvalues and % variance for the functions for the clusters of the Pusillum Complex showing the amount of variation attributable to each function.	217
D.3	Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the functions used in separating the clusters of the Pusillum Complex.	218
D.4	Structure matrix indicating the correlations of each character to the functions used in separating the clusters of the Pusillum Complex.	219
D.5	Wilks lambda illustrating the significance of the functions calculated for the taxa of the Pusillum Cluster of the Pusillum Complex.	220
D.6	Eigenvalues and % variance for the functions used in separating the taxa of the Pusillum Cluster of the Pusillum Complex showing the amount of variation attributable to each function.	220
D.7	Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the functions used in separating the taxa of the Pusillum Cluster of the Pusillum Complex.	221
D.8	Structure matrix indicating the correlations of each character to the functions used in separating the taxa of the Pusillum Cluster of the Pusillum Complex.	222
D.9	Eigenvalues and % variance for the functions used in separating the taxa of the Ozarkanum Cluster of the Pusillum Complex.	223
D.10	Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the function used in separating the taxa of the Ozarkanum Cluster of the Pusillum Complex.	223
D.11	Structure matrix indicating the correlations of each character to the function used in separating the taxa of the Ozarkanum Cluster of the Pusillum Complex.	224
D.12	Wilks lambda illustrating the significance of the functions calculated for the taxa of the Virginianum Cluster of the Pusillum Complex.	225
D.13	Eigenvalues and % variance for the functions used in separating the taxa of the Virginianum Cluster of the Pusillum Complex showing the amount of variation attributable to each function.	225

D.14 Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the functions used in separating the taxa of the Virginianum Cluster of the Pusillum Complex.	226
D.15 Structure matrix indicating the correlations of each character to the functions used in separating the taxa of the Virginianum Cluster of the Pusillum Complex.	227
D.16 Wilks lambda illustrating the significance of the functions calculated for the taxa of the Pusillum Complex	228
D.17 Eigenvalues and % variance for the functions used in separating the taxa of the Pusillum Complex showing the amount of variation attributable to each function.	228
D.18 Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the functions used in separating the taxa of the Pusillum Complex.	229
D.19 Structure matrix indicating the correlations of each character to the functions used in separating the taxa of the Pusillum Complex.	230

LIST OF FIGURES

1.	Putative taxa within <i>Delostylis</i> .	2
2.	County-level distribution maps for putative members of <i>Delostylis</i> .	6
3.	The earliest known specimen of <i>Trillium pusillum</i> , from the Clayton Herbarium.	8
4.	Type specimen of <i>Trillium pusillum</i> Michx.	10
5.	County-level distribution maps for members of the Pusillum Complex at the beginning and at the conclusion of this study.	20
6.	Strict consensus trees from preliminary analyses of Trilliaceae with different data sets and different outgroups.	30
7.	Fifty percent majority rule consensus tree of 85,280 shortest trees from the parsimony analysis of the ITS sequence data of <i>Trillium</i> .	40
8.	Fifty percent majority rule consensus tree of 56,895 shortest trees from the parsimony analysis of the <i>matK</i> sequence data of <i>Trillium</i> .	41
9.	Fifty percent majority rule consensus tree of 5,346 shortest trees from the parsimony analysis of the <i>psbA-trnH</i> sequence data of <i>Trillium</i> .	43
10.	Fifty percent majority rule consensus tree of 280 shortest trees from the parsimony analysis of the combined sequence data of <i>Trillium</i> .	44
11.	Graphs of the first 50,000 generations of sump values from the bayesian analyses of <i>Trillium</i> .	48
12.	Consensus tree from the bayesian analysis of the ITS sequence data of <i>Trillium</i> .	49
13.	Consensus tree from the bayesian analysis of the <i>matK</i> sequence data of <i>Trillium</i> .	50
14.	Consensus tree from the bayesian analysis of the <i>psbA-trnH</i> sequence data of <i>Trillium</i> .	51
15.	Consensus tree from the bayesian analysis of the combined sequence data of <i>Trillium</i> .	53
16.	<i>Delostylis</i> from phylogenetic analysis of the <i>psbA-trnH</i> sequence data with diagnostic molecular markers mapped onto the tree.	54
17.	Diagnostic areas of the 5' <i>trnK</i> - <i>matK</i> spacer region, the <i>matK</i> coding region and the <i>matK</i> -3' <i>trnK</i> spacer region.	56
18.	Diagnostic areas of <i>psbA</i> (part) and the <i>psbA-trnH</i> spacer region.	57
19.	Two-dimensional plot of function scores of the individual specimens and cluster centroids from the discriminant analysis of the clusters of the Pusillum Complex.	65
20.	Two-dimensional plot of function scores of the individual specimens and taxon centroids from the discriminant analysis of the Pusillum Cluster of the Pusillum Complex.	69
21.	Plots of function scores of the individual specimens and taxon centroids from the discriminant analysis of the Ozarkanum Cluster of the Pusillum Complex.	71
22.	Two-dimensional plot of function scores of the individual specimens and taxon centroids from the discriminant analysis of the Virginianum Cluster of the Pusillum Complex.	77
23.	Two-dimensional plot of function scores of the individual specimens and taxon centroids from the discriminant analysis of the taxa of Pusillum Complex.	82
24.	Three-dimensional plot of taxon centroids from the discriminant analysis of the Pusillum Complex.	83

25. Tree from the parsimony analysis of the combined sequence data of <i>Trillium</i> with morphological characters defining <i>Delostylis</i> mapped onto the tree.	86
26. <i>Linaria Cærulea</i> . The Blew Linnet. <i>Solanum</i> &c. <i>floræ carnoe</i>	89
27. County-level distribution map for clusters of the Pusillum Complex.	93
28. Sister species complexes within <i>Trillium</i>	94

1.0 INTRODUCTION

Delostylis is one of the most enigmatic groups within *Trillium*, a genus that is known for “containing and concealing many species” (Elliott 1817). The distinct morphological feature that is characteristic of this group is the presence of a style with three slender stigmatic branches; prior to this study, the group (if recognized at all) was considered to consist of four species: *Trillium pusillum* Michx. (1803), *T. catesbaei* Ell. (1817), *T. nivale* Riddell (1835), and *T. persistens* W.H. Duncan (1971) (Fig. 1). There are several reasons to study *Delostylis*; most notable of which is the need to ascertain proper conservation status for these taxa. Not only does *Delostylis* contain one of the rarest of all *Trillium* species (*T. persistens*), but *T. pusillum*, the most widespread of the group, is considered locally rare in many places. The focus of this study was to determine the circumscription and phylogenetic status of this group as a whole and to address the taxonomic nature and phylogenetic status of *T. pusillum*.

1.1 Introduction to Trilliaceae and *Trillium*

Trilliaceae is a family of herbaceous liliaceous, monocots comprising six genera in two tribes. Tribe Parideae comprises two monotypic genera *Trillidium* and *Kinugasa*, *Paris* (five species), and *Daiswa* (ca. 20 species); tribe Trillieae comprises the monotypic genus *Pseudotrillium* and *Trillium* (ca. 40-50 species).

The traditional view of generic limits in Trilliaceae has been the classification into the two Linnaean genera based on floral merosity: *Trillium* is trimerous, whereas *Paris* is 4- 11-merous. Within *Trillium*, a major issue is whether the two subgenera (subgen. *Trillium* having a flower pedicel, subgen. *Phyllantherum* lacking a pedicel [Freeman 1969, 1975]) are monophyletic (summary in Zomlefer 1996). A full formal hierarchy was proposed by Murray (1983, 1984) for *T.* subgen. *Phyllantherum* from section to subseries, but it has not been adopted. Formal subgroups have not been proposed for *Trillium* subgen. *Trillium*, but two subgroups are often mentioned (e.g., Patrick 1984) based on the anthocyanin chemistry exhibited in aging petals: the Erectum Group (where the white petals just turn brown) and the Grandiflorum Group (where the petals turn a vivid pink). Within *Paris*, the debate is whether to recognize a single broad genus (Hara 1969; Li 1984, 1998; Ji et al. 2006) or split it into three genera (Takhtajan 1983; Dahlgren et al. 1985; Tamura 1998; Farmer and Schilling 2002; Farmer 2006).

Trilliaceae are plants of North Temperate forests with a holarctic distribution (Takhtajan 1986) and are thus hypothesized to be Arcto-Tertiary in origin (Tamura 1998), referring to high latitude, Northern Hemisphere areas having an abundance of Tertiary fossils (Bufford & Spongberg 1983; see Engler 1879). Most contemporary botanists agree that this distribution consists of four disjunct areas: eastern North America, western North America, eastern Asia, and southeastern Europe/Asia Minor (Wood 1972; Tiffney 1985b). Members of Trilliaceae occur in each of these regions as well as



a.



b.



c.



d.

Figure 1. Putative taxa within *Delostylis*. a. *Trillium nivale*, b. *T. persistens*, c. *T. catesbaei*, d. *T. pusillum* s.l. (image of *T. nivale* by Dr. Kenneth Robertson and used with permission).

throughout most of Europe. Of these Tertiary relict disjunctions, the most widely studied is that of eastern Asia and North America particularly eastern North America (Gray 1846; White 1983; Tiffney 1985a; Hong 1993; Wen 1999; see Bufford and Spongberg 1983 for summary). Another pattern of distribution, that of the southern Appalachians and western North America (Wood 1971), has also been noted in Trilliaceae (Farmer 2006).

Within Trilliaceae there are some additional interesting distributional patterns: there are no *Trillium* in Europe; no members of Parideae are found in the New World; polyploids are restricted to the Old World; the only documented hybrids are in Asia; and the only sessile-flowered species are New World. In addition, there is only one globally widespread species, *Paris quadrifolia* (which is found from Ireland to Mongolia and Siberia); there are two widespread genera, *Paris* and *Trillium*; but there are three narrowly endemic genera, *Pseudotrillium*, *Kinugasa*, and *Trillidium*. The traditional sister species groups that are often found in Arcto-Tertiary distributions also exist: the Erectum Group exhibits the traditional eastern North America–Asia pattern (Fernald 1931; Raven 1972; Thorne 1972; Wood 1972; Xiang et al. 1998), while both the Grandiflorum Group and the sessile flowered species of *Trillium* (subgen. *Phyllantherum* Raf.) exhibit the western North America–eastern North America pattern (Wood 1971).

1.2 *Delostylis* and its Distribution

Formal infrageneric names have been used only sporadically in *Trillium*. Rafinesque was the first to propose subgeneric names for *Trillium*, which were subsequently relegated to synonymy until Freeman (1975). Beginning in 1819 Rafinesque proposed subgen. *Delostylis*, pedicellate species with a style; followed by subgen. *Phyllantherum* (1820), the sessile-flowered species; and in 1830 he proposed subgeneric names: *Delostylium*, the species with a common style, *Anthopium*, the pedicellate species, and *Sessilium*, the sessile-flowered species. Rafinesque's taxonomy has not been completely adopted with the result that *Phyllantherum* (Freeman 1975) and, by extension, the autonym *Trillium*, are the only currently recognized subgeneric, supraspecific names. In 1983, Murray proposed formal section names for *T.* subgen. *Phyllantherum* based on Freeman (1975); in 1984, he erected an elaborate schema for the subgenus including subsections, series, and subseries. Formal subgroups have not been proposed for *Trillium* subgen. *Trillium*, but two subgroups are often mentioned based on the visible differences in anthocyanin chemistry exhibited in aging petals: the Erectum Group (where the white petals turn brown) and the Grandiflorum Group (where the petals turn a vivid pink) (Patrick, 1984). Gleason (1906), Barksdale (1938), and Ihara & Ihara (1978, 1982) each proposed dividing *T.* subgen. *Trillium* into three different subgroups (Table 1); both Ihara & Ihara (1978, 1982) and Gleason (1906) recognized a group corresponding to *Delostylis* whereas Barksdale (1938) included them in his Grandiflorum Group.

Table 1. Subgeneric groups within *Trillium* subgen. *Trillium*.

author	group	criteria	members
Barksdale (1938)	Erectum Group	rhombic leaves and globose, 6-angled ovaries	<i>T.cernuum</i> , <i>T.erectum</i> , <i>T.gleasoni</i> (= <i>T.flexipes</i>), <i>T.rugelii</i> , <i>T.vaseyi</i>
	Grandiflorum Group	elliptic to ovate leaves and green, 6-angled ovaries	<i>T.catesbaei</i> , <i>T.grandiflorum</i> , <i>T.pusillum</i>
	Undulatum Group	petiolate leaves and 3-angled ovary	<i>T. undulatum</i>
Gleason (1906)	Erectum Group	a sharply 6-angled ovary and recurved, subulate stigmas	<i>T.cernuum</i> , <i>T.erectum</i> , <i>T.declinatum</i> (= <i>T.flexipes</i>), <i>T.kamtschaticum</i> (= <i>T.camtschatcense</i>) , <i>T.rugelii</i> , <i>T.simile</i> , <i>T.smallii</i> , <i>T.tschonoskii</i> , <i>T.vaseyi</i>
	Grandiflorum Group	a less deeply angled 3-lobed ovary and slender, cylindrical stigmas	<i>T.govanianum</i> , <i>T.grandiflorum</i> , <i>T.nivale</i> , <i>T.ovatum</i> , <i>T.rivale</i> , <i>T.undulatum</i> , <i>T.scouleri</i> (= <i>T.ovatum</i>)
	Catesbaei Group	a slender and cylindrical stigma and style	<i>T.catesbaei</i> , <i>T.pusillum</i> , <i>T.affine</i>
Ihara and Ihara (1978, 1982)	Erectum Group	karyotype and microsporogenesis as well as the variability in pistil characters – even though that variability helps categorize this group: “Their gynoeceum forms are variable in the ovary shape as well as in the stigmatic portion, though the variability <i>per se</i> characterizes the present assemblage”	<i>T.cernuum</i> , <i>T.erectum</i> (with var. <i>simile</i> , <i>sulcatum</i> , <i>vaseyi</i>), <i>T.flexipes</i> , <i>T.rugelii</i> , <i>T.affine</i> , <i>T.latifolium</i>
	Grandiflorum Group	slender stigmas	<i>T.grandiflorum</i> , <i>T.nivale</i> , <i>T.ovatum</i> , <i>T.rivale</i> , <i>T.undulatum</i>
	Pusillum Group	a pale green ovary with a style	<i>T.catesbaei</i> , <i>T.persistens</i> , <i>T.pusillum</i>

Farmer (2000) used a phylogenetic analysis of morphological data to show a group of taxa that fit within the circumscription of *Delostylis* to be potentially distinct within *Trillium* and potentially from *Trillium* itself. Even though this name has not been widely adapted, the presence of a style, rare in *Trillium*, makes *Delostylis* an easily recognizable one.

Based on the style character, there are four species that potentially make up *Delostylis* (Table 2). *Trillium nivale* (Fig. 1a; Fig. 2a), the only member of the group not found in the southeastern United States, is primarily found in the north central United States with outlying populations in OH, PA, KY, WV and VA. Morphologically it is the most distinct of the four species. It has the shortest stem; typically it has a blue-green cast to the distinctly petioled leaves; it has the longest stigma/style ratio; and the petals are white and remain so throughout the life of the flower. *Trillium persistens* (Fig. 1b; Fig. 2b), the only species in the group that is currently listed as endangered by the US Fish and Wildlife Service (Kral 1983; USFWS 1984; 1991), is known from seven populations in and around the Tallulah Gorge in Habersham and Rabun Cos. in GA and Oconee and Stephens Cos. in SC (Patrick, pers comm.). Typically *T. persistens* has the narrowest petals of the four species; it is also one of the tallest species in the group with stems of 20-30 cm. With age, the acute, non-recurved petals turn pink as do the remaining members of *Delostylis*, but in *T. persistens*, the petal bases remain white. *Trillium catesbaei* (Fig. 1c; Fig. 2c) is found primarily in the southern Appalachians and the Piedmont of the southeastern US. Distinctive with its nodding flowers, the petals are also recurved as are the bright yellow stamens. In addition to the petals aging to pink, the petals are sometimes hot pink in color even at the time that the flower first opens. *Trillium pusillum* (Fig. 1d; Fig. 2d) as circumscribed prior to the current study has the most scattered distribution within *Delostylis*; and a number of the widely isolated, geographically defined, and ecologically distinct populational aggregates have been proposed for varietal or even species status. The taxonomic history of *T. pusillum* is further described in section 1.3. Prior to this study, considerable variability has been observed within *T. pusillum*, ranging from diminutive plants similar in stature to those of *T. nivale* to large plants of similar size to *T. catesbaei* and *T. persistens*. The sepals and petals are similar to each other in size and shape, unlike the other members of the group. The range of variation coupled with the confusing taxonomy has made the study of this species one of the primary goals of this study.

1.3 Taxonomic History of *Trillium pusillum*

Gronovius in 1742 was the first to describe a member of the *Trillium pusillum* complex with the name "*Paris foliis ternis subovatis obtusis: flore sessili, erecto, univo, foliis dimidium minore.*" (Paris, foliage in three, subovate, obtuse; flower sessile, erect, solitary, half the size of the leaves) based on Clayton n. 536 (Fig. 3). This specimen was determined to be *T. pusillum* by Rendle in 1901 and more accurately to be *T. pusillum* var. *virginianum* by Fernald in 1943. Because the date of this protolog is

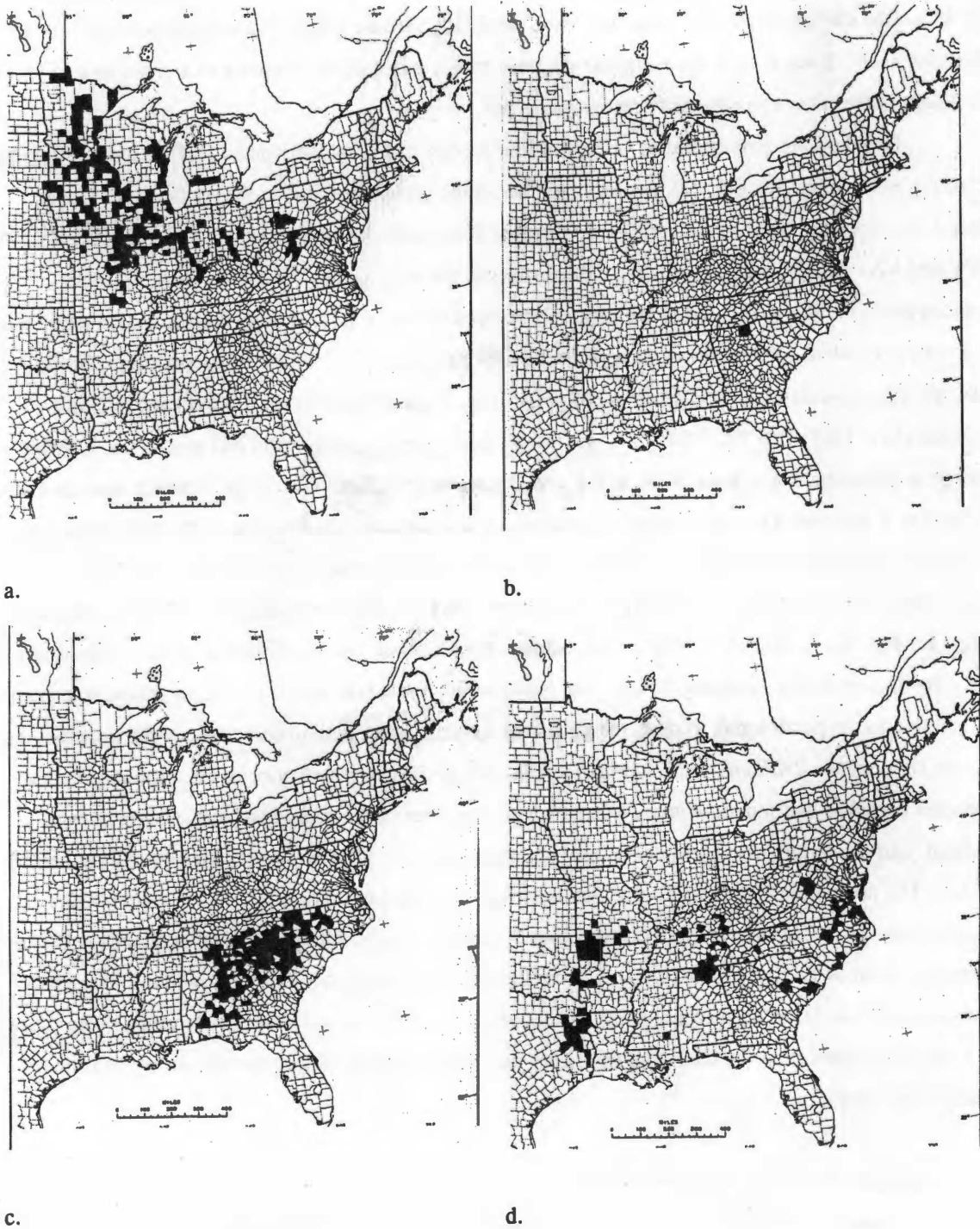


Figure 2. County-level distribution maps for putative members of *Delostylis*. a. *Trillium nivale*. b. *T. persistens*. c. *T. catesbaei* d. *T. pusillum s.l.*

Table 2. Character differences between putative members of *Delostylis*. Diagnostic characters are **bolded**.

	<i>T. catesbaei</i>	<i>T. nivale</i>	<i>T. persistens</i>	<i>T. pusillum</i>
stem	20-45 cm	3-5 cm at anthesis to 4.5-8 cm at fruit maturity	20-30 cm	6-28 cm
leaves	[sessile to] petioled	distinctly petioled	sessile	petiolate, subsessile or sessile
	green often with purplish-maroon undertones	bluish-green	green with maroon undertones	dark green with maroon undertones
	65-150 x 40-80 mm	15-45 x 7-34 mm veins and margins "clear"	31-95 x 16-33 mm	25-80 x 10-30 mm
pedicel	20-40 (-50) mm; deflexed or recurved, angled	5-20 mm; recurving in fruit	12-34 mm; arched to nearly erect	0-20 mm erect to nearly erect
sepals	reflexed or curled	spreading, flat,	spreading	in same plane with petals
petals	white or pink aging to pink or rose	white; typically remaining so	white fading to deep pink	white aging to deep rosy pink
	35-50 x 10-20 mm	13-35 x 8-15 mm	20-35 x 5-10 mm	15-30 x 5-15 mm
pistil	deeply 6-angled ovary; inconspicuous, angular-ovoid, style 2-6 mm stigma 4-10 mm	round, 3-lobed ovary; style 0.5-1.5 mm stigma 4-12 mm +	sharply 6-angled ovary; style 2-6 mm stigma 3-7.5 mm	obscurely 6-angled ovary; conspicuous style 2.5-8 mm stigmas 3-12 mm
stamens	recurved 16-26 mm long, anthers 5-14 mm filaments 11-12 mm	straight 5-18 mm anther 2.5-11 mm filament 2.5-7 mm	erect to divergent 9.5-14 mm anther 5.6-7.2 mm filaments 4.8-6 mm	erect-spreading 8-10 mm anther 2.5-11 filaments 2.5-8 mm
habitat	Acid soils, open dry or rich woods.	limestone-derived soils	humus soils in mixed deciduous-pine woodlands; rhododendron thickets	low swampy woodlands, spring-wet floodplains, upland rocky slopes
flowering	late Mar. - early June	early Mar. - early Apr.	early Mar. - mid Apr.	late Feb. to early May

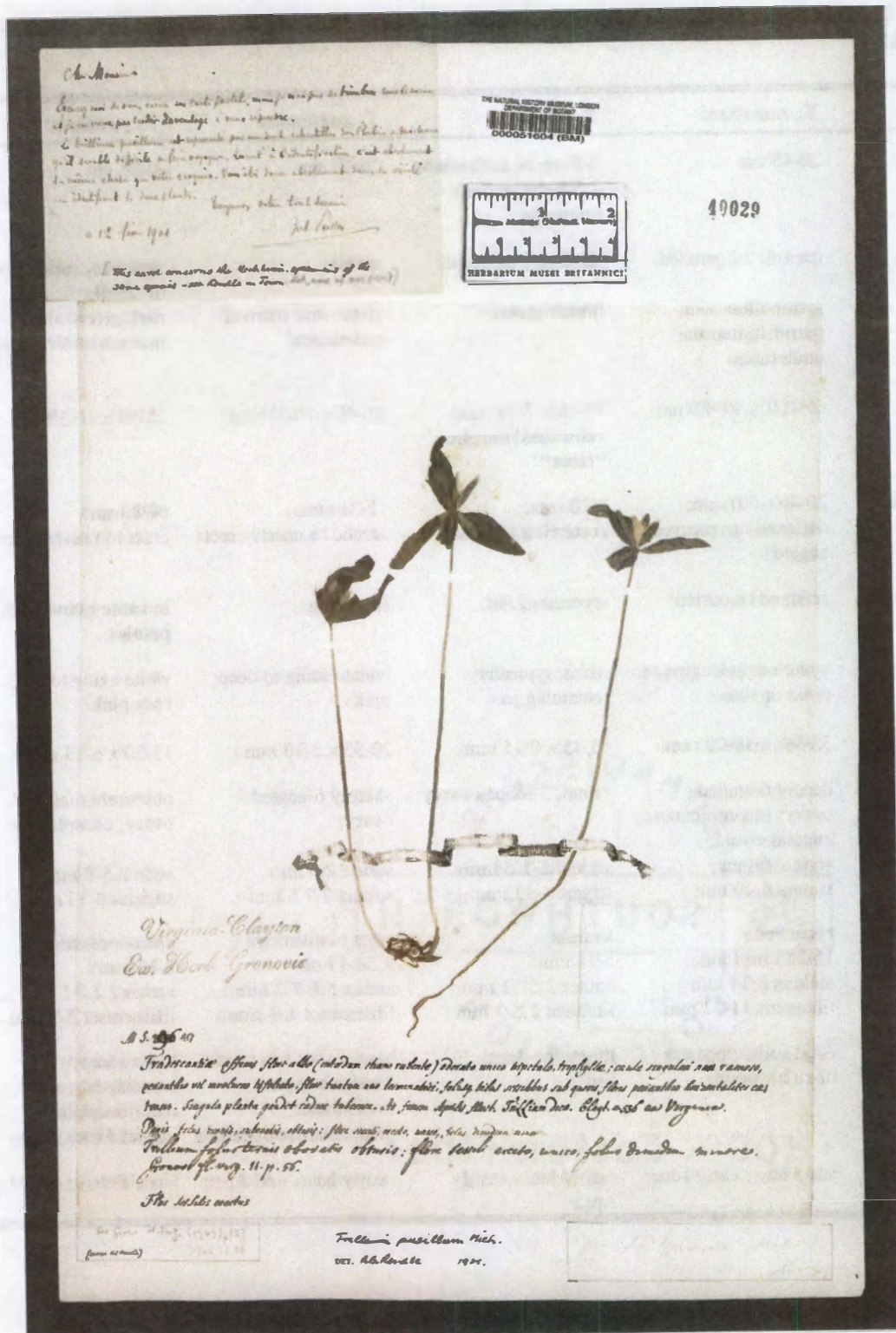


Figure 3. The earliest known specimen of *Trillium pusillum*, from the Clayton Herbarium.

before the “start date” of 1753 for botanical nomenclature, it is of historical interest only. However in 1762, Gronovius transferred the species from *Paris* to *Trillium* with the sentence epithet remaining the same, resulting in an invalid name under the ICBN (art. 23.1; Greuter et al. 2000).

Michaux in 1803 proposed the first valid name for *Trillium pusillum* from the pine woods of lower Carolina (Fig. 4). It appears that the publication of *T. pumillum* by Pursh in 1814 is an unfortunate typographical error as his description closely mimics that of Michaux (albeit slightly rearranged).

Rafinesque noted the range of *T. pusillum* as from Pennsylvania to Arkansas in 1830, and included western Kentucky in 1840. Prior to this study, *T. pusillum* could be considered to have one of the widest ranges of any of the southeastern *Trillium* species (Fig. 2d) from Texas north to Arkansas to Maryland; yet within this range, populations are scattered. It is locally abundant at some sites, but is considered rare in others.

In 1860, Buckley extended the known range of the Pusillum Complex by describing *Trillium texanum* from Panola Co., Texas. For the next 75 years, most botanists included *T. texanum* in *T. pusillum* (Watson 1879, Gleason 1906, Gates 1917, and Peattie 1927), but other botanists (Rendle 1901, Small 1913, 1933) made no mention of either *T. texanum* or included Texas in their range of *T. pusillum*. In 1977, the range of *T. texanum* was extended to Louisiana by a find in Caddo Parish (MacRoberts 1977). Palmer & Steyermark in 1935 were the first to use *T. texanum* as distinct from *T. pusillum* since its proposal by Buckley; but in 1981, Reveal & Broome officially made it a variety of *T. pusillum* (as did Reed in 1982, evidently unaware of the Reveal & Broome publication). Over the last 75 years, *T. texanum* was variously recognized as a species distinct from *T. pusillum* (Freeman 1970; Case 1981; and Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002b), as *T. pusillum* var. *texanum* (Bodkin & Reveal 1982, 1983; Garrett 1982; Mitchell 1990; Freeman undated, 1994, 1995; Case & Case 1997; Jacobs & Jacobs 1997; Singhurst et al. 2002), or as synonymous with *T. pusillum* (Barksdale 1938; Ihara & Ihara 1978; Samejima & Samejima 1987; Henderson 2000; Case 2002; Timmerman-Erskine et al. 2002a, 2003).

Rafinesque (1830) was the first to note that the range of *T. pusillum* extended to Arkansas, but it was not until 1935 that this taxon was described as *T. ozarkanum* by Palmer & Steyermark. Barksdale (1938) included it within *T. pusillum* as did Fernald (1950). *Trillium ozarkanum* was not recognized as distinct again until Steyermark (1960) made it a variety in preparation for the Flora of Missouri. The range of *T. ozarkanum* was extended to Kentucky (as *T. pusillum* var. *ozarkanum*) by Browne (1961). Since that time, *T. ozarkanum* has been recognized as distinct from *T. pusillum* at the species level by Case (1981), as *T. pusillum* var. *ozarkanum* (Brown, 1961; Reed 1962; Steyermark 1963; Freeman 1970; Roe 1978; Reveal & Broome 1981; Garrett 1982; Reed 1982; Bodkin & Reveal 1983; Mitchell 1990; Medley 1991; Freeman undated, 1994, 1995; Case & Case 1997; Jacobs & Jacobs 1997; Henderson 2000), or as synonymous with *T. pusillum* (Gleason & Cronquist 1963; Ihara & Ihara 1978; Samejima &

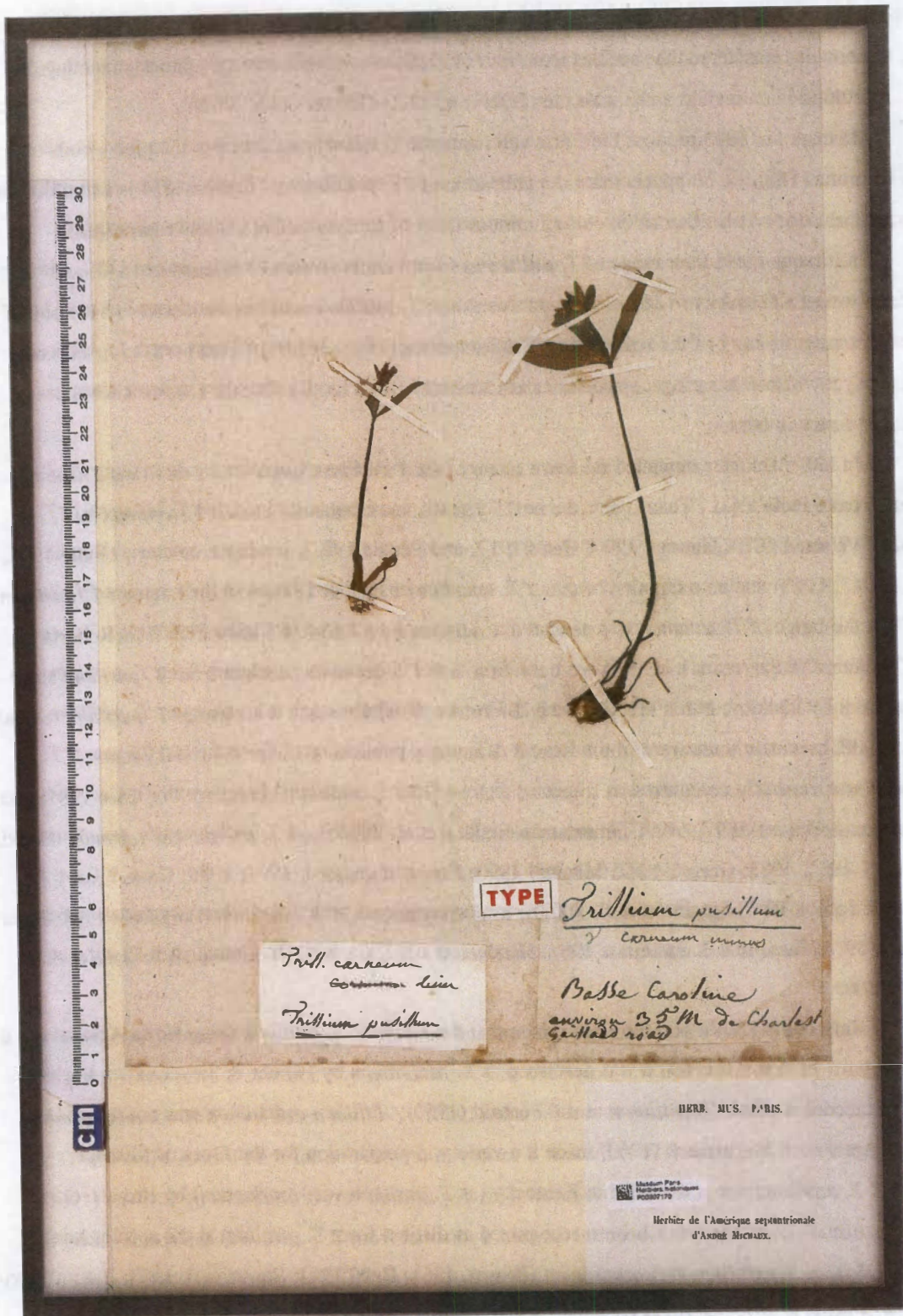


Figure 4. Type specimen of *Trillium pusillum* Michx.

Samejima 1987; Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a, 2002b, 2003; Case 2002).

Fernald was the first to describe an actual variety of *T. pusillum* when he described *T. pusillum* var. *virginianum* in 1943 after having earlier determined the plants to be specimens of *T. lanceolatum* (sic) or *T. sessile* (Norton & Brown 1946); the other taxa associated with *T. pusillum* were first described as species. Wherry (1949) extended its range to Maryland; with the publication of *T. virginianum*, Reed (1982) extended the range to North Carolina. In the interim, the varietal name was accepted by Wherry (1949), Fernald (1950), Reed (1956), Steyermark (1963), Roe (1978), Case (1981), Reveal & Broome (1981), and Garrett (1982); but not by Gleason & Cronquist (1963), or Ihara & Ihara (1978). Despite Reed's publication as a species (1982), subsequent botanists either retained varietal status, including Bodkin & Reveal (1983), Mitchell (1990), Freeman (undated, 1994, 1995), Cabe (1995), Cabe & Werth (1995), Case & Case (1997), Jacobs & Jacobs (1997), Case (2002); or included it within a broader *T. pusillum*, including Samejima & Samejima (1987), Timmerman-Erskine (1997, 1999); Timmerman-Erskine et al. (2002a, 2002b, 2003), Henderson (2000), and Hill (2005).

The last variety to be validly described, *T. pusillum* var. *monticola*, was described by Bodkin & Reveal in 1982 from the West Virginia/Virginia mountains. Originally published as variety *monticulum*, the orthography has been corrected to *monticola* (Greuter et al. 2000). Until 1997, Freeman in his undated proposal, the preliminary status report (1994), and a survey of *Trillium* in the southeastern US (1995) was the only taxonomist to recognize this variety. In 1995, Cabe published the results of a morphological and isozymatic study in an attempt to determine if the two sessile-flowered varieties were taxonomically "good" and found insufficient evidence to recognize var. *monticola* as distinct from var. *virginianum*. Since that date, most treatments have considered *T. pusillum* var. *monticola* to be included within *T. pusillum* (Samejima & Samejima 1987; Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a, 2002b, 2003; Henderson 2000; Hill 2005); included within *T. pusillum* var. *virginianum* (Mitchell 1990; Jacobs & Jacobs 1997; Case 2002), or as a distinct variety (Case & Case 1997).

Trillium pusillum var. *alabamicum* was published in a thesis by Garrett in 1982 which is not considered valid publication under the ICBN (Art. 29.1; Greuter et al. 2000); formal publication of the name is in preparation (Garrett, pers. comm.). Despite its invalid nomenclatural status, this name has been used by Freeman (undated, 1994, 1995), Case & Case (1997), and Jacobs & Jacobs (1997).

Trillium pusillum vars. *georgianum* and *kentuckianum* were used by Jacobs & Jacobs (1997) to describe plants in a spring-wet floodplain in Whitfield Co., GA, and upland populations in KY and TN respectively. As these names were published without a Latin diagnosis or description, and without declaring type specimens, neither of these names has any nomenclatural standing.

Even though *T. pusillum* does not have as many described subspecific taxa as some others in the genus (e.g., *T. erectum* with 39 named varieties and forms, or *T. grandiflorum* with 33) its

nomenclatural/taxonomic history is almost as long and certainly as confusing as any. Table 3 contains the publication data for each of the associated taxa; Tables 4-7 summarize the differing treatments within the species. Of the four validly published taxa associated with *T. pusillum* (originally published as *T. texanum* Reveal & C.R. Broome [Table 4], *T. ozarkanum* Steyerm. [Table 5], *T. pusillum* var. *virginianum* Fernald [Table 6], and *T. pusillum* var. *monticola* N.L. Bodkin & J.L. Reveal [Table 7]), one (*T. virginianum*) has been elevated to species status, and two (*T. pusillum* vars. *ozarkanum* and *texanum*) have been reduced to varietal rank at one or more times. In addition, there are three more invalidly published names (var. *alabamicum* Garrett, var. *georgianum* Jacobs & Jacobs, and var. *kentuckianum* Jacobs & Jacobs).

1.4 Goals of this Project.

Three specific questions were addressed in this study. The first question concerned the monophyly of *Delostylis*. As described by Rafinesque (1819), this group is characterized by a common style formed by basal fusion of the stigmatic branches, unlike the other *Trillium* species in which the stigmatic branches are sessile and sit directly on the ovary. Does the presence of this character delineate a natural and monophyletic group, or is this a homoplasious trait? Phylogenetic analysis of DNA sequence data was used to evaluate the monophyly of the group.

The second question concerned the phylogenetic placement of *Delostylis*. Is the basal position suggested by a previous morphological analysis (Farmer 2000; Farmer & Schilling 2002) an accurate representation of the phylogenetic placement of the group in relation to *Trillium* or might it be an artifact of long-branch attraction? Phylogenetic analysis of DNA sequence data was used to address the placement of the group.

The third question concerned the species-level taxonomy of the *Pusillum* Complex (e.g., *T. pusillum* and closely related taxa). Is this best regarded as a single species with multiple subspecific taxa, a small group of species each with two or more varieties, or is this a larger group of closely related species each of which is distinct? Traits that were examined to address this question included molecular evidence, geographic distribution, physiography, habitat preference, and morphology.

Table 3. Taxonomic and nomenclatural history for *Trillium pusillum* and varieties.

authority	year	name	action	type
Gronovius	1743	<i>Paris foliis ternis subovatis obtusis : flore sessili, erecto, unico, foliis dimidium minore</i>	sp. nov. (ined.)	BM
Gronovius	1762	<i>Trillium foliis ternis subovatis obtusis : flore sessili, erecto, unico, foliis dimidium minore</i>	comb. nov. (ined.)	
Michaux	1803	<i>Trillium pusillum</i>	sp. nov.	P
Pursh	1814	<i>T. pumilum</i>	sp. nov.	unknown
Buckley	1860	<i>T. texanum</i>	sp. nov.	here designated
Palmer & Steyermark	1935	<i>T. ozarkanum</i>	sp. nov.	MO
Fernald	1943	<i>T. pusillum</i> var. <i>virginianum</i>	var. nov.	GH
Steyermark	1960	<i>T. pusillum</i> var. <i>ozarkanum</i>	stat. nov.	
Reveal & Broome	1981	<i>T. pusillum</i> var. <i>texanum</i>	stat. nov.	
Reed	1982	<i>T. pusillum</i> var. <i>texanum</i> ; <i>T. virginianum</i>	stat. nov. stat. nov.	
Bodkin & Reveal	1982	<i>T. pusillum</i> var. <i>monticola</i>	var. nov	MARY
Garrett	1982	<i>T. pusillum</i> var. <i>alabamicum</i>	var. nov. (ined.)	AUA ¹
Jacobs & Jacobs	1997	<i>T. pusillum</i> var. <i>georgianum</i> , <i>T. pusillum</i> var. <i>kentuckianum</i>	var. nov. (ined.) var. nov. (ined.)	none none

1. The name *T. pusillum* var. *alabamicum* was published in a thesis, and not validly published under the ICBN. However, all other requirements of the code were met including the designation of type specimens.

Table 4. Taxonomic treatments for entities included within *Trillium pusillum: texanum*. The boldfaced reference indicates the first publication at that rank.

as <i>T. pusillum</i> s.l.	as <i>T. pusillum</i> var. <i>texanum</i>	as <i>T. texanum</i>
		Buckley 1860
Watson 1879		
Gleason 1906		
Gates 1917		
Peattie 1927		
		Palmer & Steyermark 1935
Barksdale 1938		
		Freeman 1970
Ihara & Ihara 1978		
	Reveal & Broome 1981	Case 1981
	Reed 1982	
	Bodkin & Reveal 1982	
	Garrett 1982	
	Bodkin & Reveal 1983	
Samejima & Samejima 1987		
	Mitchell 1990	
	Freeman undated	
	Freeman 1994	
	Freeman 1995	
	Case & Case 1997	Timmerman-Erskine 1997
	Jacobs & Jacobs 1997	
		Timmerman-Erskine 1999
Henderson 2000		
Case 2002	Singhurst et al. 2002	Timmerman-Erskine 2002b
Timmerman-Erskine 2002a		
Timmerman-Erskine 2003		

Table 5. Taxonomic treatments for entities included within *Trillium pusillum*: *ozarkanum*. The boldfaced reference indicates the first publication at that rank.

as <i>T. pusillum</i> s.l.	as <i>T. pusillum</i> var. <i>ozarkanum</i>	as <i>T. ozarkanum</i>
		Palmer & Steyermark 1935
Barksdale 1938		
Fernald 1950		
	Steyermark 1960	
	Reed 1962	
Gleason & Cronquist 1963	Steyermark 1963	
	Freeman 1970	
Ihara & Ihara 1978	Roe 1978	
		Case 1981
	Reed 1982	
	Garrett 1982	
	Bodkin & Reveal 1983	
Samejima & Samejima 1987		
	Mitchell 1990	
	Medley 1991	
	Freeman undated	
	Freeman 1994	
	Freeman 1995	
Timmerman-Erskine 1997	Case & Case 1997	
	Jacobs & Jacobs 1997	
Timmerman-Erskine 1999		
	Henderson 2000	
Timmerman-Erskine 2002a		
Timmerman-Erskine 2002b		
Case 2002		
Timmerman-Erskine 2003		

Table 6. Taxonomic treatments for entities included within *Trillium pusillum*: *virginianum*. The boldfaced reference indicates the first publication at that rank.

as <i>T. pusillum</i> s.l.	as <i>T. pusillum</i> var. <i>virginianum</i>	as <i>T. virginianum</i>
	Fernald 1943	
	Wherry 1949	
	Fernald 1950	
	Reed 1956	
Gleason & Cronquist 1963	Steyermark 1963	
	Roe 1978	
	Case 1981	
	Reveal & Broome 1981	
	Garrett 1982	Reed 1982
	Bodkin & Reveal 1983	
Samejima & Samejima 1987		
	Mitchell 1990	
	Freeman undated	
	Freeman 1994	
	Freeman 1995	
	Cabe 1995	
	Cabe & Werth 1995	
Timmerman-Erskine 1997	Case & Case 1997	
	Jacobs & Jacobs 1997	
Timmerman-Erskine 1999		
Timmerman-Erskine 2002a	Case 2002	
Timmerman-Erskine 2002b		
Hill 2005		

Table 7. Taxonomic treatments for entities included within *Trillium pusillum*: *monticola*. The boldfaced reference indicates the first publication at that rank.

as <i>T. pusillum</i> s.l.	as <i>T. pusillum</i> var. <i>monticola</i>	as <i>T. pusillum</i> var. <i>virginianum</i>
	Bodkin & Reveal 1982	
Samejima & Samejima 1987		Mitchell 1990 ¹
	Freeman undated	
	Freeman 1994	
	Freeman 1995	Cabe 1995
		Cabe & Werth 1995
Timmerman-Erskine 1997	Case & Case 1997	Jacobs & Jacobs 1997
Timmerman-Erskine 1999		
Henderson 2000		
Timmerman-Erskine 2002a		Case 2002
Timmerman-Erskine 2002b		
Hill 2005		

¹ Mitchell discusses var. *monticola* in the text, but does not include it in his list of varieties.

2.0 MATERIALS AND METHODS

2.1 Sampling Strategy

2.1.1 Taxon sampling.— Because of the taxonomic quagmire surrounding *T. pusillum*, taxonomy at the outset follows that of Garrett (1982) in which several sub-specific taxa were recognized rather than the single taxon of Timmerman-Erskine (1997, 1999). I selected these more finely drawn taxonomic boundaries to help ensure adequate sampling.

Samples from each of the species putatively included in *Delostylis* were included in the study. Initially, only one accession each of *T. nivale* and *T. persistens* was sampled because of their apparent lack of morphological variation; *Trillium catesbaei* was sampled from north Georgia and south Alabama. As the project progressed, additional samples of *T. nivale*, *T. persistens*, and *T. catesbaei* were included in the analysis including two morphologically distinct specimens of *T. catesbaei*.

There are eight previously described taxa (five validly published) that collectively comprise *Trillium pusillum*: *pusillum*, *texanum*, *ozarkanum*, *virginianum*, *monticola*, *alabamicum*, *kentuckiense* and *georgianum* each occurring in widely scattered populations from Texas to Maryland (Fig. 5; see also section 1.2). Because of the nature of the distribution of *T. pusillum*, (widely isolated, geographically defined, and ecologically distinct populations), it was essential to adequately define the limits of variation. To ensure that any variation seen in the DNA sequences was taxonomically or phylogenetically significant, rather than populational or individual variation, wide sampling was required. As each of the varieties accepted in Garrett (1982) spanned more than one state, I decided that, rather than attempt to sample each discrete population, a sample would be taken from each taxon/state pair (e.g., *T. pusillum* var. *virginianum* from Maryland, Virginia, and North Carolina), disjunct population (e.g., Grayson Co., VA) or populations of questionable identity (e.g., Cumberland Co., TN or Wake Co., NC) for DNA analysis. The only such population that was not sampled was the population of *T. pusillum* var. *ozarkanum* occurring in Oklahoma. Taxa sampled, their *a priori* and *post priori* identities, and their locales are given in Table 8.

For the morphological analysis, an herbarium sheet from each vouchered county was selected for measurements. All plants on a given sheet were measured. Taxa sampled, their identities, their locales, and the herbarium designation are given in Table 9.

2.1.2 Molecular markers.— Phylogenetic analysis of DNA sequence data was used to assess the phylogenetic placement and monophyly of *Delostylis*. In addition, insights into the taxonomic composition of the Pusillum Complex would be gained by these analyses.

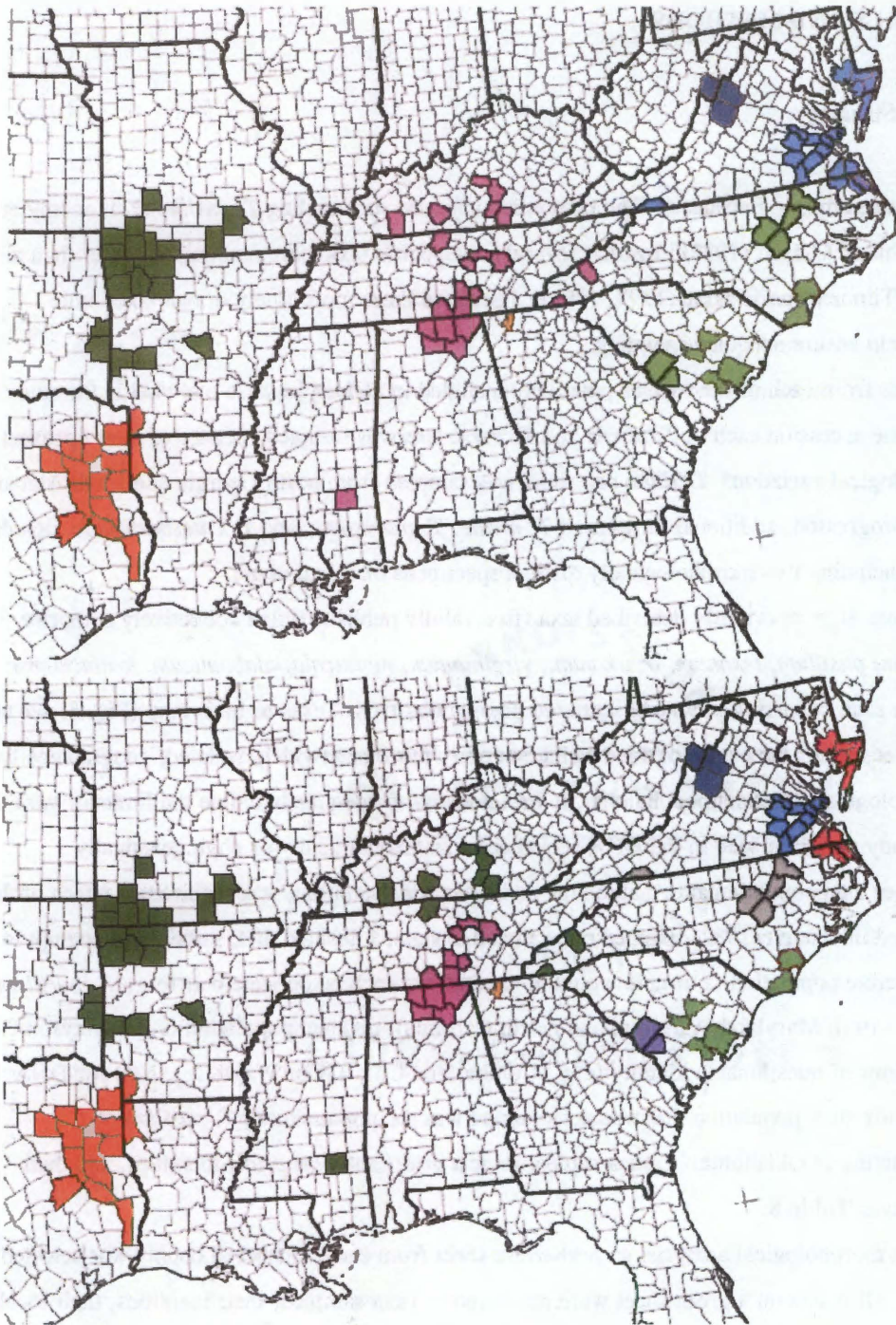


Figure 5. County-level distribution maps for members of the Pusillum Complex at the beginning and at the conclusion of this study. Top after Garrett (1982); dark green: *T. ozarkanum*, orange: *T. texanum*, lavender: *T. alabamicum*, gold: *T. georgianum*, light green: *T. pusillum*, blue: *T. virginianum*, dark blue-purple: *T. monticola*. Bottom this study; in addition to the above colors, purple: *T. telmacola*, grey: *T. carolinianum*, red: *T. palustris*.

Table 8. Taxon sampling for the molecular analyses of *Delostylis*. Populations will be vouchered at TENN and sequences will be deposited with GenBank at the completion of the project. LMC = leaf material collected; PV = population vouchered.

Taxon	Locality	Voucher Information	Sample	region
<i>Pseudotrillium rivale</i> (S. Wats.) S.B. Farmer	OR, Polk Co.-in cultivation	Graham s.n. April 2001 Graham s.n. April 2006	792 2319	ITS ITS
<i>T. alabamicum</i> stat. nov.	AL, Limestone Co. TN, Lincoln Co.	Farmer et al. s.n. 13 Mar 2002 LMC: Farmer & Simmons s.n. 18 April 2001; PV: Patrick & Simmons 1113. 29 April 1980 TENN	793 735	ITS ITS
	TN, Cumberland Co.	Wofford & Ma. 86-44. 8 May 1986 TENN	817 2161	ITS ITS
<i>T. carolinianum</i> noni. nov. (<i>T. virginianum</i>)	NC, Nash, Co. NC, Wake Co. VA, Grayson Co.	Farmer & Rhinehart s.n. 10 April 2005 Farmer et al. s.n. 3 Mar 2005 Farmer et al. s.n. 3 Mar 2005	2134 2114 2108	 ITS
<i>T. catesbaei</i> Elliott	AL, Escambia Co. AL, DeKalb Co.; cultivated TN, Knox Co.	Farmer & Wieboldt s.n. 16 May 2002 Farmer & Searcy s.n. 19 Mar 2002, Farmer & Searcy s.n. 19 Mar 2002 LMC: Farmer & Walker s.n. 14 May 1996; PV: Sherman 227. 10 May 1959 TENN	783 780 781 807	ITS ITS ITS ITS
	GA, Whitfield Co. NC, Franklin Co. SC, Jones Gap State Park	Jones s.n. 11 April 2001 Farmer s.n. April 2005 Farmer s.n. 21 April 2006	808 2135 2331	ITS ITS ITS
<i>T. cuneatum</i> Raf. <i>T. foetidissimum</i> J.D. Freeman <i>T. georgianum</i> nom. nov.	TN, Knox Co. GA, in cultivation from St. Francisville LA GA, Whitfield Co.	Farmer s.n. 5 April 2001 Mellard s.n. 22 Mar 2001 Farmer & Noble s.n. 11 April 2001	802 748 736	ITS ITS ITS
<i>T. grandiflorum</i> (Michx.) Salisb. <i>T. lancifolium</i> Raf. <i>T. ludovicianum</i> Harbison	GA, Whitfield Co. VA, Wise Co. GA, in cultivation from FL GA, in cultivation from LeCompte LA GA, in cultivation from Leesville LA	Farmer s.n. 5 April 2002 Farmer s.n. 2 May 1996 Mellard s.n. 22 Mar 2001 Mellard s.n. 22 Mar 2001 Mellard s.n. 22 Mar 2001	809 812 2113 747 749	ITS ITS ITS ITS ITS
<i>T. luteum</i> (Muhl.) Harbison <i>T. maculatum</i> Raf. <i>T. monticola</i> Bodkin & Reveal <i>T. nivale</i> Riddell	TN, Cumberland Co. GA, in cultivation from Summerville SC VA, Rockingham Co. IN, Putnam Co. PA, Westmoreland Co.	Farmer & Rhinehart s.n. 10 Apr 2005 Mellard s.n. 22 Mar 2001 Farmer & Clevinger s.n. Apr. 27, 2002 Steven s.n. Apr. 13, 2003 Kluse s.n. 30 Mar 2006 Gund s.n. 6 April 2006	2139 746 785 789 2290 2330	 ITS ITS ITS ITS ITS

Table 8. continued.

Taxon	Locality	Voucher Information	Sample	region
<i>T. ovatum</i> Pursh var. <i>ovatum</i>	OR, Washington Co.; cultivated TN, Knox Co.	<i>Farmer s.n.</i> 7 April 2002	779	ITS <i>matK</i> <i>psbA-trnH</i>
	Canada: British Columbia, Westbank	<i>Karstad s.n.</i> May 2002	799	ITS <i>matK</i> <i>psbA-trnH</i>
	OR, Polk Co.-cultivated	<i>Graham s.n.</i> April 2001	801	ITS <i>matK</i> <i>psbA-trnH</i>
	CA, Humboldt Co.	<i>Lee 19.</i> 13 April 2004 HSC	2119	ITS <i>psbA-trnH</i>
	WA, Skagit Co.	<i>Legler 288.</i> 29 April 2003 WTU	2120	ITS <i>psbA-trnH</i>
(= <i>T. scouleri</i> Ryd. in Gleason)	MT, Flathead Co.	<i>Ream s.n.</i> 25 May 2005	2133	ITS <i>psbA-trnH</i>
(= <i>T. scouleri</i> Ryd. in Gleason)	CO, Routt Co.	<i>Proctor s.n.</i> 13 April 2004	2137	<i>psbA-trnH</i>
<i>T. ovatum</i> var. <i>oettingeri</i> Munz & Thorne	CA, Trinity Co.	<i>Ferlatte 2101.</i> 28 June 1983 HSC	2117	<i>psbA-trnH</i>
<i>T. ovatum</i> var. <i>hibbersonii</i>	Canada: Vancouver-cultivated	LMC: <i>Gundlandsen s.n.</i> April 2001	796	ITS <i>matK</i> <i>psbA-trnH</i>
(T.M.C. Taylor & Szczaw.)	Canada: Vancouver-cultivated	LMC: <i>Thimble Farms s.n.</i> May 2001	800	ITS <i>matK</i> <i>psbA-trnH</i>
G.W. Douglas & P. Pojar	Canada: Vancouver Island	<i>Cesca et al. 5109.</i> 10 May 1981 UBC	2116	<i>psbA-trnH</i>
<i>T. ozarkanum</i> E.J. Palmer & Steyermark	AR, Montgomery Co.	<i>Farmer & Whitsell s.n.</i> 22 Mar 2002	791	ITS <i>matK</i> <i>psbA-trnH</i>
	KY, Casey Co.	<i>Farmer & Estes s.n.</i> 31 May 2005	2112	<i>psbA-trnH</i>
	KY, Russell Co.	<i>Farmer & Estes s.n.</i> 31 May 2005	2132	<i>psbA-trnH</i>
	MO, Barry Co.	<i>Hollis s.n.</i> 27 June 2001	734	ITS <i>matK</i> <i>psbA-trnH</i>
	MS, Jones Co.	LMC: <i>Farmer & Wieland s.n.</i> 20 Mar 2002; PV: <i>Morgan 468</i> 9 May 1978 MNHP	790	ITS <i>matK</i> <i>psbA-trnH</i>
		LMC: <i>Farmer & Wieland s.n.</i> 20 Mar 2002	2141	ITS <i>psbA-trnH</i>
	NC, Clay Co.	<i>Freeman et al. s.n.</i> 16 April 1988 AUA	2115	<i>psbA-trnH</i>
<i>T. palustris</i> nom. nov.	MD, Worcester Co.	<i>Frye s.n.</i> 26 April 2002	811	ITS <i>matK</i> <i>psbA-trnH</i>
<i>T. persistens</i> Duncan	GA, Habersham Co.	<i>Patrick s.n.</i> April 2000	806	ITS <i>matK</i> <i>psbA-trnH</i>
	in cultivation at UGA Botanical Garden	<i>Chafin s.n.</i> 20 May 2005	2107	ITS <i>matK</i> <i>psbA-trnH</i>
<i>T. pusillum</i> Michx.	NC, Brunswick Co.	<i>Galloway s.n.</i> 4 April 2005	2110	<i>psbA-trnH</i>
	SC, Dorchester Co.	LMC: <i>Brunswick s.n.</i> 25 May 05; PV: <i>Porcher 1246C</i> 11 Mar 1977 CITA	2106	ITS <i>matK</i> <i>psbA-trnH</i>
<i>T. recurvatum</i> Beck	TN, Cumberland Co.	<i>Farmer & Rhinehart s.n.</i> 10 April 2005	2111	<i>psbA-trnH</i>
<i>T. species 1</i>	TN, Hamilton Co.	<i>Farmer & Mellard s.n.</i> 29 Mar 2001	750	ITS <i>matK</i> <i>psbA-trnH</i>
<i>T. species 2</i>	SC, Kershaw Co.	<i>Farmer & Gaddy s.n.</i> 1 April 2005	2109	<i>psbA-trnH</i>
<i>T. telmacola</i> nom. nov.	SC, Aiken Co.	<i>McMillan & Porcher 2241.</i> 12 April 1997	816	ITS <i>matK</i> <i>psbA-trnH</i>
(<i>T. pusillum</i>)		USCH		
<i>T. texanum</i> Buckley	LA, Caddo Parish	<i>Reynolds LRR1950.</i> 16 April 2002	778	ITS <i>matK</i> <i>psbA-trnH</i>
	TX, Cass Co.	LMC: <i>Singhurst s.n.</i> 22 Mar 2002; PV: <i>Correll 36935</i> 9 April 1969 NCU	786	<i>psbA-trnH</i>
	TX, Smith Co.	<i>Farmer et al. s.n.</i> 22 Mar 2002	794	ITS <i>matK</i> <i>psbA-trnH</i>

Table 8. continued.

Taxon	Locality	Voucher Information	Sample	region	
<i>T. undulatum</i> Willd.	VA, Rockingham Co.	Farmer & Clevinger s.n. 27 April 2002	784	ITS	matK psbA-trnH
<i>T. virginianum</i> (Fernald) Reed	VA, James City Co.	LMC: Ware & Grubbs s.n. 16 May 2002; PV: Ware & Ware 7322 8 April 1979 VPI	782	ITS	matK psbA-trnH
<i>T. viridescens</i> Nutt.	MO, Barry Co.	Hollis s.n. 27 June 2001	810	ITS	matK psbA-trnH
			2105	ITS	matK psbA-trnH

Table 9. Reported county-level distribution and taxon sampling of *Trillium pusillum* for the morphological analyses (adapted from Freeman [undated]). The DNA sample was not necessarily taken from the same specimen that was measured for the morphological analysis. Acronyms in bold indicate specimens borrowed.

Taxon	state	county	sheet ref. no.	herbarium information (after Freeman 1994)	DNA sample #
<i>alabamicum</i>	AL	Jackson Co.	0067	AUA, TENN, TROY, VDB	793
		Limestone Co.	0068	AUA, JSU, TENN, TROY, UNA, VDB	
		Madison Co.	0072	AUA, GA, GH, JSU, MO, NY, PH, SMU, TROY, UNA, VDB	
	TN	Marshall Co.	0074	AUA, JSU, TROY, VDB	817, 2134
		Morgan Co.	0986	AUA, TENN, TROY, VDB	
		Coffee Co.	1626	TENN, UOS, VDB	
		Cumberland Co.	2499	AUA, NY, TENN, VDB	
		DeKalb Co.		TNHP; population not vouchered	
		Franklin Co.		TENN-photo, UOS	
		Grundy Co.		UOS	
		Lincoln Co.	1646	AUA, TENN, VDB	
		Putnam Co.	0299	TENN	735
<i>carolinianum</i>	NC	Halifax Co.		DUKE	2114
		Johnston Co.		NCNHP; population not vouchered	
		Nash Co.	2500	NCSC; TENN	
	VA	Wake Co.	1989	DUKE, GA, GH, MO, TENN	2108
		Grayson Co.	1243	AUA, TENN, VPI	740, 783
<i>georgianum</i>	GA	Whitfield Co.	1120	GA, TENN, USCH	736, 809
<i>monticola</i>	VA	Augusta Co.	1295	JMU, VPI, WILLI	785
		Rockingham Co.	1272	CAS, GH, JMU, MARY, NY, TENN, US, VPI, WILLI, WVA	
	WV	Pendleton Co.	1155	AUA, JMU, TENN, WILLI	
<i>ozarkanum</i>	AR	Benton Co.	1607	APCR, NLU, NY, TEX, UARK, US, VDB, VPI, WIS	791
		Boone Co.	1156	AUA, UARK	
		Carroll Co.	1157	AUA, UARK	
		Madison Co.	0100	GH, KANU, MO, NCU, NY, SMSU, UARK, UMO, US, VDB, WILLI	
		Montgomery Co.	1115	CM. GA, TENN, UARK	
		Newton Co.	1161	APCR, AUA, CLEMS, CM, DHL, FLAS, FSU, GA, GEO, GH, KE, NCU, RSA, SMS, SMSU, SMU, TENN, TROY, UARK, UMO, UNC, US, VDB, WIS, WVA	
		Polk Co.	1575	ASTC, AUA, BRIT, CM, UARK	
		Pulaski Co.	2502	ASTC	
		Washington Co.	0250	MO, NY, OKL, OKLA, P, PENN, SMU, UARK, UMO, VDB	
	KY	Casey Co.	1252	EKY, KY, NCU	2112
		Christian Co.	1142	ASPC, AUA, VDB	
		Marion Co.	0301	EKY, KY, TENN	
		Russell Co.	2501	EKY, TENN	
		Warren Co.		KYNHP; population not vouchered	2132
		Wayne Co.	1143	AUA, EKY, TENN	

Table 9. continued.

Taxon	state	county	sheet ref. no.	herbarium information (after Freeman 1994)	DNA sample #
<i>ozarkanum</i>	MO	Barry Co.	1261,	AUA, CM, F, GH, MO, SMSU,	734
			1262	TENN, UMO, US, VPI, WILLI	
		Howell Co.	1992	MO	
		Lawrence Co.	1165	AUA, F, SMU, SO, UMO, WIS;	
		McDonald Co.		NODC: population not vouchered	
		Newton Co.		map dot (Garrett 1982)	
		Polk Co.		SMS	
		Shannon Co.		F, MO	
		Stone Co.		map dot (Cabe & Werth 1995)	
		Taney Co.		SO	
<i>palustris</i>	NC	Clay Co.	1149	AUA	2115
		Haywood Co.		GA, NCU, US (specimens deemed to fragile for measurements)	
	OK	Le Flore Co.		OKNHI; population not vouchered	
	TN	Sumner Co.	1610	VDB	
	MD	Dorchester Co.		map dot (Garrett 1982)	
		Somerset Co.		map dot (Timmerman-Erskine 1999)	
		Wicomico Co.		map dot (Garrett 1982)	
		Worcester Co.	1227, 2262	BRIT, DUKE, GH, JMU, LCU, NCU, NY, PH, SRH, VPI, WILLI	811
	NC	Camden Co.		NCNHP; population not vouchered	
		Currituck Co.		NCNHP; population not vouchered	
	VA	Gates Co.	0116	NCU	
		Pasquotank Co.		map dot (Timmerman-Erskine 1999)	
		Accomack Co.		VANHP; population not vouchered	
		Chesapeake, City		GH	
<i>pusillum</i>	NC	Norfolk Co.		GH (on sheet with type; did not measure)	
		Suffolk, City of/ Nansemond Co.	1247	NCU, VDB, VPI, WILLI	
	SC	Brunswick Co.		TENN	
		Pender Co.	1166	AUA, NCU, TENN, UC, VDB	2110
	SC	Berkeley Co.	1340	type locality; CU, GH, MO, NCU	
		Calhoun Co.	0114	FSU, GA, GH, NCU, NY, USF, VDB	
<i>telmacola</i>	SC	Dorchester Co.	0115	AUA, CITA, NCU, TENN	2106
		Sumter Co.		map dot (Cabe & Werth 1995)	
<i>texanum</i>	SC	Aiken Co.	0057	USCH	816
		Barnwell Co.		TENN	
<i>texanum</i>	AR	Miller Co.		map dot (Garrett 1982)	
	LA	Caddo Parish	0247	ASTC, AUA, LSU, NLU, NY, TENN, VDB	778
				ASTC, TEX	
	TX	Angelina Co.	1596	AUA, GH, LL, NCU, TEX UC, VDB	786
		Cass Co.	1591	TPWD	
		Cherokee Co.		ASTC	
		Harrison Co.		BKL, MO, UMO, US, VDB, WIS	
		Houston Co.	1967	ASTC	
		Jasper Co.		ASTC	
		Nacogdoches Co.	1581	ASTC, AUA, BAYLU, BRIT, LL, SMU, TAMU, TEX, VDB	

Table 9. continued.

Taxon	state	county	sheet ref. no.	herbarium information (after Freeman 1994)	DNA sample #
<i>texanum</i>	TX	Panola Co.		type locality; population not vouchered; no longer extant	
		Rusk Co.		ASTC, BAYLU	
		Smith Co.	0251	BAYLU, TENN	794
		Upshur Co.		map dot (Timmerman-Erskine 1999)	
		Van Zandt Co.		map dot (Timmerman-Erskine 1999)	
		Wood Co.		TXNHP; population not vouchered	
<i>virginianum</i>	DC	Washington DC		map dot (Garrett 1982)	
	VA	Charles City Co.	1231	NCU, VDB, VPI, WILLI	
		Dinwiddie Co.		GH; type – did not measure	
		Gloucester Co.	1238	BRIT, VDB, VPI, WILLI	
		Greensville Co.	1244	CM, VPI	
		Hampton City		VANHP; population not vouchered	
		Henrico Co.	1233	GH, URV, VPI	
		James City Co.	1239	AUA, BRIT, CM, GA, GH, KANU, MIN, NCSC, NCU, NY, OKL, PH, POM, SMU, US, VDB, VPI, WILLI, WIS, WVA	782
				NCU, VPI	
		Richmond, City of/ Chesterfield Co.	1232		
		Surry Co.	0984	AUA, GH, TENN, TROY, VDB	
		Sussex Co.	1269	VPI, WILLI	
		Westmoreland		TENN	
		York Co.	1237	BRIT, VPI, WILLI	

At the start of this project, DNA sequence data from GenBank were used as the basis for the molecular component of this analysis. Initially, the two regions available from GenBank (Table 10) 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. Because these sequence data were already available for species within the family, these two regions were chosen for initial sequencing for this study. A third gene region, the cpDNA intergenic spacer *psbA-trnH*, was selected as a result of a sampling strategy developed by Shaw et al. (2005) which indicated that it was the most variable and thus the most potentially informative plastid region for Trilliaceae of the 22 regions sampled. The *psbA-trnH* DNA sequence was generated for each populational entity (see also Table 8). Additionally, using a similar sampling strategy, four additional members of the Pusillum Complex were used to sample the most informative regions for variation within this complex. However, even the next most informative regions (*rpL16* and *trnD-trnT*) did not provide the same degree of resolution as seen in *psbA-trnH*. Because of the lack of variation within both the nrITS and plastid *matK* data, ITS and *matK* sequences were generated for an exemplar for each taxon. Near the conclusion of this project, additional sequences for other members of Trilliaceae for the *psbA-trnH* intergenic spacer became available from GenBank and were incorporated into the analyses.

2.1.3 Outgroup selection.— Despite the fact that molecular analyses have suggested that Xerophyllaceae are the nearest relatives of Trilliaceae, (Davis 1995; Chase et al. 1995, 2000; Davis et al. 1998; Fuse & Tamura 2000; Rudall et al. 2000; Zomlefer et al. 2001), taxa within Melanthiaceae sensu APG are not satisfactory outgroups for Trilliaceae (Farmer 2000) due to both morphological and molecular divergence making assessment of character homologies difficult. Based on Farmer & Schilling (2002), *Pseudotrillium rivale* is the logical choice as a functional outgroup for the remainder of the family (Fig. 6).

2.1.4 Morphology.— The validity of taxonomic boundaries suggested by the molecular analysis for the Pusillum Complex were evaluated using morphological data.

A single herbarium sheet of *Trillium pusillum* s.l. was selected from each county (Table 9). Measures traditionally considered to be most important in differentiating the varieties of *T. pusillum* as well as other *Trillium* taxa were used for this study. Measurements were made of stem length, leaf length and width, pedicel length, sepal length and width, petal length and width, filament length, anther length, ovary length, style length and stigma length. Anther length was calculated based on filament and stamen lengths; pistil length was calculated based on ovary, style and stigma lengths; ratios indicative of organ shape were also calculated including leaf length/width, sepal length/width, and petal length/width. Ratio characters, traditionally useful in delimiting taxa (Hooker 1840; Gleason 1906; Gates 1917; Peattie 1927;

Table 10. DNA sequence data available from GenBank used in these analyses. Accession numbers are listed ITS, *matK*, *psbA-trnH*. Publication notes: A) published in Kazempour Osaloo and Kawano (1999); B) Kress et al. (2005); C) Ji et al. 2006; D) Kazempour Osaloo et al (1999); VIU = voucher information unknown.

Taxon	Locality	Voucher Information	Source	GenBank numbers	
<i>Pseudotrillium rivale</i> (S. Wats.) S.B. Farmer	USA: OR, Josephine Co.	Hayashi et al. VIU	A	AB018822	AB017404
	USA: CA, Del Norte Co.	Kawano et al. VIU	A	AB018822	AB017404
	USA: OR, Josephine Co.	Kawano et al. VIU	A	AB018822	AB017404
<i>Trillium albidum</i> Freeman	USA: CA, Sonoma Co.	Ohara et al. VIU	A		AB017376
	UK: RBG, Edinburgh (in cultivation)	19623094B	C	DQ404198	DQ404232
<i>T. angustipetalum</i> (Torr.) Freeman	USA: CA, Stanislaus Nat. Forest	Kawano et al. VIU	A		AB017377
<i>T. apetalum</i> Makino	Japan: Toyama, Echu-miyazaki	Yoshida et al. VIU	A		AB017378
<i>T. camschatcense</i> Ker Gawl.	Japan: Hokkaido, Samani-cho	Kato VIU	A	AB018808	AB017379
	UK: RBG, Edinburgh (in cultivation)	19741690	C	DQ404197	DQ404231
<i>T. cateabaei</i> Elliott (sample = <i>T. simile</i>)	USA: TN, Knox Co. (in cultivation)	Kawano et al. VIU	A		AB017380
<i>T. cernuum</i> L. (ITS sample = <i>T. cuneatum</i>)	USA: WI, Iron Co.	Kawano et al. VIU	A		AB017381
	UK: RBG, Edinburgh (in cultivation)	19370524B	C	DQ404193	DQ404227
<i>T. chloropetalum</i> (Torr.) Howell	USA: CA, Santa Cruz Co.	Ohara et al. VIU	A	AB018809	AB017382
<i>T. chloropetalum</i> var. <i>giganteum</i> (Hook. & Arn.) Munz	USA: CA, Santa Cruz Co.	Kawano et al. VIU	A		AB017383
<i>T. cuneatum</i> Raf.	USA: GA, Rabun Co.	Ohara et al. VIU	A		AB017384
			C	DQ404199	DQ404233
<i>T. decipiens</i> Freeman	USA: FL, Jackson Co.	Ohara et al. VIU	A	AB018810	AB017385
<i>T. decumbens</i> Harb.			A		AB017386
<i>T. discolor</i> Wray ex Hooker	USA: SC, McCormick Co.	Ohara et al. VIU	A	AB018811	AB017387
<i>T. erectum</i> L.	USA: PA, Westmoreland Co.	Kawano et al. VIU	A	AB018812	AB017388
	UK: RBG, Edinburgh (in cultivation)	19653535A	C	DQ404196	DQ404230
<i>T. flexipes</i> Raf.	USA: IL, Jackson Co.	Ohara et al. VIU	A		AB017389
<i>T. foetidissimum</i> Freeman	USA: LA, West Feliciana Parish	Ohara et al. VIU	A		AB017390
<i>T. grandiflorum</i> (Michx.) Salisb.	USA: PA, Westmoreland Co.	Kawano et al. VIU	A	AB018814	AB017392
	UK: RBG, Edinburgh (in cultivation)	19685241	C	DQ404195	DQ404229
<i>T. kurabayashii</i> Freeman	USA: CA, Del Norte Co.	Ohara et al. VIU	A		AB017393
<i>T. lancifolium</i> Raf.	USA: SC, McCormick Co.	Ohara et al. VIU	A	AB018815	AB017394
<i>T. ludovicianum</i> Harb.	USA: LA, Rapides Parish	Ohara et al. VIU	A		AB017395

Table 10. continued.

Taxon	Locality	Voucher Information	Source	GenBank numbers	
<i>T. luteum</i> Harb.	USA: NC, Graham Co.	<i>Ohara et al.</i> VIU	A	AB017396	
	UK: RBG, Edinburgh (in cultivation)	19841338	C	DQ404200	DQ404234
<i>T. maculatum</i> Raf.	USA: GA, Early Co.	<i>Ohara et al.</i> VIU	A	AB018816	AB017397
<i>T. nivale</i> Riddell (sample = <i>T. grandiflorum</i>)	USA: PA, Westmoreland Co.	<i>Utech</i> VIU	A		AB017398
<i>T. ovatum</i> Pursh var. <i>ovatum</i>	USA: CA, Del Norte Co.	<i>Kawano et al.</i> VIU	A	AB018817	AB017399
	UK: RBG, Edinburgh (in cultivation)	19360567C	C	DQ404194	DQ404228
<i>T. pusillum</i> E.J. Palmer & Steyermark (sample = <i>T. ozarkanum</i>)	USA: NC, Sokes Co. (sic.)	<i>Kawano et al.</i> VIU	A	AB018819	AB017401
<i>T. petiolatum</i> Pursh	USA: WA, Chelan Co.	<i>Ohara et al.</i> VIU	A	AB018818	AB017400
<i>T. recurvatum</i> Beck	USA: AR, Newton Co.	<i>Ohara et al.</i> VIU	A	AB018820	AB017402
<i>T. reliquum</i> Freeman	USA: GA, Columbia Co.	<i>Ohara et al.</i> VIU	A	AB018821	AB017403
<i>T. rugelii</i> Rendle	Japan: Cult. in Bot. Gard. of Hokkaido Univ.	VIU	A	AB018823	AB017405
<i>T. sessile</i> L.	USA: PA, Westmoreland Co.	<i>Kawano et al.</i> VIU	A	AB018824	AB017406
	unknown	<i>Wells</i> 4879 US	B	DQ006019	DQ006020
<i>T. simile</i> Gleason (sample = <i>T. grandiflorum</i>)	USA: TN, GRSMNP	<i>Kawano</i> VIU	A		AB017407
<i>T. smallii</i> Maxim.	Japan: Hokkaido, Kojima Island	<i>Kawano et al.</i> VIU	A		AB017408
<i>T. stamineum</i> Harb.	USA: MS, Lauderdale Co.	<i>Ohara et al.</i> VIU	A		AB017409
<i>T. sulcatum</i> Patrick	USA: NC, Burke Co.	<i>Kawano et al.</i> VIU	A		AB017410
<i>T. tschonoskii</i> Maxim.	Japan: Hokkaido, Hakodate-yama	<i>Kawano et al.</i> VIU	A		AB017411
	unknown	<i>GLGS Exp.</i> 20202 KUN	C	DQ404201	DQ404235
<i>T. underwoodii</i> Small	USA: FL, Gaden Co.	<i>Ohara et al.</i> VIU	A		AB017412
<i>T. undulatum</i> Willd.	USA: PA: Westmoreland Co.	<i>Kawano et al.</i> VIU	A	AB018825	AB017413
<i>T. vaseyi</i> Harb.	Sweden: Göteborg Bot. Gard. (in cultivation)	unknown, VIU	A		AB017414
<i>T. viridescens</i> Nutt.	USA: AR, Washington Co.	<i>Kawano et al.</i> VIU	A		AB017416
<i>T. viride</i> Beck	USA: MO, Washington Co.	<i>Ohara et al.</i> VIU	A		AB017415
<i>Veratum maackii</i> Regel	Japan: Nyukawa-mura	<i>Kato</i> VIU	A	AB018826	AB017417

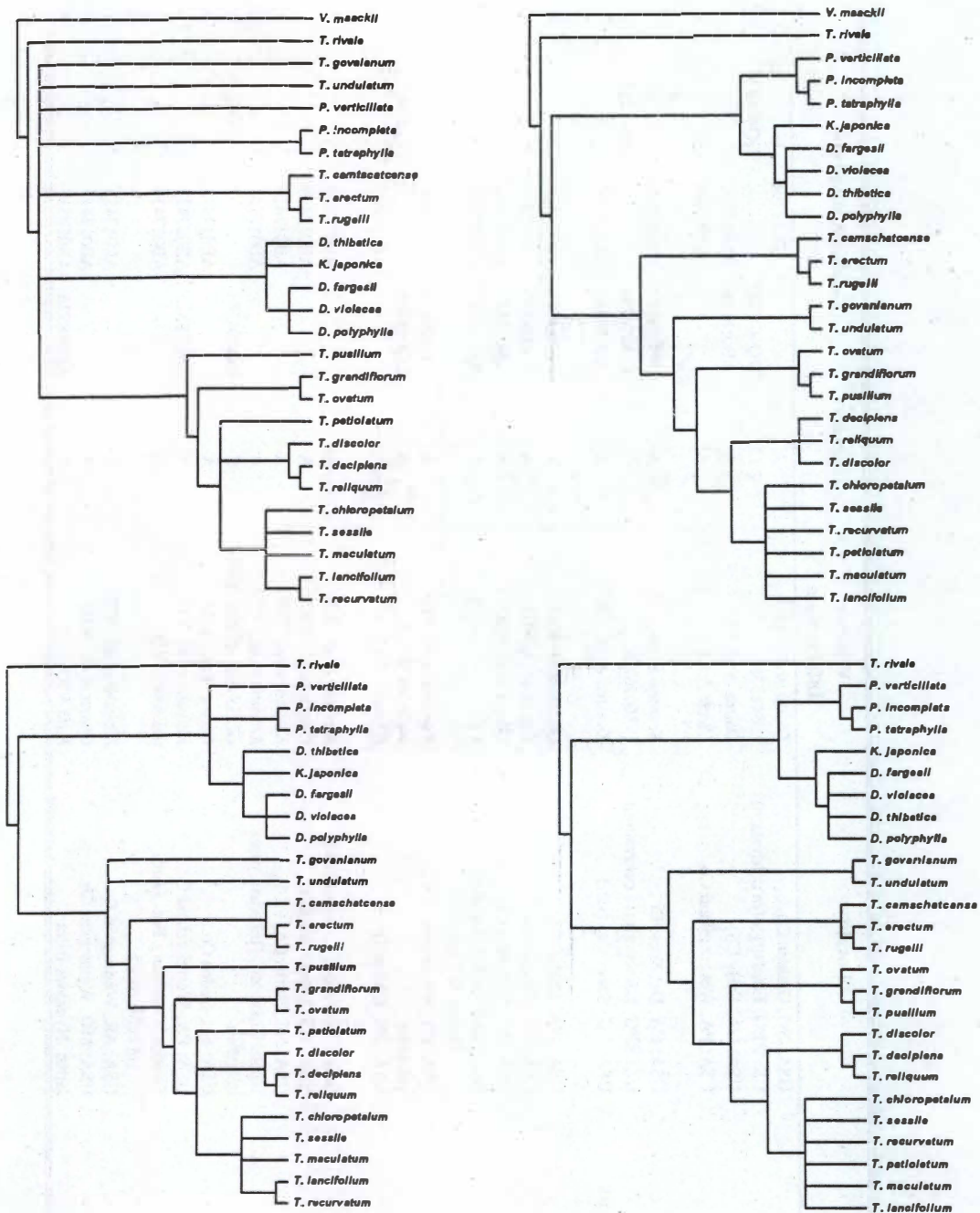


Figure 6. Strict consensus trees from preliminary analyses of Trilliaceae with different data sets and different outgroups. a,c. Tree produced using *matK* data. b,d. Tree produced using ITS data. a. *Veratrum maackii* as the outgroup. Strict consensus of 1326 trees; length 285. b. Strict consensus of 6 trees; length 748. c. *Trillium rivale* (now *Pseudotrillium rivale*) as the outgroup. Strict consensus of 6 trees; length 107. d. Tree produced using ITS data. Strict consensus of 6 trees; length 331 (Farmer & Schilling 2002).

Small 1933; Anderson 1934; Barksdale 1938; Fernald 1950; Gleason & Cronquist 1963; Serota & Smith 1967; Freeman 1975), were also calculated, such as stem length/leaf length, stem length/pedicle length, pedicle length/sepal length, sepal length/petal length, filament length/anther length, filament length/ovary length, ovary length/stigma length, stigma length/anther length, stigma length/style length, and stamen length/pistil length. Stamen and pistil measurements were made using a binocular dissecting microscope with an ocular micrometer. When necessary, sepal widths, petal widths, and pedicle lengths were measured with the dissecting microscope as well. Measurements made with the dissecting microscope were made to the nearest 0.1 mm. All other measurements were made to the nearest mm.

2.2 DNA Extraction, PCR, and Sequencing Protocols

For the analysis of *Delostylis*, genomic DNA was extracted from plant material using the DNeasy Plant Kit (QIAGEN Inc., Valencia, California, USA) from frozen, desiccated, or herbarium specimens (Table 8). PCR amplification and sequencing were carried out using the primers in Table 11. The PCR reactions were performed in 20 μ l reactions containing 13.5 μ l sterile water, 2.0 μ l 10X PCR buffer, 1.8 μ l 25 mM MgCl₂, 0.4 μ l 0.2 mM dNTPs in equimolar ratio, 1 unit *Taq* polymerase (Eppendorf North America, Westburg, New York, USA), 0.4 μ l 0.5 mM each primer, 0.4 μ l BSA and 1 μ l genomic DNA.

The PCR protocol for the ITS nuclear gene region followed Sun et al. (1994), used primers ITS4 and ITS5, and proceeded as follows: initial denaturing at 94°C for 3 min, followed by 35 cycles of a 94°C denaturing step for 60 sec, a 58°C annealing step of 60 sec, and a 72°C extension step for 120 sec followed by an additional extension step for 5 min at 72°C to complete any unfinished DNA strands.

The PCR protocol for the *matK* gene (Fuse & Tamura 2000) used primers 710F and matK-1 and proceeded as follows: initial denaturing at 94°C for 2 min, followed by 40 cycles of a 94°C denaturing step for 60 sec, a 50°C annealing step of 60 sec, and a 72°C extension step for 90 sec followed by an additional extension step for 3 min at 72°C to complete any unfinished DNA strands.

The PCR protocol for the *psbA-trnH* intergenic spacer (Shaw et al. 2005) used primers psbA and trnH for most material and proceeded as follows: initial denaturing at 80°C for 5 min, followed by 35 cycles of a 94°C denaturing step for 30 sec, a 50-56°C annealing step of 30 sec, and a 72°C extension step for 60 sec followed by an additional extension step for 5 min at 72°C to complete any unfinished DNA strands.

After PCR, the samples were purified with ExoSAP-IT (USB Corp., Cleveland, Ohio, USA) before sequencing. In all cases, both strands of the PCR products were sequenced using the ABI PRISM Dye Terminator cycle Sequencing Ready Reaction Kit (Perkin-Elmer/Applied Biosystems, Foster City, California, USA) per manufacturers instructions. To sequence the ITS region, primers

Table 11. PCR and sequencing primers used in the molecular analyses of *Delostylis*. Amplification primers are denoted PCR; sequencing primers SEQ.

Region	Reference	Primer	5'- Primer sequence -3'										Use
ITS	White et al., 1990	ITS5	GGA	AGT	AAA	AGT	CGT	AAC	AAG	G			PCR, SEQ PCR
		ITS4	TCC	TCC	GCT	TAT	TGA	TAT	GC				
	Sun et al., 1994	17SE	ACG	AAT	TCA	TGG	TCC	GGT	GAA	GTG	TTC	G	PCR, SEQ
		26SE	TAG	AAT	TCC	CCG	GTT	CGC	TCG	CCG	TTA	C	PCR, SEQ
	Nickrent 2000	79R	GAT	GGT	TCA	CGG	GAT	TCT	GC				PCR, SEQ
		79F	GCA	GAA	TCC	CGT	GAA	CCA	TC				PCR, SEQ
	Small 2004	5.8F	TCG	ATG	AAG	AAC	GTA	GCG	AAA	T			PCR, SEQ
		5.8R	TGA	CAC	CCA	GGC	AGA	CGT	GC				PCR, SEQ
	<i>matK</i> Fuse & Tamura 2000	710F	GTA	TCG	CAC	TAT	GTA	TCA	TTT	GA			PCR, SEQ
		1470R	AAG	ATG	TTG	ATC	GTA	AAT	GA				SEQ
		1470F	TCA	TTT	ACG	ATC	AAC	ATC	TT				SEQ
		8R	AAA	GTT	CTA	GCA	CAA	GAA	AGT	CGA			PCR, SEQ
<i>psbA-trnH</i>	Tate & Simpson 2003	trnH	CGC	GCA	TGG	TGG	ATT	CAC	AAT	CC			PCR, SEQ
	Sang et al., 1997 generated for this project	psbA	GTT	ATG	CAT	GAA	CGT	AAT	GCT	C			PCR, SEQ
		421F	CTC	CGT	CCT	CGA	GCT	CTA	GG				PCR, SEQ
		565R	GCG	TTA	TCG	GGC	ATC	TTA	T				PCR, SEQ
		1100F	ATG	TTC	CTT	TCC	ATT	ATG	AAT	AGC			PCR, SEQ
		1130R	AAT	CCT	TTT	GTA	GCT	AAT	CAT	TTA	T		PCR, SEQ

ITS4 and 17SE were used and 79R, 79F, and/or 5.8R were also used with recalcitrant samples (e.g., DNA extracted from herbarium material); to sequence the *matK* coding region primers 710F, 1470R, 1470F, 8R, 8F, and *matK*-1 were used; to sequence the *psbA-trnH* region primers *psbA* and *trnH* were used and 565R, 421F, 1130R, and 1100F were also used with recalcitrant DNA. After the sequencing reaction, the samples were purified with Sephadex G-50 (Sigma-Aldrich, St. Louis, Missouri, USA) using Centri-Sep tubes (Princeton Separations, Adelphia, New Jersey, USA) and vacuum dried. The dried samples were sequenced on an ABI Prism 3100 automated sequencer (Perkin-Elmer/Applied Biosystems, Foster City, California, USA) at the University of Tennessee Molecular Biology Resource Facility.

2.3 Sequence Manipulation and Alignment

The sequences were trimmed and contiged with Sequencher 4.2 (Gene Codes Corp., 2004) and aligned using BioEdit (Hall 1997-2005, 1999). The data matrices from these analyses will be deposited at TreeBase upon publication of this project; DNA sequences will be deposited at GenBank upon publication of this project.

2.4 Phylogenetic Analyses

Bayesian and parsimony analyses were performed on each of the individual data sets as well as the combined data set. Before data sets are combined, congruence must be assessed (Bull et al. 1993; Huelsenbeck et al. 1996; Johnson & Soltis 1998) in order to determine if the data have similar phylogenetic histories. If the data are congruent, characters from different data sets can strengthen weak phylogenetic signal to the point that it can overcome noise (Sullivan 1996). The incongruence length difference test (ILD) implemented in PAUP* as the Homogeneity of Partitions test was used to assess congruence of data sets.

Parsimony analysis of DNA sequence data was performed using PAUP* (Swofford 2001) vers. 4.0b10 with maximum parsimony using heuristic search methods with TBR (tree bisection reconnection) and MULPARS and a simple addition sequence. Larkin's "Bob Barker Strategy" (Larkin et al. 2006) was used to check for islands of trees (Maddison 1991) before a run to completion. The "Bob Barker" strategy is a two-step heuristic search strategy which effectively searches a large expanse of tree space while avoiding extensive swapping on suboptimal islands of trees. In the first step, 10,000 random addition replicates were conducted, saving only the 50 best trees in each; had any islands produced shorter trees, those trees would have been used as starting trees which would then be swapped to completion. Bootstrap support (Felsenstein 1985) was estimated based on 100,000 FastStep replicates with the same search strategy as simple parsimony. As gaps can be among the more informative

(McGuire et al. 2001; Applequist & Wallace 2002; Kress et al. 2005; Lunter et al. 2005; Müller 2005) and least homoplasious (Graham et al. 2000; Simmons et al. 2001; Hamilton et al. 2003; Dane & Lang 2004) characters, gaps have been coded as additional characters. For the *matK* data, gaps were simply coded as presence/absence characters; gaps for the *psbA-trnH* sequence data were coded using a slightly more complex method (Simmons & Ochoterena 2000). Gaps in the ITS data set were not coded as the majority were single base-pair indels.

Bayesian analysis of DNA sequence data was performed using MrBayes (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) vers. 3.1.2 with the GTR+I+G model as selected by MrModelTest (Nylander 2004) using the AIC criterion for the ITS and *psbA-trnH* data sets. GTR+I was selected as the proper model for each of the codon positions of the *matK* data set using the AIC criterion. The indel characters in the *matK* data set were treated as restriction-site (binary) data; the indel characters in the *psbA-trnH* data set were treated as standard (morphological) data. The *matK*, *psbA-trnH*, and combined data sets were analyzed utilizing mixed models. Three heated and one cold chain were run simultaneously for two million generations; the best tree of the four chains was sampled every 100 generations. Model parameter values (priors) were set using default values. Stationarity was assessed by plotting each estimated parameter against the generation number via the sump command and viewed in Excel; burnin value was estimated visually. A majority rule consensus tree was calculated for trees saved via the sumt command once the likelihood score and all estimated parameter values had reached stationarity; this consensus indicates the posterior probabilities of each clade.

2.5 DNA Sequence Data as Taxonomic Characters

During the alignment of the DNA sequence data, I noticed that many base pair changes and indels appeared to be taxonomically significant (e.g., a particular DNA nucleotide, series of nucleotides, or insertion/deletion events were exhibited in a particular taxon or group of taxa [Davis & Nixon 1992; Holt et al. 2004; Pons et al. 2006; see also McGuire et al. 2001; Applequist & Wallace 2002; Kress et al. 2005; Lunter et al. 2005; Müller 2005]). DNA-based taxonomy uses fixed diagnostic or uniquely derived changes in the DNA sequence (whether it is one or more base pair changes [SNPs] or insertion/deletion events [indels]) for determining species boundaries (Holt et al. 2004; Pons et al. 2006). One approach is called population aggregation analysis (PAA; Davis & Nixon 1992) in which individuals in the same population are assumed to belong to the same species, and individuals sharing fixed character traits drawn from different populations are conspecific (Davis & Nixon 1992; Sites & Marshall 2003).

2.6 Morphological Analysis

Data were analyzed in a variety of ways. The quantitative, morphological cohesiveness was assessed by treating each of the clusters indicated by phylogenetic analysis of the molecular sequence data as an Operational Taxonomic Unit (OTU). In addition, each cluster was analyzed separately where each taxon in that cluster was treated as an OTU. Finally, each taxon was treated as an OTU in a global analysis of the *Pusillum* Complex. The analyses were performed using SPSS 14.0.0 for Windows (SPSS 2005).

Standard simple descriptive statistics, which would provide a basis for the morphological descriptions of the taxa, were used to define the basic distributional characteristics of each of the selected variables. Mean, median and mode provide measures of central tendency; standard deviation and variance indicate the degree of dispersion around the mean; and skewness and kurtosis more precisely define the shape of the distribution. Box plots were produced to illustrate the distribution and dispersion of the variables by displaying the median and quartiles, outliers, and extreme values. Extreme values were double-checked against the herbarium sheets for recording errors.

Discriminant analysis, a kind of factor analysis, tests an a priori classification based on DNA analyses by observing whether cases are classified into groups as predicted, investigates differences between those groups, and assesses the relative importance of characters used to separate those groups. Included in discriminant analysis are: an F-test of differences between group multivariate means (centroids), statistics of the discriminant functions (eigenvalues, relative percent variance explained, canonical correlation and group membership); stepwise summery tables (indicates the variables most useful in separating the groups); the standardized canonical discriminant function coefficients (indicates the relative importance of each of the original variables); plots of the individuals in discriminant space; and classification of the individuals into groups, regardless of prior group designation. In addition, group centroids were plotted on three-dimensional projection graphs in which the first three discriminant functions are used as the axes.

3.0 RESULTS

Sequence lengths of the ITS region in *Trillium* ranged from 645 to 662 nucleotides. The aligned matrix (Appendix B.1) consisted of 72 *Trillium* samples (including 28 *Delostylis* samples of which 20 were Pusillum Complex samples) with 26 of those sequences from GenBank (Table 12). The aligned matrix was 683 base pairs long, included 20 indel regions, and was aligned by hand. Of the 683 characters, 163 were variable and 141 of those were parsimony informative. Indels were not coded as separate characters for the ITS region because of the preponderance of single base indels.

Sequence lengths of the *matK* coding region ranged from 1542 to 1554 nucleotides. The *matK* data set used in phylogeny reconstruction consisted of only the *matK* coding region. The aligned matrix (Appendix B.2) consisted of 76 *Trillium* samples (including 22 *Delostylis* samples of which 15 were Pusillum Complex samples) with 39 of those sequences from GenBank (Table 12). The aligned data matrix was 1586 characters in length including 8 added characters representing indels and was aligned by hand. Of the 1586 characters, 86 were variable and 72 of those were parsimony informative. When this region was examined for taxonomic markers, both the 3' *matK-trnK* spacer region and the 5' *trnK-matK* spacer region were used in addition to the coding region; they were not used for phylogenetic analysis because they were not sequenced for all members of *Trillium*.

Sequence lengths of the *psbA-trnH* intergenic spacer ranged from 628 to 1287 nucleotides including two groups of excluded characters: 10 characters that served as markers for minisatellite regions and the minisatellite region itself which consisted of 373 characters. The *psbA-trnH* data set used in phylogeny reconstruction consisted of only the *psbA-trnH* intergenic spacer region. The aligned matrix (Appendix B.3) consisted of 69 *Trillium* samples (including 31 *Delostylis* samples of which 24 were Pusillum Complex samples) with ten of those from GenBank (Table 12). The matrix was 1808 characters long including 47 added characters representing indels and was aligned by hand. Of the 1808 characters, 383 were an excluded minisatellite region present only within certain members of *T. subgen. Phyllantherum*, 146 of the remaining characters were variable and 121 of those were parsimony informative. When the *psbA-trnH* intergenic spacer region was examined for taxonomic markers, parts of *psbA* and *trnH* were also used in addition to the intergenic spacer; the portions of *psbA* and *trnH* were not used for phylogenetic analysis because they were not sequenced for all members of *Trillium*.

The combined data set used in phylogeny reconstruction in *Trillium* consisted of nuclear DNA (ITS region) as well as plastid coding (*matK*) and non-coding (*psbA-trnH* intergenic spacer) DNA. The homogeneity of partitions indicated that the data are congruent ($p = 0.10$), and are thus combinable (Cunningham 1997). The aligned data matrix (Appendix B.4) consisted of 45 samples and was 4077 characters long including 56 characters representing indels (Table 12). Of the 4077 characters, 383 were excluded, 362 were variable and 301 of those were parsimony informative. In order to allow four members of the Erectum Complex (two Asian and two North American) to be included in the combined

Table 12. Summary statistics for the four data sets used in the phylogenetic analyses of *Delostylis*.
Numbers in parentheses refer to sequence data retrieved from GenBank.

data set	unaligned range	aligned length	# <i>Trillium</i>	# <i>Delostylis</i> Group	# <i>Pusillum</i> Complex	# Gap Characters	# excluded characters
ITS	645-662	683	72 (26)	28 (1)	20 (1)	n/a	n/a
<i>matK</i>	1542-1566	1586	76 (39)	22 (1)	15 (1)	8	n/a
<i>psbA-trnH</i>	628-1287	1808	69 (10)	31 (0)	24 (0)	48	383
combo	n/a	4077	43 (10)	19 (0)	13 (0)	56	383

data set, sequence data from *T. rugelii* (ITS) and *T. cernuum* (*psbA-trnH*) were combined into a single taxon called “rugellium;” the sequence data for *matK* is the same in both taxa. Because of the lack of variation in DNA sequence data in the Erectum Complex (Millam 2006), this was deemed an acceptable solution.

3.1 Parsimony Analyses

3.1.1 ITS sequence data.– Parsimony analysis of the ITS data produced 85,280 shortest trees of length 235 in one island with a consistency index (CI) of 0.78; the 50% majority-rule consensus tree is shown in Fig. 7. The spine of the bootstrap tree was a polytomy consisting of *T. undulatum* (100%), *T. ovatum* var. *hibbersonii* (100%), the Erectum Group (98%), *T. ovatum* (89%), *T.* subgen. *Phyllantherum* (96%), *T. nivale* and *T. grandiflorum* (91%), and *Delostylis* (99%). Within *Delostylis*, the clade consisting of *T. catesbaei* and *T. persistens* was shown to be sister group to a clade containing the Pusillum Complex. The Catesbaei Clade was supported at 99%; *T. catesbaei* was not found to be monophyletic. The Pusillum Clade was supported at 73%. *Trillium telmacola* (the Aiken Co. SC population of *T. pusillum*) was the basal member in this group with the remaining taxa forming sister clades with only 53% support. The clade containing the rest of the *T. pusillum*, *texanum*, and *georgianum* samples was supported at 62%, and the clade containing *T. ozarkanum*, *alabamicum*, *virginianum*, *carolinianum*, *palustris* and *monticola* was supported at 89%.

3.1.2 *matK* sequence data.– Parsimony analysis of the *matK* data produced 56,895 shortest trees of length 120 in one island with a CI of 0.77. The 50% majority-rule consensus tree is shown in Fig. 8. The backbone of the bootstrap tree was a polytomy which consisted of *T. undulatum* (100%), *T. ovatum* var. *hibbersonii* (100%), the Erectum Group (72%), and the rest of *Trillium* supported at 69%. The backbone of the clade containing the rest of *Trillium* was also a polytomy consisting of *T. nivale*, *T. ovatum* (94%), *Delostylis* (55%), and *T.* subgen. *Phyllantherum* (78%). The backbone of *Delostylis* was also a polytomy consisting of *T. texanum* (52%), *ozarkanum* and *alabamicum* (52%) with *alabamicum* supported at 59%, and *T. catesbaei* and *T. persistens* supported at 74%; as with the ITS data, *T. catesbaei* was not shown to be monophyletic. Within *Delostylis* a clade containing *T. catesbaei* and *T. persistens* was supported at 74%; *T. catesbaei* was not shown to be monophyletic. Within the Pusillum Complex, resolution present in the 50% majority rule tree showed the Pusillum Cluster present 87% of the time, the Ozarkanum Cluster present 83% of the time, and the Virginianum Cluster is shown as a polytomy.

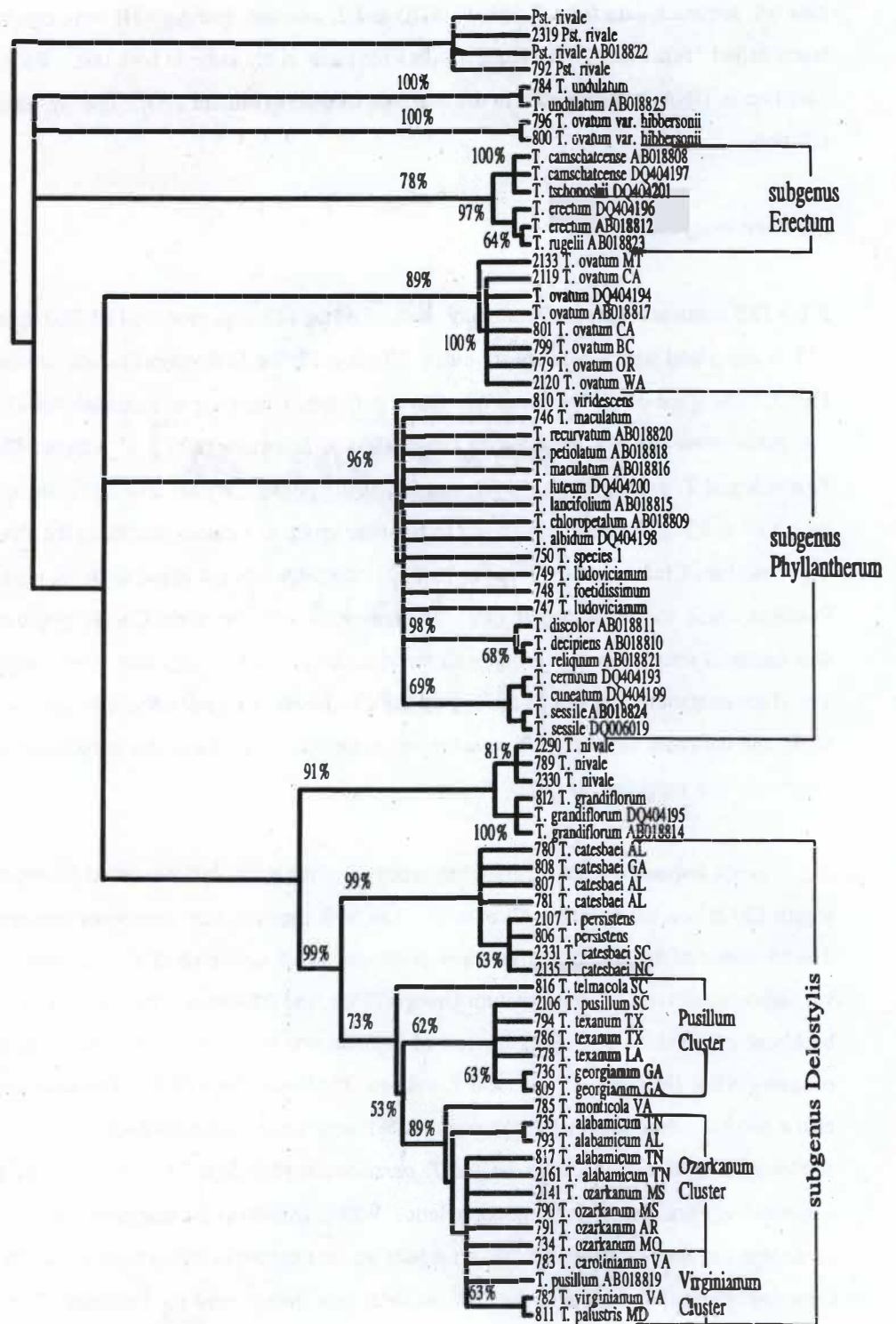


Figure 7. Fifty percent majority rule consensus tree of 85,280 shortest trees from the parsimony analysis of the ITS sequence data of *Trillium*. Percentages are bootstrap values.

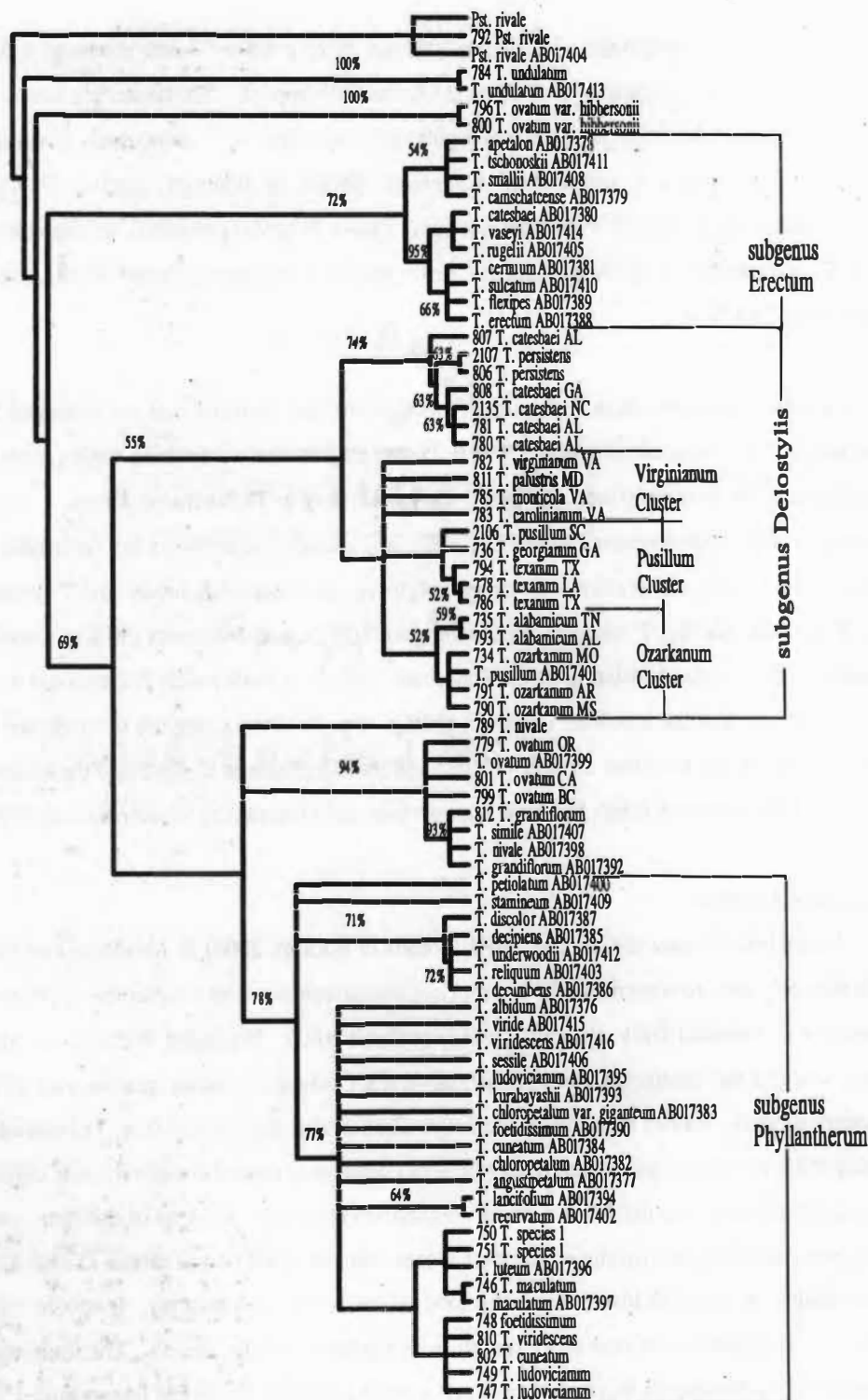


Figure 8. Fifty percent majority rule consensus tree of 56,895 shortest trees from the parsimony analysis of the *matK* sequence data of *Trillium*. Percentages are bootstrap values.

3.1.3 *psbA-trnH* sequence data.— Parsimony analysis of the *psbA-trnH* data produced 5,346 shortest trees of length 232 in one island with a CI of 0.68; the 50% majority-rule consensus tree is shown in Fig. 9. The backbone of the bootstrap tree was a polytomy consisting of *T. undulatum*, *T. grandiflorum* (100%), *T. nivale* (100%), *T. ovatum* var. *hibbersonii* (98%), the Erectum Complex (92%); *T. catesbaei* and *T. persistens* (61%) with *T. catesbaei* not being shown to be monophyletic, the Pusillum Cluster (100%), *T. ovatum* (88%), the Virginianum Cluster and the Ozarkanum Cluster (96%), and *T. subgen. Phyllantherum* (100%).

3.1.4 Combined sequence data.— Parsimony analysis of the combined data set produced 280 shortest trees of length 528 in one island with a CI of 0.75; the majority rule consensus tree is shown in Fig. 10. The backbone of the bootstrap tree was a polytomy consisting of *Trillium undulatum*, *T. ovatum* var. *hibbersonii* (100%), the Erectum Complex (100%), and a clade consisting of the remainder of the *Trillium* species (73%). That clade was also a polytomy consisting of *T. nivale* and *T. grandiflorum* (71%), *T. ovatum* (100%), *T. subgen. Phyllantherum* (100%), and *Delostylis* (99%). *Delostylis* is composed of sister clades consisting of the Catesbaei Complex (100%) with *T. catesbaei* not being shown to be monophyletic and the Pusillum Complex (94%). The Pusillum Complex is composed of sister clades consisting of the Pusillum Cluster (100%) and the Virginianum Cluster and the Ozarkanum Cluster (99%). The Virginianum Cluster has 83% support with the Ozarkanum Cluster having 99% support.

3.2 Bayesian Analyses

ModelTest (Posada & Crandall 1998; Posada & Buckley 2004) or MrModelTest (Nylander 2004) is typically used to determine the appropriate evolutionary model for running MrBayes (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Normally, the user sets up a PAUP block that will test the likelihood score for a Jukes-Cantor neighbor-joining tree for each of the different evolutionary models. Rather than utilize the Jukes-Cantor neighbor-joining tree, I prepared a tree-file containing three parsimony generated trees (the strict consensus tree, the majority-rule consensus tree, and the bootstrap tree), and the Jukes-Cantor neighbor-joining tree. In three of the four cases, the majority-rule consensus tree produced the best scores with the GTR+I+G model (Table 13); the neighbor-joining tree had the lowest log-likelihood scores for the ITS data set. It should also be noted that GTR+I+G produced the best score for all trees regardless of the source. The individual nucleotide positions of the *matK* data set were also analyzed individually with MrModelTest resulting in the GTR+I model being chosen using the AIC criterion for each of the individual codon positions for this data set.

3.2.1 ITS sequence data.— Bayesian analysis produced trees with a similar topology to the 50% majority rule parsimony trees (Fig. 7). After 2,000,000 generations, the average standard deviation of

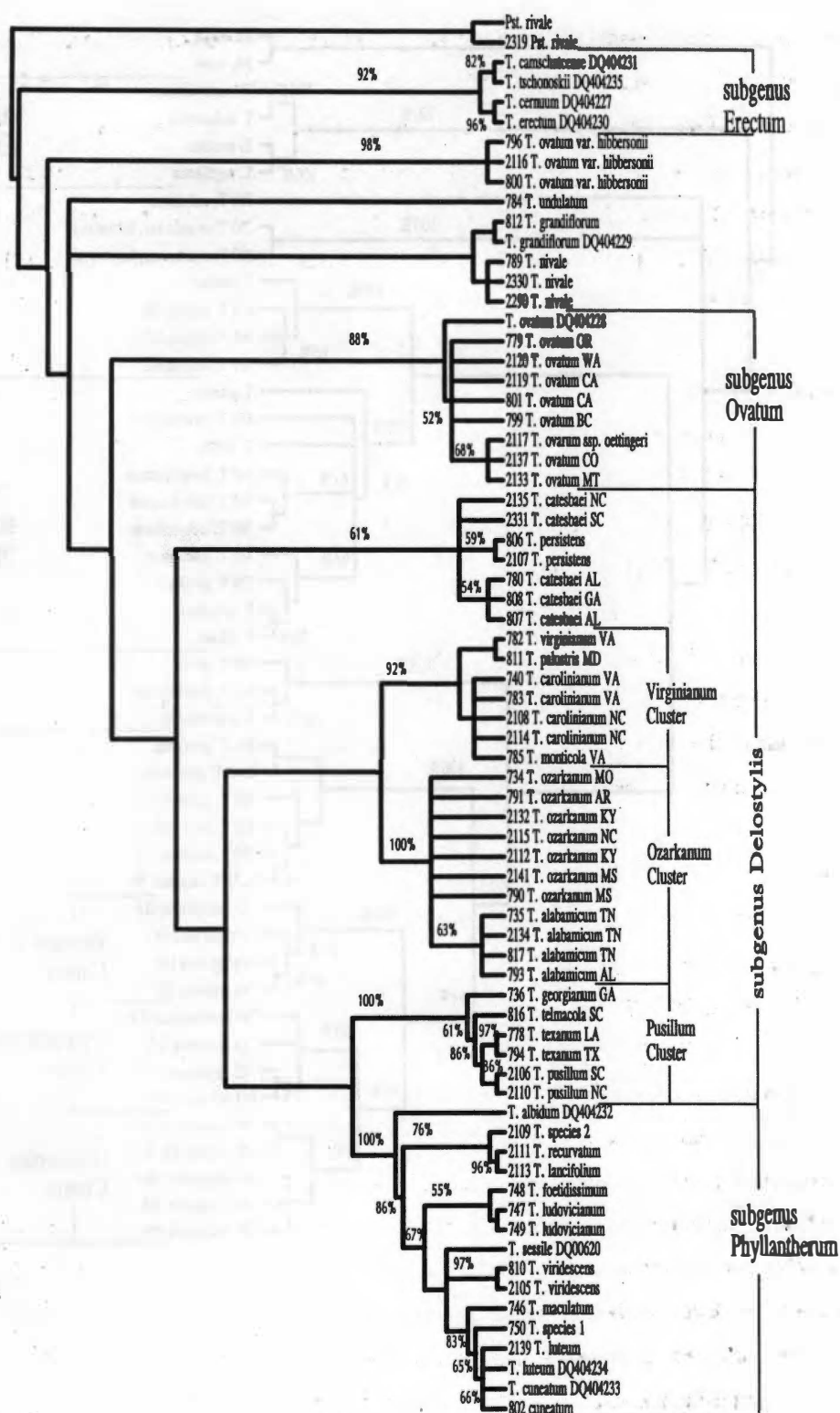


Figure 9. Fifty percent majority rule consensus tree of 5,346 shortest trees from the parsimony analysis of the *psbA-trnH* sequence data of *Trillium*. Percentages are bootstrap values.

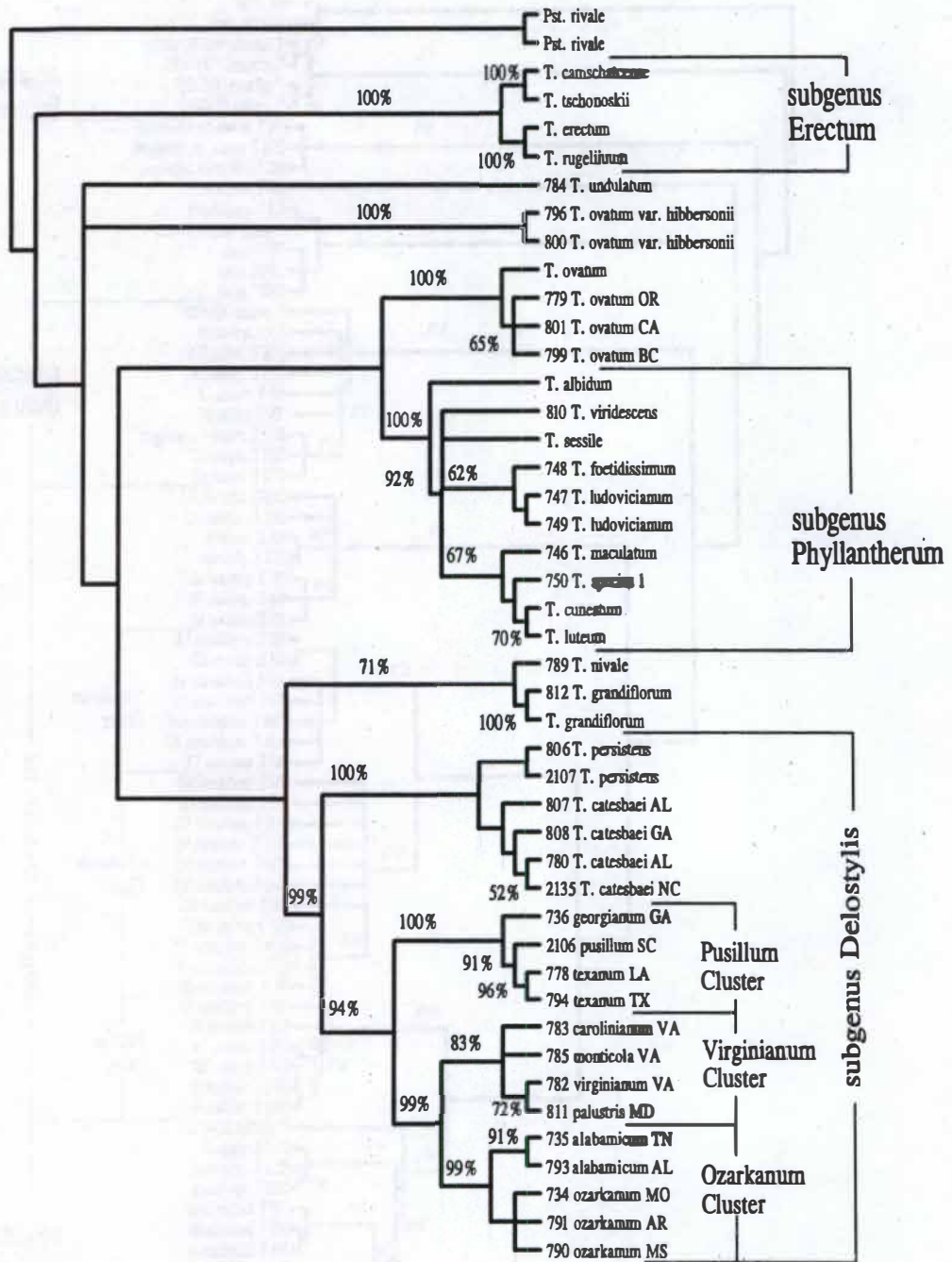


Figure 10. Fifty percent majority rule consensus tree of 280 shortest trees from the parsimony analysis of the combined sequence data of *Trillium*. Percentages are bootstrap values.

Table 13. Scores from MrModeltest analyses with different starting trees. All scores are log likelihood scores. The best score in each analysis is italicized.

data set	tree	model		+I	+G	+I+G
ITS	bootstrap	JC	4869.9961	4681.4355	4636.1470	4629.9551
		F81	4859.6450	4673.9746	4629.0327	4622.7637
		K80	4635.0439	4444.9619	4399.3901	4393.0688
		HKY	4624.1948	4438.3413	4395.4360	4389.5488
		SYM	4611.8135	4429.0220	4383.5503	4378.8770
		GTR	4603.0620	4425.4072	4380.2700	4375.8960
	majority rule	JC	5372.8491	5101.6646	5067.3745	5062.8921
		F81	5361.3052	5091.6621	5057.6416	5053.0952
		K80	5110.6001	4834.8662	4799.2612	4794.3032
		HKY	5096.5703	4823.2690	4791.9795	4787.1289
		SYM	5083.7256	4817.9814	4782.9868	4777.1123
		GTR	5072.9663	4810.2847	4777.2856	4771.1436
	neighbor joining	JC	4619.9175	4482.4722	4454.6123	4451.2036
		F81	4606.9507	4471.5571	4444.2188	4440.8833
		K80	4419.3408	4279.2764	4250.1875	4246.5312
		HKY	4405.3232	4269.3120	4242.9683	4239.8330
		SYM	4396.2754	4262.3672	4233.3613	4230.8696
		GTR	4383.6611	4256.3145	4228.9736	4226.8228
	strict consensus	JC	5472.2261	5186.0923	5146.7739	5141.5742
		F81	5460.6230	5176.0200	5136.8120	5130.8799
		K80	5208.6538	4917.8970	4877.0938	4871.3003
		HKY	5194.5259	4906.6777	4870.7769	4865.0112
		SYM	5182.1821	4901.0317	4861.8140	4855.0898
		GTR	5171.3311	4893.2466	4856.3413	4849.4028
matK	bootstrap	JC	4529.9053	4111.1431	4233.0132	4055.4219
		F81	4411.1235	4006.6111	4120.2651	3951.3738
		K80	4523.9868	4105.5332	4227.2451	4049.6267
		HKY	4401.2573	4001.9458	4112.5298	3946.1113
		SYM	4476.8999	4057.9829	4180.6631	4001.9617
		GTR	4334.1313	3953.2158	4047.2095	3895.4019
	majority rule	JC	3829.7761	3646.5491	3696.9336	3630.8608
		F81	3729.4473	3552.8625	3598.8459	3536.8940
		K80	3825.3074	3642.4038	3692.5798	3626.9250
		HKY	3722.3232	3548.6687	3592.7422	3532.7986
		SYM	3795.9082	3612.0933	3663.1377	3596.7683
		GTR	3681.1047	3514.5923	3552.5930	3497.6675
	neighbor joining	JC	4095.1663	3832.2905	3909.1282	3810.2305
		F81	3982.6555	3729.8013	3800.0525	3709.4832
		K80	4091.9541	3829.2344	3905.9949	3807.5134
		HKY	3976.7754	3726.9900	3795.2307	3706.6733
		SYM	4065.4573	3802.4402	3880.0740	3780.9067
		GTR	3934.4978	3694.4111	3753.9275	3670.3184
	strict consensus	JC	3899.4060	3691.9502	3750.6201	3673.6838
		F81	3798.3896	3598.1240	3651.9998	3579.4800
		K80	3895.5774	3688.4343	3746.9026	3670.3374
		HKY	3792.0466	3594.6787	3646.6592	3576.1035
		SYM	3869.3467	3660.5283	3720.4067	3642.3318
		GTR	3754.4683	3564.5222	3610.1045	3544.7480

Table 13. continued.

data set	tree	model		+I	+G	+I+G
<i>psbA-trnH</i>	bootstrap	JC	3133.3350	3019.7341	3040.0320	3012.8601
		F81	3049.7639	2937.0066	2956.6150	2930.4692
		K80	3131.0422	3017.4790	3037.7585	3010.6309
		HKY	3045.8557	2933.6218	2952.8213	2927.0684
		SYM	3104.6641	2994.1716	3012.4875	2986.7266
		GTR	3011.6624	2903.0662	2918.2051	2896.1663
	majority rule	JC	3029.7676	2962.3086	2970.7542	2959.7783
		F81	2945.9041	2879.1450	2887.1541	2876.9036
		K80	3028.8745	2961.3845	2969.8438	2958.8552
		HKY	2943.9702	2877.4077	2885.2764	2875.1665
		SYM	3007.2139	2941.2708	2948.8677	2938.2388
		GTR	2913.9829	2849.0581	2855.0952	2846.6946
	neighbor joining	JC	3032.8037	2963.3452	2972.2671	2960.9375
		F81	2950.4160	2881.5193	2890.0259	2879.4082
		K80	3031.7656	2962.2793	2971.2139	2959.8540
		HKY	2948.1931	2879.5920	2887.9434	2877.4507
		SYM	3010.5425	2942.5852	2950.6907	2939.7534
		GTR	2918.5188	2851.5017	2857.9734	2849.3831
	strict consensus	JC	3104.3896	3008.5344	3024.0229	3005.1956
		F81	3017.9041	2923.0107	2937.8201	2920.0845
		K80	3103.2615	3007.3665	3022.8748	3004.0068
		HKY	3015.5889	2921.0024	2935.5745	2918.0361
		SYM	3080.9297	2987.1011	3001.2952	2982.9465
		GTR	2983.8782	2892.0271	2903.3677	2888.7444
combo	bootstrap	JC	7788.7593	7672.2651	7681.8120	7666.8521
		F81	7656.8232	7545.0571	7552.8330	7539.4766
		K80	7754.1382	7637.8394	7647.3008	7632.2988
		HKY	7617.2935	7509.0298	7515.5229	7503.1230
		SYM	7723.5508	7607.8071	7617.0977	7602.2544
		GTR	7578.9243	7475.4380	7480.1265	7469.3506
	majority rule	JC	7769.6978	7661.9780	7669.8472	7656.7310
		F81	7636.7129	7533.6655	7539.9199	7528.3516
		K80	7735.9150	7628.3345	7636.1426	7622.9692
		HKY	7598.0688	7498.3198	7503.4194	7492.7324
		SYM	7706.4014	7599.2749	7606.9580	7593.8057
		GTR	7560.7773	7465.5825	7469.0684	7459.7759
	neighbor joining	JC	7793.0586	7676.3657	7685.9878	7670.9873
		F81	7661.3613	7549.2344	7557.1343	7543.6538
		K80	7758.4048	7641.7856	7651.3647	7636.3047
		HKY	7621.7891	7513.0605	7519.6973	7507.2261
		SYM	7727.7891	7611.7266	7621.1313	7606.2471
		GTR	7583.3535	7479.4297	7484.2295	7473.4116
	strict consensus	JC	7780.1855	7668.8604	7677.4277	7663.4692
		F81	7647.7817	7541.1851	7548.0430	7535.7056
		K80	7747.1030	7635.9111	7644.4175	7630.3931
		HKY	7609.9038	7506.6050	7512.2998	7500.8223
		SYM	7716.4907	7605.7100	7614.0947	7600.1143
		GTR	7571.4854	7472.7793	7476.8062	7466.7930

split frequencies was 0.008445. The sump burnin data is illustrated in Fig. 11a. The consensus tree (Fig. 12) is generated using the “sumt” command with a burnin value of 116 as determined by the generation number at which the likelihood values for the trees became asymptotic (Fig. 11). The ingroup of the tree is supported with a posterior probability of 1.0. The backbone of the ingroup is a polytomy consisting of *T. undulatum* (posterior probability of 1.0), *T. ovatum* var. *hibbersonii* (1.0), The Erectum Group (1.0), and the rest of *Trillium* (0.93). The backbone for the remainder of *Trillium* was also a polytomy consisting of *T. ovatum* (0.99), *T. subgen. Phyllantherum* (1.0), and the rest of *Trillium* (0.97). The remainder of *Trillium* consisted of sister clades containing *T. nivale* and *T. grandiflorum* (1.0) and *Delostylis* (1.0). *Delostylis* consisted of sister clades with *T. catesbaei* and *T. persistens* (1.0) sister to The Pusillum Complex (0.94); once again, *T. catesbaei* is not shown to be monophyletic. *Trillium telmacola* is the basal taxon in the Pusillum Complex. The remainder of the Pusillum Complex (supported with a posterior probability of 0.80) consists of sister clades consisting of *T. pusillum*, *T. texanum* and *T. georgianum* (0.81) and the rest of the Pusillum Complex (1.0). The wetland populations of *T. alabamicum* are sister to the clade containing the upland populations of *T. alabamicum* as well as *T. ozarkanum*, *T. virginianum*, and *T. monticola* (0.90).

3.2.2 matK sequence data.— Bayesian analysis produced trees with the same topology as the parsimony trees (Fig. 8). After 2,000,000 generations, the average standard deviation of split frequencies was 0.017429. The sump burnin data is illustrated in Fig. 11b. The consensus tree (Fig. 13) is generated using the “sumt” command with a burnin value of 183 as determined by the generation number at which the likelihood values for the trees became asymptotic (Fig. 11). The ingroup of the tree is supported with a posterior probability of 1.0. The remainder of the tree is a series of successive sister clades. The most basal group in the ingroup is *T. undulatum* (posterior probability of 1.0) with the rest of the taxa supported at 0.83. Next is *T. ovatum* var. *hibbersonii* (1.0) and the rest of the taxa (0.74). Next is the Erectum Group (0.83) as sister to the rest of the taxa (1.0). The next pair of clades consists of *Delostylis* (0.96) and the rest of the taxa (0.92) which is composed of a polytomy consisting of *T. nivale*, sister taxa *T. ovatum*, and *T. grandiflorum* (1.0), and *T. subgen. Phyllantherum* (1.0). Within *Delostylis*, *T. catesbaei* and *T. persistens* (1.0) form a clade that is sister to the Pusillum Complex (0.72). Again, *T. catesbaei* is not shown to be monophyletic. The Pusillum Complex consists of a polytomy with *T. alabamicum* (0.89), *T. georgianum* and *T. texanum* (0.55), and *T. ozarkanum* (0.78).

3.2.3 psbA-trnH sequence data.— Bayesian analysis produced trees with a similar topology to the parsimony trees (Fig. 9). After 2,000,000 generations, the average standard deviation of split frequencies was 0.007285. The sump burnin data is illustrated in Fig. 11c. The consensus tree (Fig. 14) is generated using the “sumt” command with a burnin value of 134 as determined by the generation

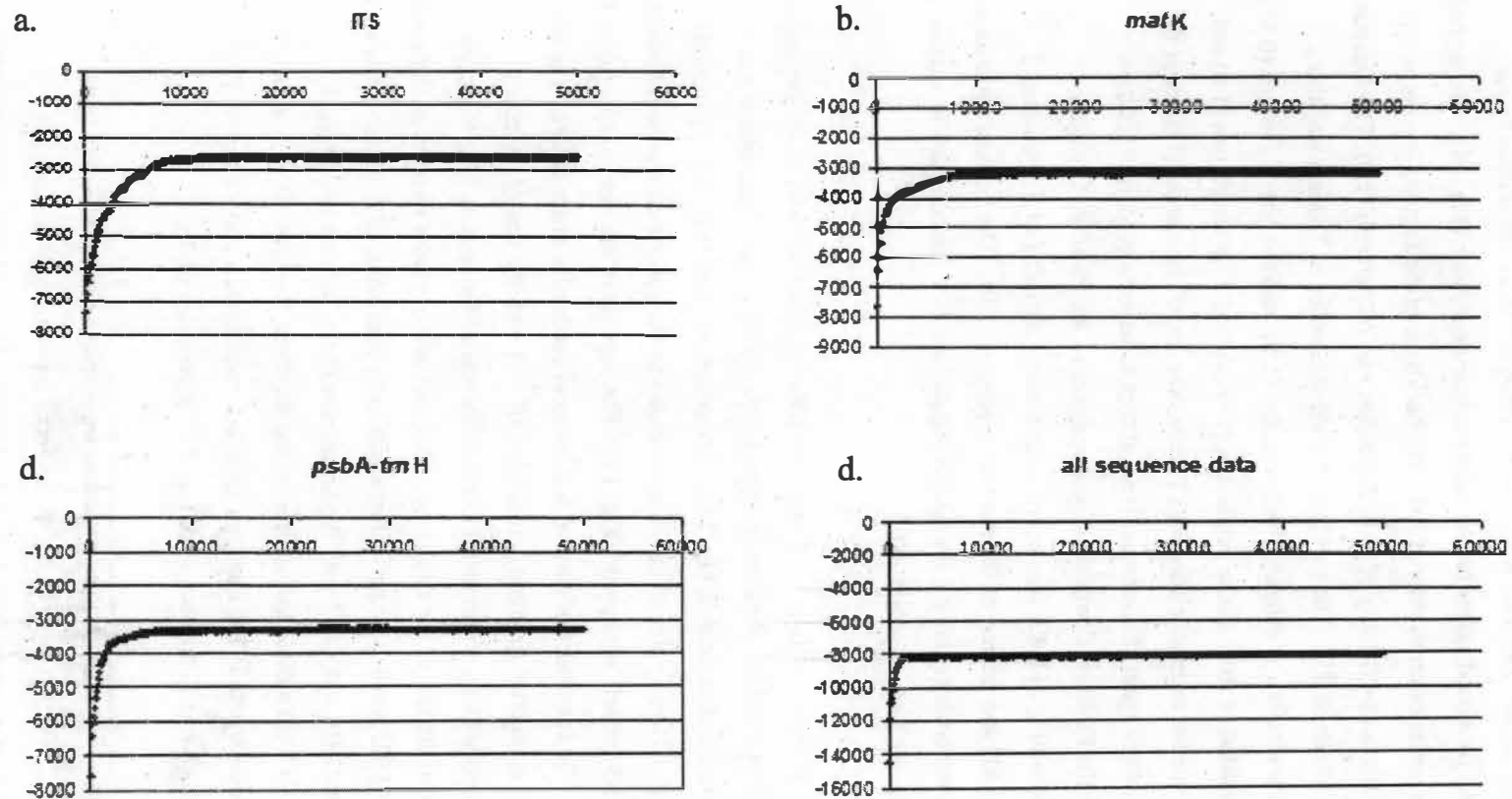


Figure 11. Graphs of the first 50,000 generations of sump values from the Bayesian analyses of *Trillium*.

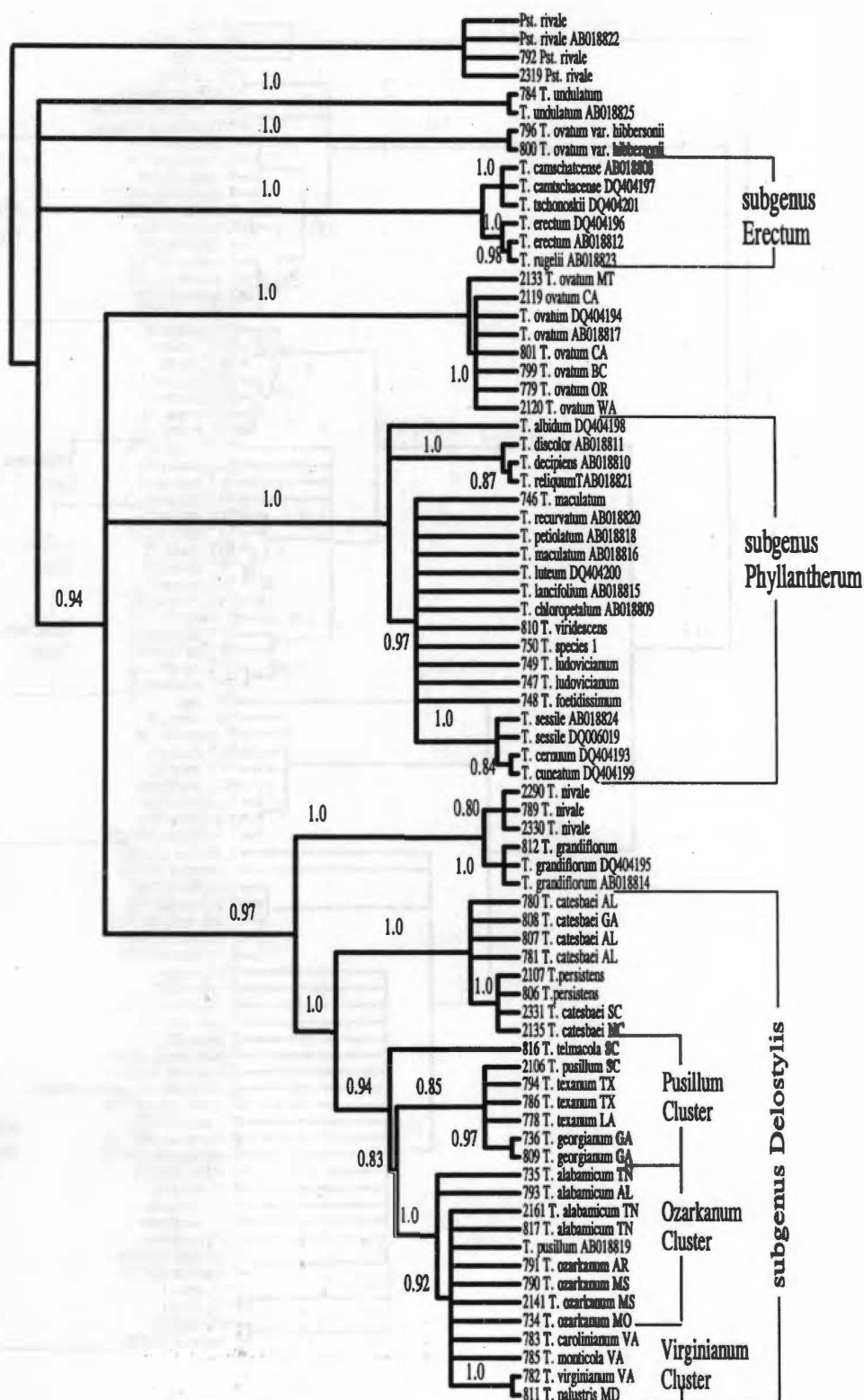


Figure 12. Consensus tree from the bayesian analysis of the ITS sequence data of *Trillium*.

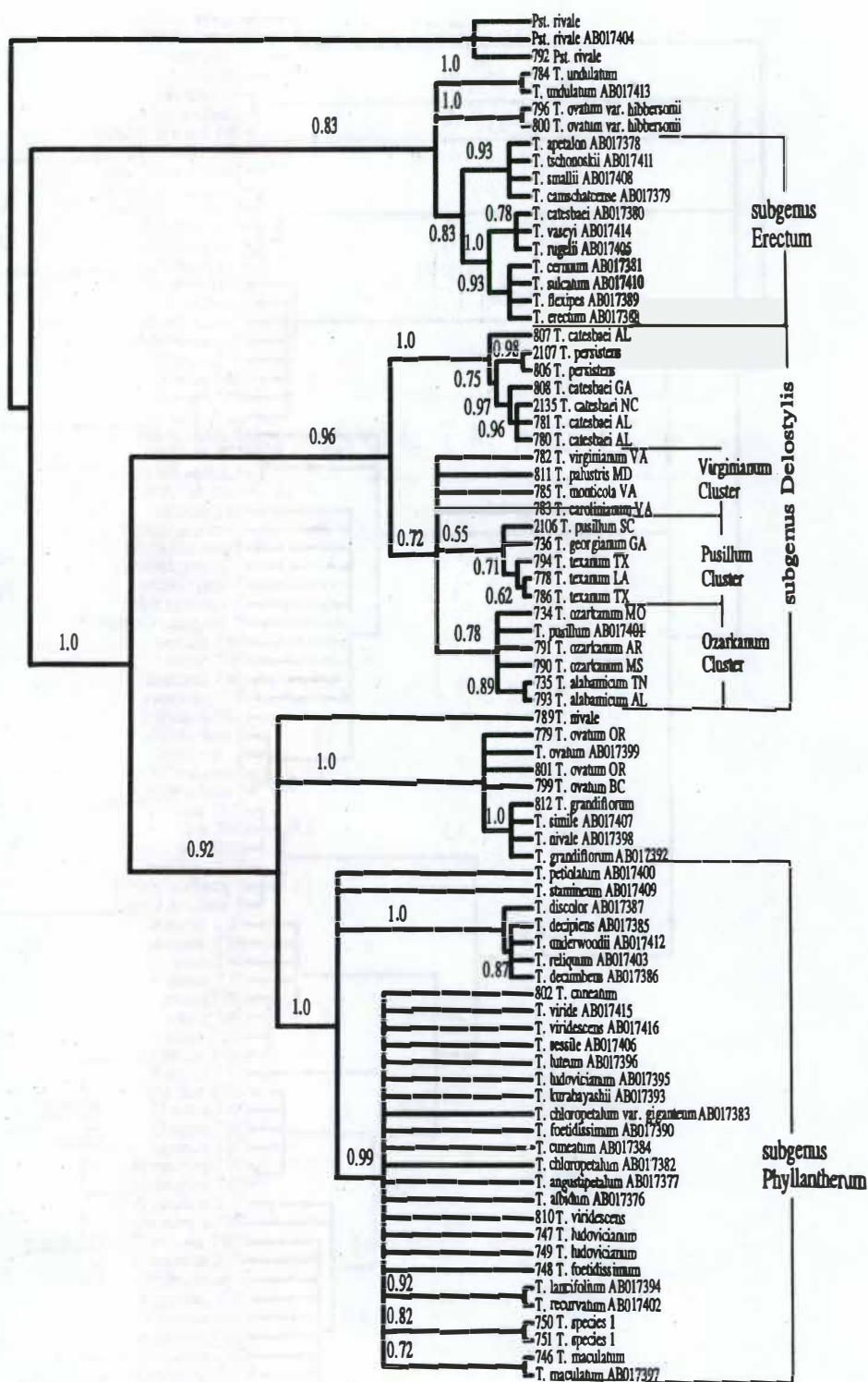


Figure 13. Consensus tree from the bayesian analysis of the *matK* sequence data of *Trillium*.

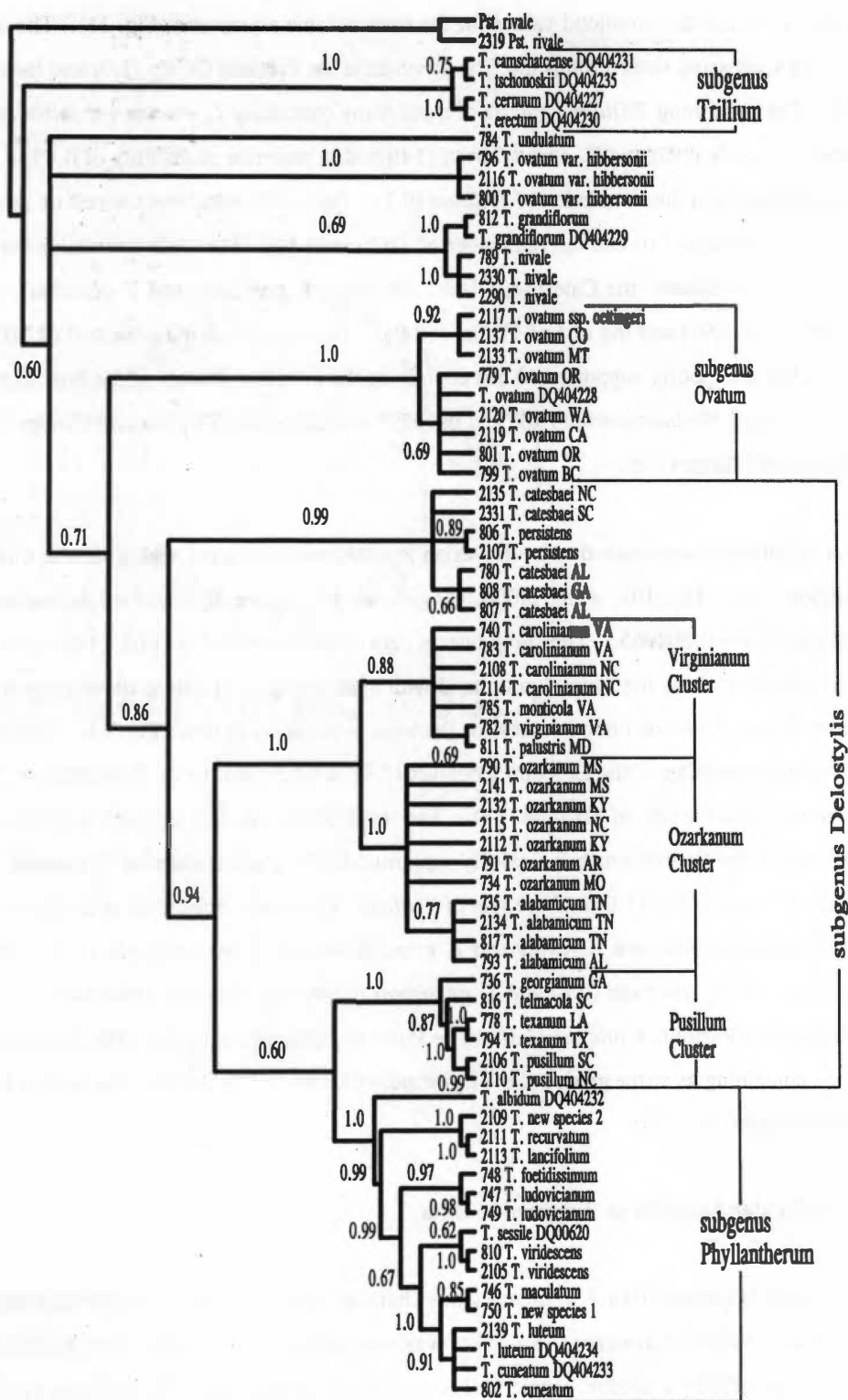


Figure 14. Consensus tree from the bayesian analysis of the *psbA-trnH* sequence data of *Trillium*.

number at which the likelihood values for the trees became asymptotic (Fig. 11). The tree consists of a series of successive sister groups the first of which is the Erectum Group (1.0) and the rest of *Trillium* (0.6). The remaining *Trillium* consists of a polytomy containing *T. ovatum* var. *hibbersonii* (1.0), paired species *T. nivale* (1.0) and *T. grandiflorum* (1.0) with a posterior probability of 0.69, a single sample of *T. undulatum*, and the remainder of *Trillium* (0.7). The clade containing the rest of *Trillium* contains two clades: *T. ovatum* (1.0) and the remainder of *Trillium* (0.86). The clade containing the rest of *Trillium* consists of two clades: the Catesbaei Clade (containing *T. persistens* and *T. catesbaei*; posterior probability of 0.99) and the rest of *Trillium* (0.93). The clade containing the rest of *Trillium* consists of two clades, one poorly supported (0.59) containing the Pusillum Cluster of the Pusillum Complex (1.0) and *T. subgen. Phyllantherum* (1.0), and the other containing the Virginianum Cluster (0.87) and the Ozarkanum Cluster (1.0).

3.2.4 Combined sequence data.— Bayesian analysis produced trees with a similar topology to the parsimony trees (Fig. 10). After 2,000,000 generations, the average standard deviation of split frequencies was 0.004965. The sump burnin data is illustrated in Fig. 11d. The consensus tree (Fig. 15) is generated using the “sumt” command with a burnin value of 240 as determined by the generation number at which the likelihood values for the trees became asymptotic (Fig. 11). The spine of the tree is a polytomy consisting of the Erectum Complex (1.0), a single sample of *T. undulatum*, *T. ovatum* var. *hibbersonii*, and the rest of *Trillium* (1.0). The remainder of the tree consists of a series of successive sister groups the first of which is a poorly supported (0.57) clade containing *T. ovatum* (1.0) and *T. subgen. Phyllantherum* (1.0) and the rest of *Trillium* (also poorly supported with a posterior probability of 0.64). The next clades are *T. nivale* and *T. grandiflorum* (1.0) and *Delostylis* (1.0). *Delostylis* consists of a clade with *T. catesbaei* (0.6) and *T. persistens* (0.99) with posterior probability of 1.0, and a clade containing the Pusillum Complex (1.0). The Pusillum Complex consists of the Pusillum Cluster (1.0) and a clade containing as sister groups the Virginianum Cluster (0.98) and the Ozarkanum Cluster (1.0) together supported at 1.0.

3.3 Molecular Markers as Taxonomic Data

3.3.1 *matK* sequence data.— *Delostylis* was characterized by a single, unique base-pair change (SNP) at position 269 within Trilliaceae; this marker was not exhibited in *T. nivale*. The Pusillum Complex was also characterized by a unique, single SNP at position 344 (Fig. 16); other changes divided it into three taxonomic clusters: The Pusillum Cluster (consisting of samples from SC, TX, LA, and GA; e.g., *T. pusillum*, *texanum*, *georgianum*, and *telmacola*); the Ozarkanum Cluster (consisting of samples from MO, MS, AR, TN, and AL; e.g., *T. ozarkanum* and *alabamicum*); and the Virginianum Cluster (consisting of

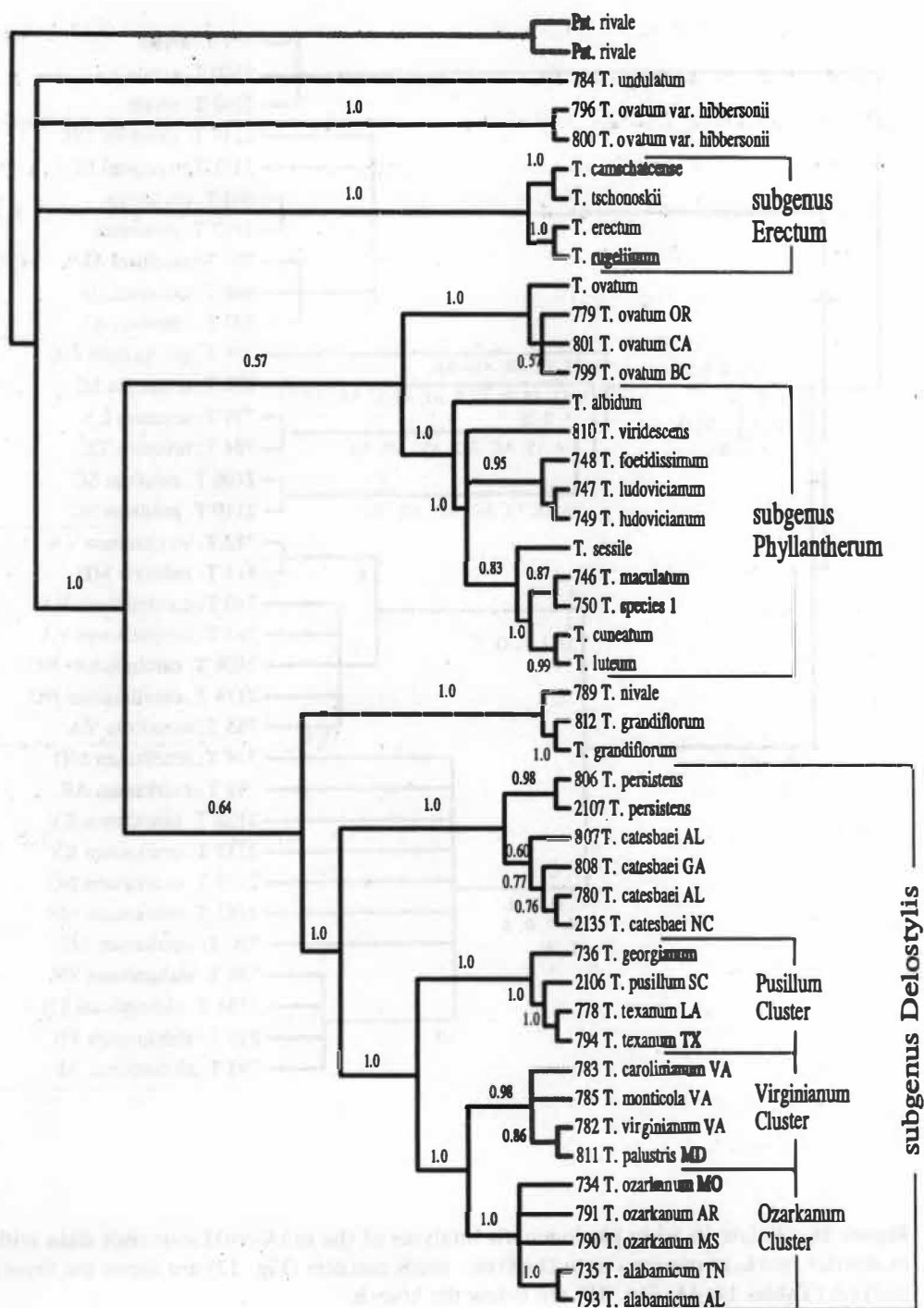


Figure 15. Consensus tree from the bayesian analysis of the combined sequence data of *Trillium*.

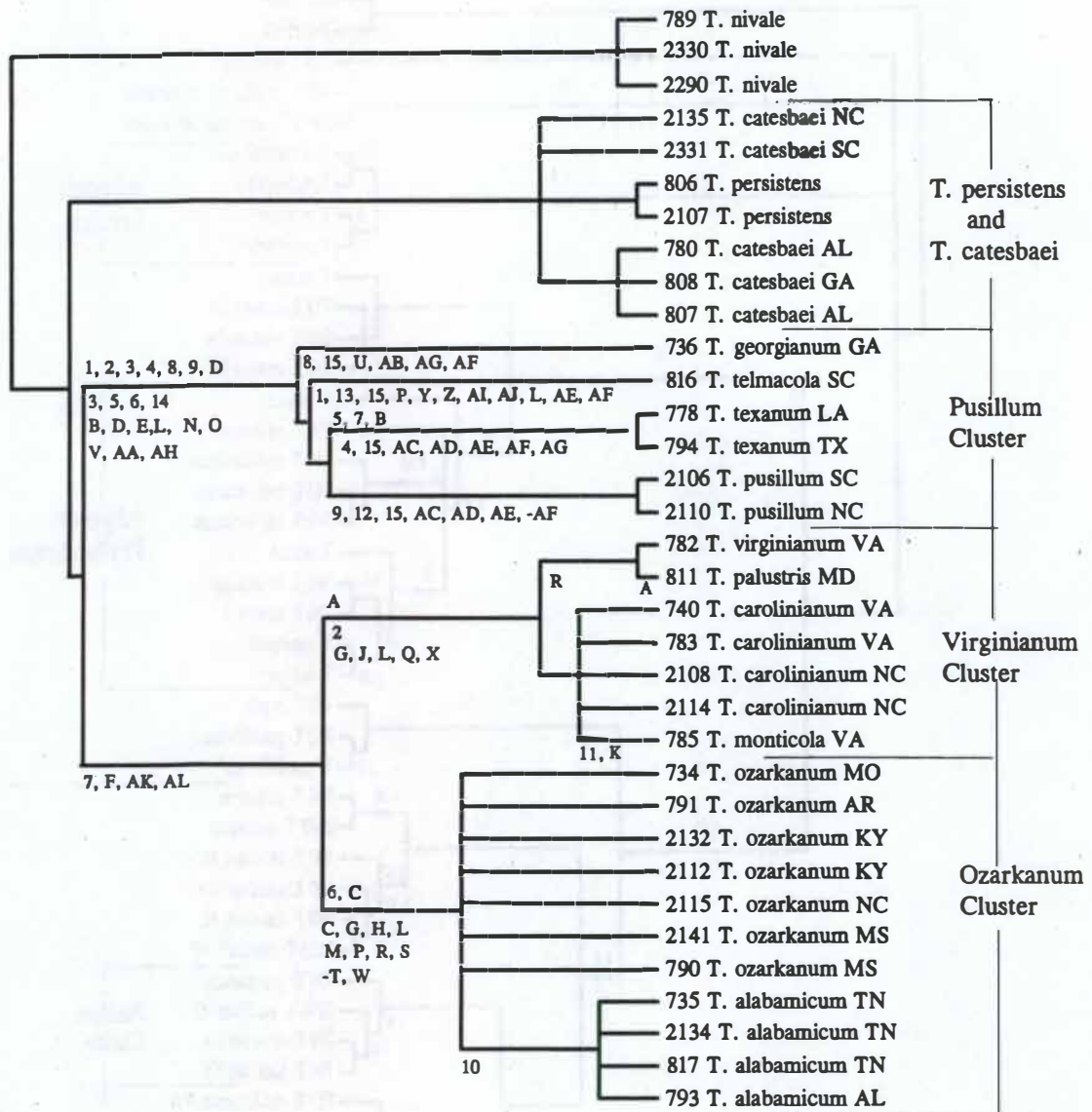


Figure 16. *Delostylis* from phylogenetic analyses of the *psbA-trnH* sequence data with diagnostic molecular markers mapped onto the tree. *matK* markers (Fig. 17) are above the branch; *psbA-trnH* markers (Tables 14, 15; Fig. 18) are below the branch.

samples from VA and MD; e.g., *T. virginianum*, *monticola*, *carolinianum*, and *palustris*) (Fig. 17). Within the *matK* coding region, the Pusillum Cluster was characterized by a 6-base pair deletion which is shared with *T. subgen. Phyllantherum* and a SNP resulting in an amino acid change; in addition, *T. texanum* was characterized by an additional 6-base pair deletion and another SNP. Other markers identifying both the Pusillum Cluster and *T. texanum* were present in the 5'*trnK*-*matK* spacer region, and a deletion identifying the Pusillum Cluster was present in the *matK*-3'*trnK* spacer region. The Ozarkanum Cluster was characterized by a 6-base pair deletion and a SNP; these markers allowed the Kazempour-Osaloo et al. (1999) sample to be identified as belonging to the Ozarkanum Cluster.

3.3.2 *psbA-trnH* sequence data.— The *psbA-trnH* sequence data showed variation down to the taxon level for all members of the Pusillum Complex (Fig. 18). There were 2 insertions (a unique insertion of 6 nucleotides [position 1161] and an insertion of 11 nucleotides [position 131] which has an 8 base-pair motif that is apparently homoplasious with some members of *Paris*) and one unique SNP (location 912) that characterized *Delostylis*; these markers were not exhibited in *T. nivale*. There were six characters (four SNPs and two insertions with one insertion and one SNP being unique) that characterized the Pusillum Complex.

The Pusillum Cluster was characterized by a combination of indels and SNPs which are summarized in Tables 14 and 15. Reference numbers given in parentheses refer to the specific SNP/indel in Fig. 18. The unique indels included a 6-bp deletion (6), a 7-bp insertion (14), and a 2-bp insertion (15); shared indels included a 5-bp insertion that is unique within the Pusillum Cluster (3) but shared with *T. ovatum* var. *hibbersonii*, a 7-bp deletion that is also unique within the Pusillum Cluster (5) but is shared with other *Trillium* species, and the absence of a 6-bp Virginianum/Ozarkanum Cluster insertion (7) that is shared with all other *Trillium* species. The unique SNPs included B, D, E, V; the SNPs shared with other members of the Pusillum Complex but not the Pusillum Cluster included the absence of SNPs present in the Ozarkanum/Virginianum Clusters (F, N, O and AJ), SNPs shared with other members of *Trillium* but unique within the Pusillum Complex included AA shared with *T. subgen. Phyllantherum*, AH shared with *T. grandiflorum*, and AK shared with *Pst. rivale*, *T. grandiflorum*, and *T. nivale*. Within the Pusillum Cluster, *T. texanum* is characterized by two unique indels (a 6-bp insertion [4] and a length polymorphism of 3 bp versus 2 for the rest of the cluster [16]), two unique SNPs (I and AG), and a SNP shared with *T. pusillum* (AC and AD). *T. georgianum* is characterized by one 10-bp unique deletion (8), three unique SNPs (U, AB, and AG), and two SNPs shared with *T. telmacola* (AC and AD). *T. pusillum* is characterized by two unique insertions (one of 9-bp [9] and the other of 6 [12]), and two SNPs shared with *T. texanum* (AC and AD). *Trillium telmacola* is characterized by two unique indels (a 3-bp unique insertion located in the *psbA* sequence [1], and a 5-bp deletion [13]) five unique SNPs (P, Y, Z, AI and AJ), and four shared SNPs (L shared with the

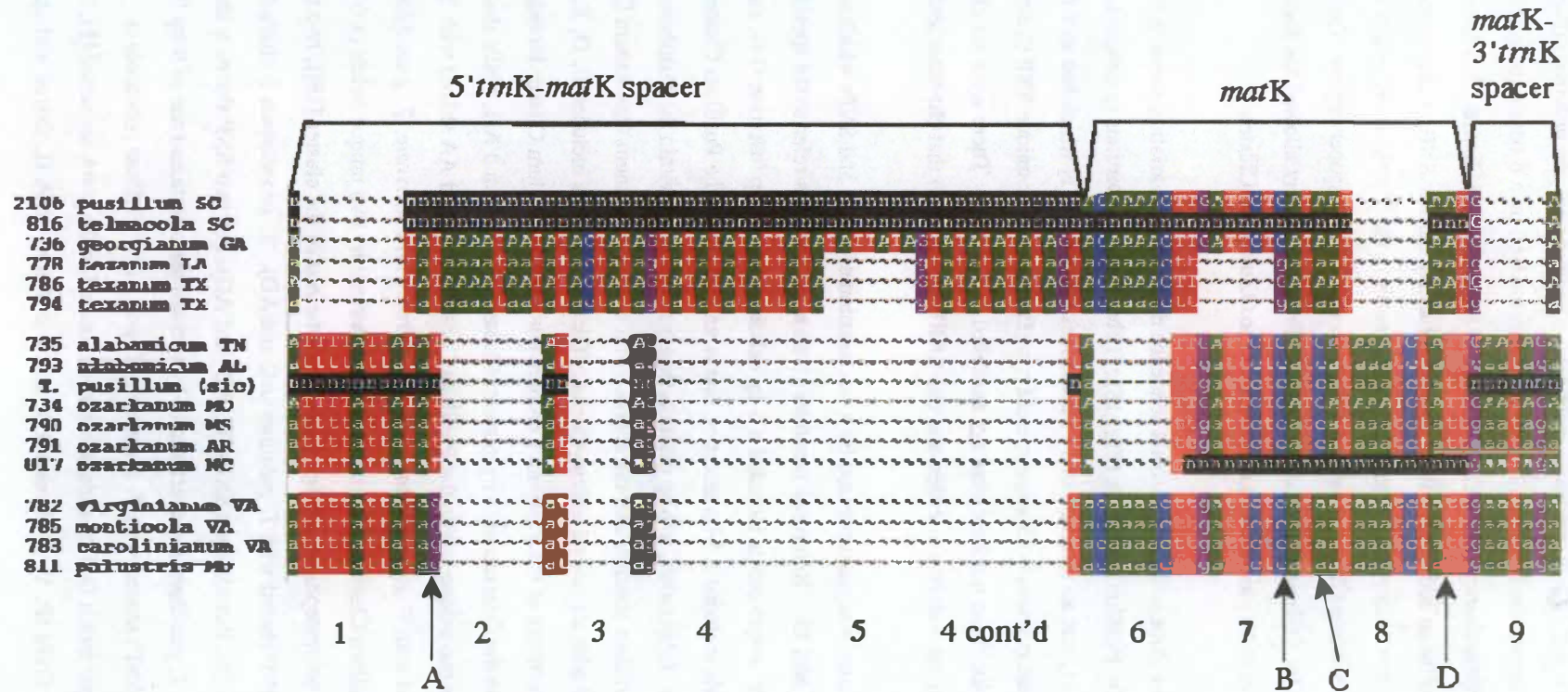


Figure 17. Diagnostic areas of the *5'trnK-matK* spacer region, the *matK* coding region and the *matK-3'trnK* spacer region. Indels are numbered; SNPs are indicated by letters; regions of variability are bounded by invariant nucleotides.

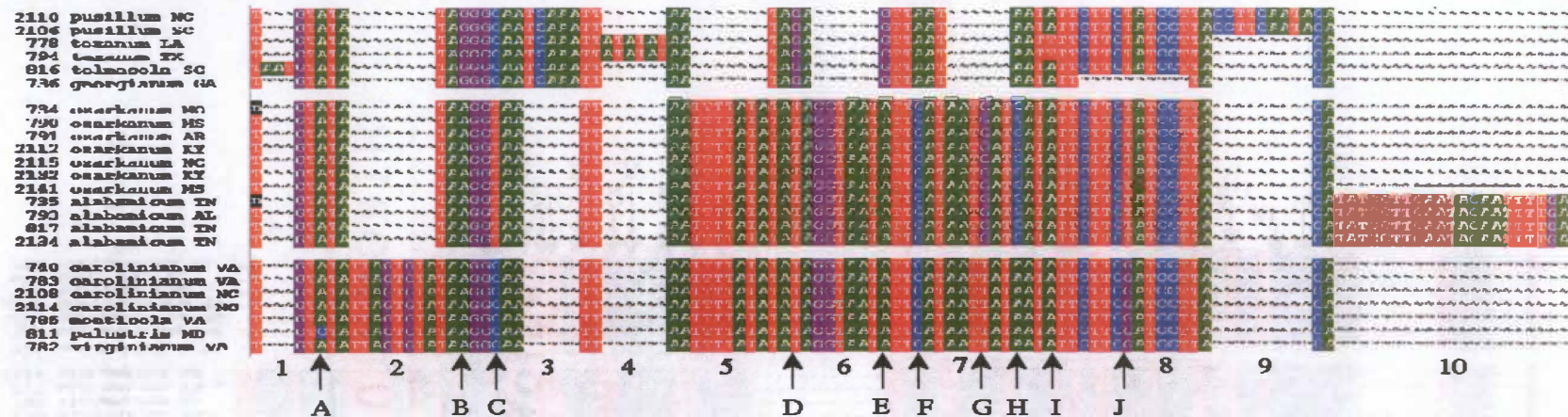


Figure 18. Diagnostic areas of *psbA* (part) and the *psbA-trnH* spacer region. Indels numbered; SNPs indicated by letters; regions of variability are bounded by invariant nucleotides.

734	osarkatum	MS
790	osarkatum	MS
791	osarkatum	AR
2112	osarkatum	KY
2115	osarkatum	NC
2132	osarkatum	KY
2141	osarkatum	MS
735	albanicum	TX
793	albanicum	AL
817	albanicum	TX
2134	albanicum	TX

740	arabitanum	VA
783	carolinianum	VA
2108	carolinianum	NC
2114	carolinianum	NC
785	monticola	VA
811	palustre	MD
782	virginianum	VA

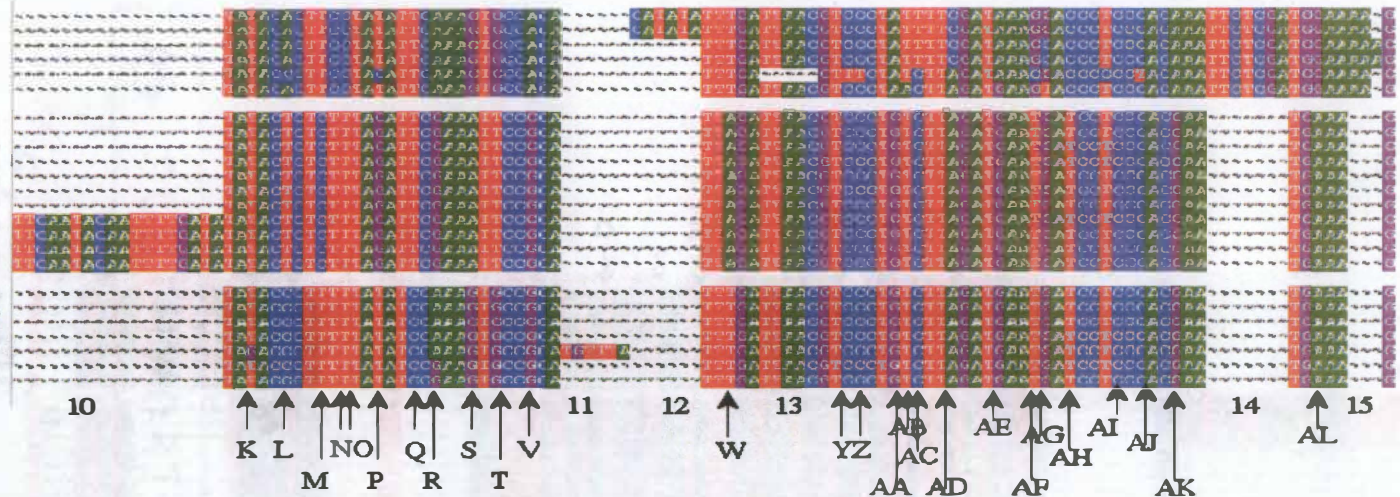


Figure 18. continued.

Table 14. *psbA-trnH* markers that are diagnostic for cluster membership within the Pusillum Complex.

marker	type	change	Cluster	shared or unique
B	SNP	a -> g	Pusillum Cluster	unique
3	insertion	5 bp	Pusillum Cluster	shared with <i>T. ovatum</i> var. <i>hibbersonii</i>
5	deletion	7 bp	Pusillum Cluster	shared with other <i>Trillium</i>
D	SNP	t -> g	Pusillum Cluster	unique
6	deletion	6 bp	Pusillum Cluster	unique
E	SNP	t -> g	Pusillum Cluster	unique
F	SNP	a -> c	absent in Pusillum Cluster	shared with rest of <i>Trillium</i>
7	insertion	6 bp	absent in Pusillum Cluster	absent in rest of <i>Trillium</i>
L	SNP	c -> a	Pusillum Cluster	unique except for <i>T. telmacola</i>
N-O	SNP	t -> c	Pusillum Cluster	shared with other <i>Trillium</i>
V	SNP	g -> a	Pusillum Cluster	unique
AA	SNP	g -> a	Pusillum Cluster	shared with <i>Phyllantherum</i>
AH	SNP	t -> c	Pusillum Cluster	shared with <i>T. grandiflorum</i>
AK	SNP	a -> g	absent in Pusillum Cluster	shared
14	insertion	7 bp	Pusillum Cluster	unique
AL	SNP	g -> a	absent in Pusillum Cluster	unique
C	SNP	c -> t	Ozarkanum Cluster	unique
7/G	SNP	t	Ozarkanum Cluster	unique
H	SNP	a -> c	Ozarkanum Cluster	unique
L	SNP	a -> t	Ozarkanum Cluster	unique
M	SNP	t -> c	Ozarkanum Cluster	unique
P	SNP	t -> g	Ozarkanum Cluster	unique
R	SNP	a -> g	Ozarkanum Cluster	shared with Coastal Plain species
S	SNP	g -> a	Ozarkanum Cluster	unique
T	SNP	t -> g	absent in Ozarkanum Cluster	shared with <i>Phyllantherum</i>
W	SNP	t -> a	Ozarkanum Cluster	unique
2	insertion	8 bp	Virginianum Cluster	unique
7/G	SNP	t	Virginianum Cluster	unique
J	SNP	t -> g	Virginianum Cluster	unique
L		a -> c	Virginianum Cluster	shared with <i>T. telmacola</i>
Q	SNP	t -> c	Virginianum Cluster	unique
X	SNP	a -> c	Virginianum Cluster	unique

Table 15. *psbA-trnH* markers that are taxon specific within the *Pusillum* Complex.

marker	type	change	taxon	shared or unique
4	insertion	6 bp	<i>T. texanum</i>	unique
I	SNP	a -> t	<i>T. texanum</i>	unique
AC	SNP	c -> t	<i>T. texanum</i>	shared with <i>T. pusillum</i>
AD	SNP	a -> c	<i>T. texanum</i>	shared with <i>T. pusillum</i> , basal <i>Phyllanthum</i> and <i>T. ovatum</i> s.l.
AE	SNP	g -> a	<i>T. texanum</i>	shared with <i>T. pusillum</i> , and <i>T. telmacola</i>
AF	SNP	c -> g	<i>T. texanum</i>	shared with <i>T. georgianum</i> and with other <i>Trillium</i>
AG	SNP	g -> c	<i>T. texanum</i>	unique
15	length polymorphism	3 bp	<i>T. texanum</i>	unique length
8	deletion	9 bp	<i>T. georgianum</i>	unique
U	SNP	g -> t	<i>T. georgianum</i>	unique
AB	SNP	t -> a	<i>T. georgianum</i>	unique
AF	SNP	c -> g	<i>T. georgianum</i>	shared with <i>T. texanum</i> and with other <i>Trillium</i>
AG	SNP	g -> t	<i>T. georgianum</i>	unique
15	length polymorphism	2 bp	<i>T. georgianum</i>	length shared with <i>T. pusillum</i> , and <i>T. telmacola</i>
9	insertion	9 bp	<i>T. pusillum</i>	unique
12	insertion	6 bp	<i>T. pusillum</i>	unique
AC	SNP	c -> t	<i>T. pusillum</i>	shared with <i>T. texanum</i>
AD	SNP	a -> c	<i>T. pusillum</i>	shared with <i>T. texanum</i> , basal <i>Phyllanthum</i> and <i>T. ovatum</i> s.l.
AE	SNP	g -> a	<i>T. pusillum</i>	shared with <i>T. texanum</i> and <i>T. telmacola</i>
AF	SNP	c -> g	<i>T. pusillum</i>	absent in <i>T. pusillum</i> ; shared w/ <i>T. georgianum</i> and other <i>Trillium</i>
15	length polymorphism	2 bp	<i>T. pusillum</i>	length shared with <i>T. georgianum</i> , and <i>T. telmacola</i>
1	insertion	3 bp	<i>T. telmacola</i>	unique
L	SNP	a -> c	<i>T. telmacola</i>	shared with Virginianum Cluster
P	SNP	t -> c	<i>T. telmacola</i>	unique
13	deletion	5 bp	<i>T. telmacola</i>	unique
Y-Z	SNP	c -> t	<i>T. telmacola</i>	unique
AF	SNP	t -> c	<i>T. telmacola</i>	unique
AI	SNP	t -> c	<i>T. telmacola</i>	unique
AJ	SNP	c -> t	<i>T. telmacola</i>	unique
AE	SNP	g -> a	<i>T. telmacola</i>	shared with <i>T. texanum</i> , and <i>T. pusillum</i>
15	length polymorphism	2 bp	<i>T. telmacola</i>	length shared with <i>T. georgianum</i> and <i>T. pusillum</i>
10	insertion	23 bp	<i>T. alabamicum</i>	unique
K	SNP	t -> g	<i>T. monticola</i>	unique
11	insertion	6 bp	<i>T. monticola</i>	unique
R	SNP	a -> g	<i>T. virginianum</i> and <i>T. palustris</i>	shared with Ozarkanum Cluster
A	SNP	a -> c	<i>T. palustris</i>	unique

Virginianum Cluster, the absence of AC and AD shared with *T. georgianum*, and AF shared with other *Trillium* species).

The Ozarkanum Cluster is characterized by a seven unique SNPs (C, G, H, absence of L, M, S, absence of T, and W) and two shared SNPs (P state shared with *T. subgen. Phyllantherum* and R shared with *T. palustris*). Within the Ozarkanum Cluster, *T. alabamicum* is characterized by the presence of a 23 base pair insertion (10).

The Virginianum Cluster is characterized by one unique 8-bp insertion (2), 4 unique SNPs (G, J, Q, and X), and one shared SNP (L shared with *T. telmacola*). Within the Virginianum Cluster, *T. virginianum* has no diagnostic markers, *per se*; it is what remains after everything else has been characterized. *T. monticola* is characterized by a unique 6-bp insertion (11) and a unique SNP (K). The coastal plain plants are characterized by SNP R which is shared with the Ozarkanum Cluster; *T. palustris* is characterized by the unique SNP A.

3.4 Morphological Analysis

Because the phylogenetic analysis combined with the DNA sequence variation clearly indicated that *Trillium pusillum* is composed of three major groups, the morphological analysis was conducted on the basis of these groups collectively as well as individually rather than the more traditionally recognized single species. Once differences were noticed in the DNA sequence data, they were compared to the taxonomic boundaries used by Garrett (1982); when novel differences were noted, ecological, geographic and physiographic data were examined to determine putative geographic boundaries for each taxon.

The standard descriptive statistics for the taxa including N, mean, standard deviation, variance, minimum, maximum, skewness, and kurtosis are provided in **Appendix C**. Input files, scripts, box plots and error-bar plots are available from the author.

3.4.1 The Pusillum Complex.— Standard descriptive statistics of the variables showed some cluster-specific trends (Table 16). The Virginianum Group had the shortest pedicels (greater than one standard deviation from the group mean). Completely non-overlapping means (95% CI) were observed for sepal width, petal width, and the pedicel to sepal ratio. For the Ozarkanum Cluster, means were different than those of the Pusillum Cluster and the Virginianum Cluster for stem length, leaf width, sepal length, petal shape, anther length, stigma length, stamen length, and pistil length. For the Pusillum Cluster, means were different than those of the Ozarkanum Cluster and the Virginianum Cluster for style length, stem to leaf length ratio, and leaf shape. For the Virginianum Cluster, means were different than those of the Ozarkanum Cluster and the Pusillum Cluster for leaf length, pedicel length, filament length, stem to pedicel ratio, petal shape, anther to filament ratio and filament to ovary ratio.

Table 16. Means for the standard morphometric characters for the clusters of the Pusillum Complex. Shape is a ratio calculated based on length/width. Bold characters are those whose means are significantly different based on 95 % confidence interval.

	ozarkanum cluster	pusillum cluster	virginianum cluster	cluster	Std. Dev.
stem	171.70	130.39	136.70	153.96	45.15
leaf length	49.55	46.46	39.58	45.67	11.64
leaf width	16.39	13.09	12.63	14.64	4.61
leaf shape	3.11	3.89	3.25	3.26	0.82
pedicel	22.45	21.11	1.67	15.06	11.90
sepal length	24.34	19.54	17.74	21.40	5.82
sepal width	8.25	6.80	5.12	6.97	2.48
sepal shape	3.05	2.93	3.57	3.21	0.68
petal length	23.46	19.57	18.81	21.32	4.47
petal width	9.15	7.48	5.43	7.63	2.61
petal shape	2.66	2.74	3.58	2.99	0.74
filament	4.01	3.77	4.23	4.06	0.74
anther	6.09	5.02	4.41	5.36	1.59
stamen	10.12	8.79	8.64	9.42	1.85
ovary	3.48	3.37	3.04	3.31	0.87
style	3.92	2.93	3.41	3.61	1.19
stigma	2.95	2.23	2.39	2.66	1.00
pistil	10.45	8.53	8.84	9.63	2.29
stem: leaf	3.52	2.80	3.57	3.44	0.83
stem: pedicel	8.40	7.38	123.39	48.17	73.21
pedicel: sepal	0.93	1.06	0.10	0.66	0.47
sepal: petal	1.04	1.01	0.95	1.00	0.15
anther: filament	1.55	1.35	1.07	1.35	0.44
filament: ovary	1.23	1.19	1.43	1.29	0.38
ovary: stigma	1.32	1.64	1.51	1.43	0.86
stigma: anther	0.51	0.45	0.56	0.52	0.20
anther: ovary	1.85	1.56	1.49	1.69	0.82
stigma: style	0.86	0.82	0.76	0.82	0.45
stamen: pistil	0.99	1.05	0.99	1.00	0.15

The results of discriminant analysis indicated that the Pusillum Cluster, the Ozarkanum Cluster, and the Virginianum Cluster were distinct from one another to varying degrees. Using the group boundaries as indicated by the DNA analysis, 91.2% of the total cases were correctly classified (Table 17); information on the misclassified samples is provided in Table 18. The group centroids (Table 19) also indicated the distinctness of the groups and indicated which clusters were separated out by which function. The discriminate analysis defined two new orthogonal multivariate variables both of which were significant (Wilks Lambda test, Table D.1) and accounted for 100% of the variation (Table D.2). In addition, these functions were relatively well correlated with group membership (canonical correlation for the first function of 92.5%; Table D.2). The first function had large negative coefficients (in order of descending magnitude) for anther length and the pedicel to sepal length ratio; it had large positive coefficients for stamen length, pedicel length and the anther to filament ratio (Table D.3) indicating the relative importance of those characters in contributing to the discriminant functions. The larger the absolute value of the coefficient, the more important that character is in separating groups with that function. Opposite signs for the coefficients indicate that the group separation can be determined by differences between the variables (Field 2005). The second function had large negative coefficients for leaf length, petal length, and pedicel length; and large positive coefficients for leaf shape and leaf width. The structure matrix (Table D.4) indicates the correlations between the characters and the functions providing information about the nature of the functions. The first function, therefore, was primarily one of pedicel characters; the second function was primarily one of lengths including petal, stem and sepal as well as leaf shape. The individual specimens and the taxon centroids are shown in discriminant space in Fig. 19.

3.4.2 The Pusillum Cluster.— Standard descriptive statistics of the variables showed some species-specific trends (Table 20). *Trillium texanum* was generally the tallest species in this group. *Trillium telmacola* had the largest leaves, widest sepals, shortest petals, shortest style, longest stigma, and shortest stem to leaf ratio (all more than one standard deviation from the group mean) and the greatest sepal to petal ratio and greatest stigma to style ratio (both more than two standard deviations from the group mean). *Trillium georgianum* had the largest leaf length to width ratio (greater than one standard deviation from the group mean). It should be noted that if looking at the Pusillum Complex as a whole, the *Trillium georgianum* leaf length to width ratio was greater than two standard deviations from the mean. Though not included in the statistical analysis, *T. texanum* and *T. georgianum* could easily be distinguished from the other members of this group by having stomates on the upper surface of the leaf; these are the only two pedicellate *Trillium* to exhibit such a character.

The results of discriminant analysis indicate that the taxa in the Pusillum Cluster (e.g., *T. pusillum*, *T. texanum*, *T. georgianum*, and *T. telmacola*) are completely distinct from one another.

Table 17. Discriminant model classification results for the clusters of the Pusillum Complex. 91.2% of original grouped cases correctly classified.

taxon	Predicted Group Membership		
	1	2	3
Ozarkanum Cluster	88.6 % 78 samples	11.4 % 10 samples	0
Pusillum Cluster	17.4 % 4 samples	78.3 % 18 samples	4.3 % 1 sample
Virginianum Cluster	0	0	100%

Table 18. Casewise statistics for misclassified cases for the clusters of the Pusillum Complex.

Case Number	Specimen	Actual Group	Predicted Group	Discriminant Scores	
				Fxn 1	Fxn 2
8	alALi_0068-10	1	2	-1.309	-0.768
19	alALms_0074-3	1	2	-2.588	-0.757
20	alALms_0074-5	1	2	-0.471	-1.322
24	alALmo_0986-5	1	2	-0.390	-1.453
26	alALja_0067-2	1	2	-3.258	-1.093
33	alTNpu_0299-2	1	2	-1.317	-1.884
42	ozTnsu_1610-2	1	2	-0.464	-1.301
46	ozKYwa_1143-4	1	2	-2.714	-0.873
57	ozARpo_1575-1	1	2	-2.493	-1.445
102	ozNCcl_1149-5	1	2	-0.116	-0.847
118	puNCpe_1166-4	2	1	-3.108	-0.264
122	puSCca_0114-1	2	1	0.286	-0.535
128	puSCdo_0115-3	2	3	0.745	-0.933
136	txTXsm_0251-1	2	1	-1.758	0.177
145	txLAca_0247-1	2	1	-1.914	-0.316

Table 19. Centroids of the clusters of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.

taxon	Function	
	1	2
Ozarkanum Cluster	-1.735	0.507
Pusillum Cluster	-1.873	-1.188
Virginianum Cluster	3.318	-0.020

Canonical Discriminant Functions

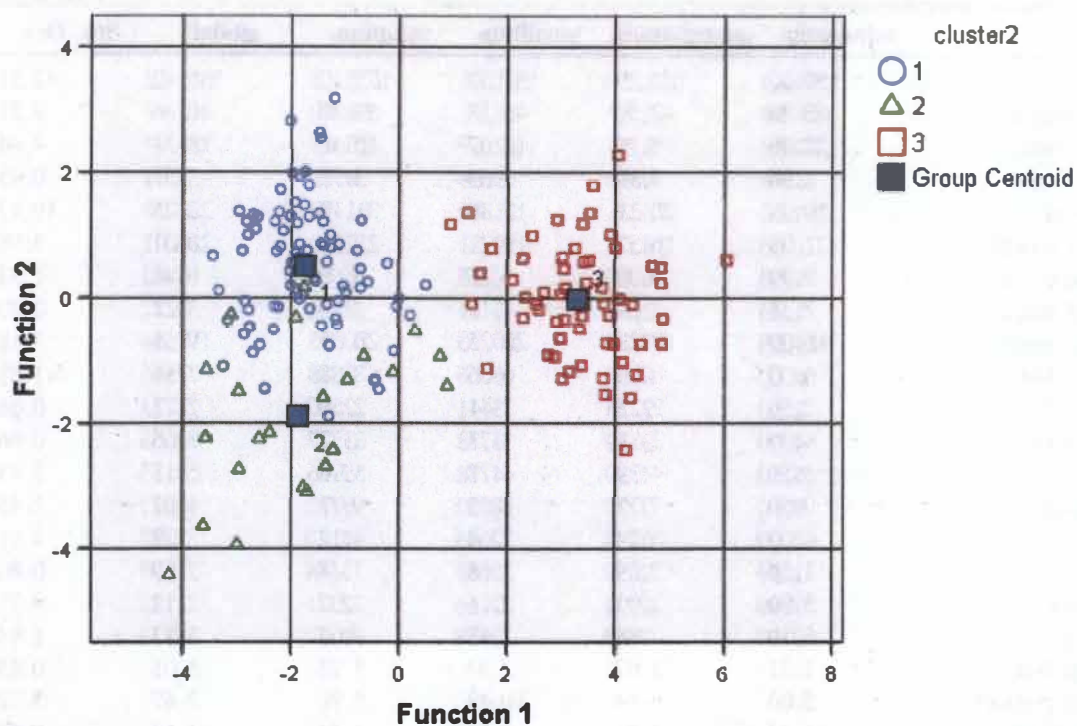


Figure 19. Two-dimensional plot of function scores of the individual specimens and cluster centroids from the discriminant analysis of the clusters of the *Pusillum* Complex. Legend: cluster2=1 is the *Ozarkanum* Cluster, cluster2=2 is the *Pusillum* Cluster, and cluster2=3 is the *Virginianum* Cluster.

Table 20. Means for the standard morphometric characters for the taxa of the Pusillum Cluster of the Pusillum Complex. Shape is a ratio calculated based on length/width. **Bold numbers** are more than one standard deviation from the global mean of the Pusillum Complex; ***bold italicized*** are more than 2 standard deviations from the global mean.

	telmacola	georgianum	pusillum	texanum	global	Std. Dev.
stem	150.00	111.29	131.33	175.13	145.42	42.31
leaf length	65.00	42.50	45.33	53.80	48.96	9.31
leaf width	22.00	8.79	12.67	15.43	13.34	4.64
leaf shape	2.96	4.89	3.63	3.73	3.91	0.93
pedicel	30.00	20.21	13.80	31.13	22.25	10.17
sepal length	21.00	16.57	19.21	22.20	20.01	3.98
sepal width	9.00	6.00	6.25	6.60	6.43	1.84
sepal shape	2.33	2.81	3.11	3.66	3.27	0.80
petal length	15.00	17.29	20.25	20.85	19.54	3.18
petal width	6.00	8.00	6.06	8.88	7.56	1.83
petal shape	2.50	2.25	3.41	2.50	2.73	0.68
filament	4.00	3.39	3.73	3.77	3.68	0.66
anther	5.50	4.39	4.78	5.96	5.13	1.13
stamen	9.50	7.77	8.52	9.73	8.81	1.45
ovary	4.00	3.24	2.64	4.12	3.32	1.01
style	1.50	2.59	2.68	3.48	2.89	0.80
stigma	3.00	2.01	2.16	2.07	2.12	0.73
pistil	8.50	7.84	7.48	9.67	8.33	1.84
stem: leaf	2.31	2.67	2.93	3.28	3.01	0.63
stem: pedicel	5.00	5.94	10.43	5.90	7.67	3.72
pedicel: sepal	1.43	1.21	0.68	1.44	1.13	0.47
sepal: petal	1.40	0.97	0.92	1.08	1.01	0.15
anther: filament	1.38	1.31	1.31	1.64	1.43	0.38
filament: ovary	1.00	1.06	1.44	0.94	1.17	0.30
ovary: stigma	1.33	1.62	1.39	2.14	1.69	0.57
stigma: anther	0.55	0.46	0.45	0.35	0.42	0.14
anther: ovary	1.38	1.36	1.86	1.53	1.62	0.44
stigma: style	2.00	0.85	0.85	0.60	0.81	0.40
stamen: pistil	1.12	0.99	1.16	1.03	1.08	0.16

Using taxonomic boundaries as defined by the DNA analysis, 100% of the total cases were correctly classified (Table 21). This can also be seen in the group centroids (Table 22). The discriminate analysis defined three new orthogonal multivariate variables of which the first two were significant (Wilks Lambda test, Table D.5) and accounted for 98.4% of the variation (Table D.6). In addition, these functions were relatively well correlated with group membership (canonical correlations of over 98.3%; Table D.6). The first function (Table D.7) had large negative coefficients (in order of descending magnitude) for filament length, sepal shape, sepal width, stigma length and leaf length; it had large positive coefficients for ovary length, sepal length, leaf width, and filament to ovary length indicating the relative importance of those characters. The second function had large negative coefficients for leaf length, petal length, and pedicel length; and large positive coefficients for leaf shape and leaf width. The structure matrix, which provides information about the nature of the functions, is shown in Table D.8. The first function, therefore, was primarily one stem and anther length; the second function was primarily one of leaf shape, sepal and petal lengths; and the third function was not significant and contributes little to the analysis. The individual specimens and the taxon centroids are shown in discriminant space in Fig. 20.

3.4.3 The Ozarkanum Cluster.— Standard descriptive statistics of the variables showed some species-specific trends (Table 23). *Trillium ozarkanum* is generally the more robust of the two taxa. Characters with non-overlapping means (95% CI) are stem, leaf length and width, pedicel length, sepal length and width, petal length and width, filament length, style length, and pistil length.

The results of discriminant analysis indicated that these taxa were generally distinct from one another (Fig. 21). Because the analysis has only 2 groups, there is only one function, and thus one axis for the graph; in order to produce a two-dimensional scatter-plot, a third taxon had to be added (*T. telmacola*). Using taxonomic boundaries as defined by the DNA analysis, 81.8% of the total cases were correctly classified (Table 24). Taxonomic membership ranged from 77.8% of *T. alabamicum* identified correctly (28 of 36 individuals) to 84.6% of *T. ozarkanum* correctly classified (44 of 52 individuals) by the discriminant model. If there were no distinctions between the 2 groups, the model would be expected to correctly classify only 51.65% of the groups by chance based on sample size. Information on misclassified samples is given in Table 25. The group centroids (Table 26) also indicated the distinctness of the groups and indicates which clusters were separated out by which function. The discriminate analysis defined one new orthogonal multivariate variable or dimension which defined a new function accounting for all of the variation. In addition, this function is relatively well correlated with group membership (canonical correlations of 65.9%; Table D.9). The standardized canonical discriminant coefficients for the function are provided in Table D.10. The function had large negative coefficients for (in order of descending magnitude) sepal length, stamen length, stem length, sepal shape, sepal width,

Table 21. Discriminant model classification results for the Pusillum Cluster of the Pusillum Complex. 100 % of original grouped cases correctly classified.

taxon	Predicted Group Membership			
	3	4	5	6
3. <i>T. georgianum</i>	100.0 % 7 samples	0	0	0
4. <i>T. pusillum</i>	0	100.0 % 8 samples	0	0
5. <i>T. telmacola</i>	0	0	100.0 % 1 sample	0
6. <i>T. texanum</i>	0	0	0	100.0 % 7 samples

Table 22. Centroids of the taxa of the Pusillum Cluster of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.

taxon	Function		
	1	2	3
<i>T. georgianum</i>	-9.816	6.551	-0.665
<i>T. pusillum</i>	-12.589	-4.803	1.130
<i>T. telmacola</i>	5.379	-7.788	-9.456
<i>T. texanum</i>	23.435	0.051	0.725

Canonical Discriminant Functions

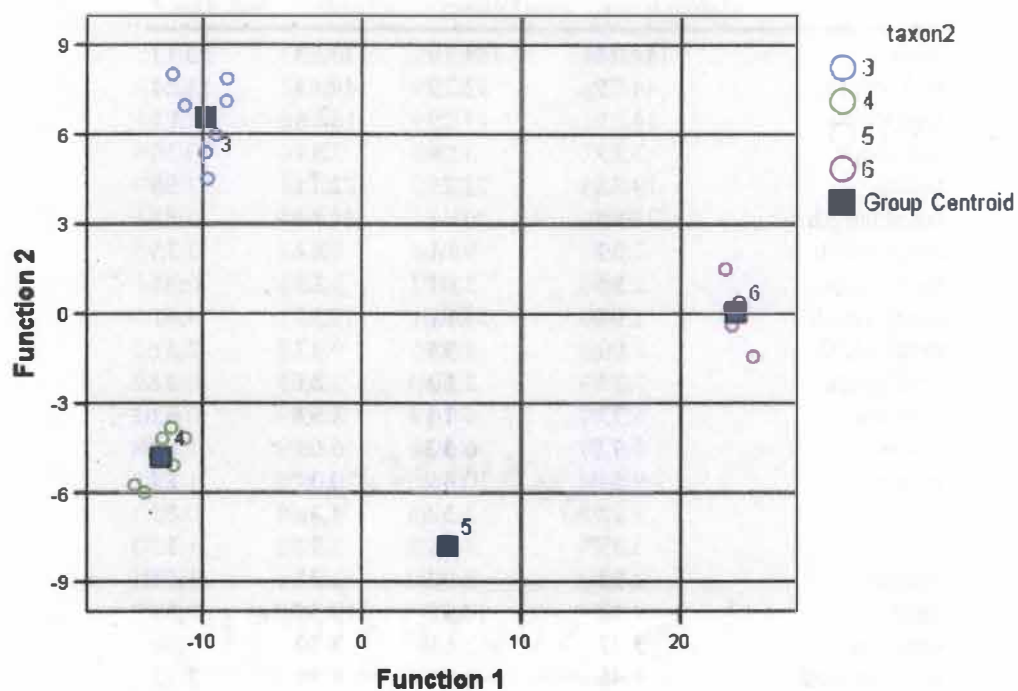
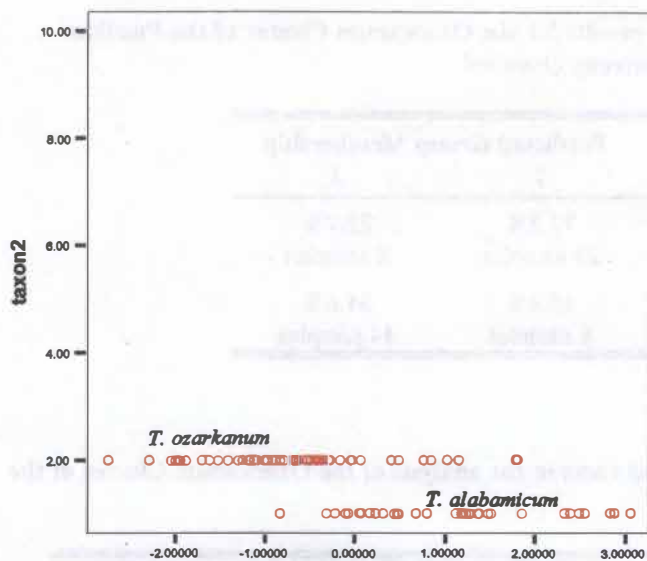


Figure 20. Two-dimensional plot of function scores of the individual specimens and taxon centroids from the discriminant analysis of the Pusillum Cluster of the Pusillum Complex. Legend: taxon2=3 is *Trillium georgianum*, taxon2=4 is *T. texanum*, taxon2=5 is *T. telmacola*, and taxon2=6 is *T. pusillum*.

Table 23. Means for the standard morphometric characters for members of the Ozarkanum Cluster of the Pusillum Complex. Shape is a ratio calculated based on length/width. **Bold characters** are those whose means are significantly different based on 95% confidence interval.

	alabamicum	ozarkanum	cluster	Std. Dev.
stem	147.24	184.19	170.33	50.13
leaf length	44.78	52.29	49.43	12.24
leaf width	14.31	17.29	16.16	4.71
leaf shape	3.23	3.09	3.14	0.56
pedicel	19.38	23.79	22.11	7.90
sepal length	21.36	26.44	24.54	6.43
sepal width	7.39	9.04	8.42	2.75
sepal shape	2.96	3.07	3.03	0.61
petal length	21.08	25.01	23.51	4.61
petal width	8.00	9.95	9.17	2.51
petal shape	2.73	2.60	2.65	0.52
filament	3.77	4.14	3.99	0.62
anther	5.77	6.30	6.09	1.68
stamen	9.54	10.46	10.09	1.88
ovary	3.28	3.59	3.46	0.83
style	3.39	4.28	3.92	1.33
stigma	2.92	2.90	2.91	1.01
pistil	9.58	10.92	10.38	2.38
stem: leaf	3.32	3.61	3.50	0.89
stem: pedicel	8.48	8.34	8.39	3.32
pedicel: sepal	0.89	0.92	0.91	0.25
sepal: petal	1.02	1.05	1.04	0.16
anther: filament	1.56	1.55	1.55	0.46
filament: ovary	1.19	1.24	1.22	0.41
ovary: stigma	1.20	1.41	1.33	0.64
stigma: anther	0.51	0.49	0.50	0.20
anther: ovary	1.77	1.92	1.86	1.02
stigma: style	0.97	0.77	0.85	0.51
stamen: pistil	1.01	0.99	1.00	0.16



Discriminant Scores from Function 1 for Analysis 1

Canonical Discriminant Functions

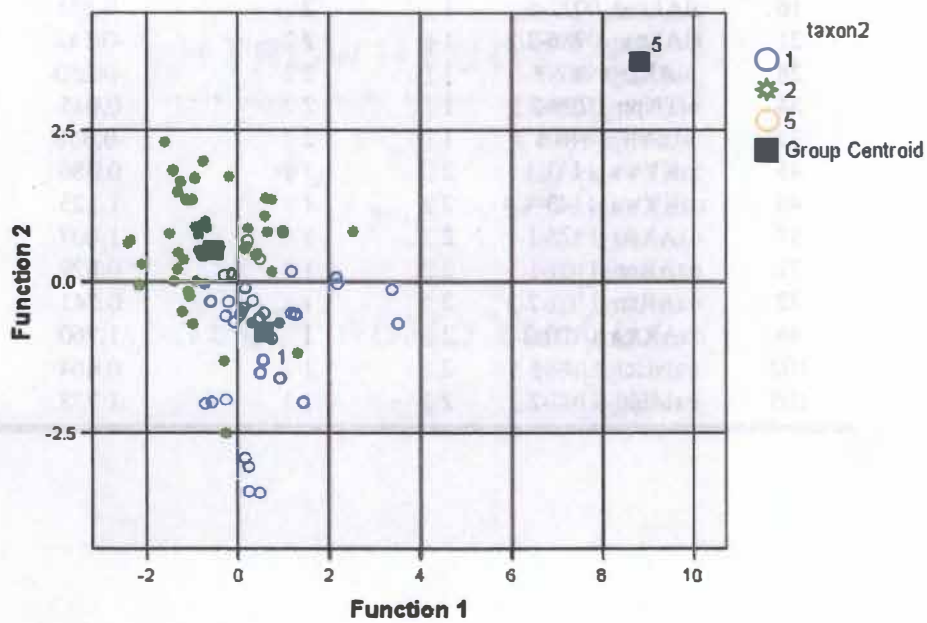


Figure 21. Plots of function scores of the individual specimens and taxon centroids from the discriminant analysis of the Ozarkanum Cluster of the Pusillum Complex. Legend: taxon2=1 is *Trillium alabamicum* and taxon2=2 is *T. ozarkanum*. Note: In order to obtain a 2-D plot, another taxon needed to be added (*T. telmacola*).

Table 24. Discriminant model classification results for the Ozarkanum Cluster of the Pusillum Complex. 81.8% of original grouped cases correctly classified.

taxon	Predicted Group Membership	
	1	2
1. <i>T. alabamicum</i>	77.8% 28 samples	22.2% 8 samples
10. <i>T. ozarkanum</i>	15.4% 8 samples	84.6% 44 samples

Table 25. Casewise statistics for misclassified cases in the analysis of the Ozarkanum Cluster of the Pusillum Complex.

Case Number	Specimen	Actual Group	Predicted Group	Discriminant Scores
				Function 1
8	alALli_0068-10	1	2	0.138
13	alALmd_0072-3	1	2	0.021
14	alALmd_0072-4	1	2	-0.109
16	alALmd_0072-6	1	2	-0.851
21	alALmo_0986-2	1	2	-0.142
28	alALja_0067-4	1	2	-0.250
34	alTNpu_0299-3	1	2	0.045
37	alTNli_1646-3	1	2	-0.358
43	ozKYwa_1143-1	2	1	0.386
46	ozKYwa_1143-4	2	1	1.125
57	ozARpo_1575-1	2	1	1.007
71	ozARne_1161-1	2	1	0.829
72	ozARne_1161-2	2	1	0.741
86	ozARma_0100-2	2	1	1.760
102	ozNCcl_1149-5	2	1	0.464
105	ozMSjo_1147-2	2	1	1.778

Table 26. Centroids of the taxa of the Ozarkanum Cluster of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means. [top table – 1 function; bottom table with addition of *T. telmacola* to get the 2-D plot.]

taxon	Function 1
<i>T. alabamicum</i>	1.040
<i>T. ozarkanum</i>	-0.720

taxon	Function 1	Function 2
<i>T. alabamicum</i>	0.567	-0.839
<i>T. ozarkanum</i>	-0.562	0.511
<i>T. telmacola</i>	8.817	3.635

and pedicel to sepal length ratio; anther length, pedicel length, sepal to petal length ratio, petal length, leaf length had large positive coefficients. The structure matrix, which provides information about the nature of the function, indicated that it is primarily one petal size and stem length and is given in Table D.11.

3.4.4 The Virginianum Cluster.— Standard descriptive statistics of the variables showed some species-specific trends (Table 27). The mean pedicel length for *Trillium carolinianum* was more than one standard deviation from the group mean. *Trillium monticola* had the widest leaves, the largest leaf length to width ratio indicating the shortest and broadest leaves, the longest filament, the longest stamens, and the longest stem to pedicel ratio all greater than one standard deviation from the group mean. *Trillium palustris* and *T. virginianum* had non-overlapping means (95% confidence interval) for leaf width, sepal length, anther length, pistil length, and sepal to petal ratio.

The results of discriminant analysis indicated that the segregate taxa associated with the Pusillum Complex were distinct from one another in varying degrees. Using taxonomic boundaries as defined by the DNA analysis, 93.2% of the total cases were correctly classified (Table 28). Taxonomic membership ranged from 88.9% for *T. palustris*, 93.5% for *T. virginianum* to 100% for *T. monticola* and *T. carolinianum*. Information on the mis-classified samples is provided in Table 29. The group centroids (Table 30) also indicated the distinctness of the groups and indicated which clusters were separated out by which function. The discriminate analysis defined three new orthogonal multivariate variables or dimensions each of which define new uncorrelated functions, two of which were significant (Wilks Lambda test, Table D.12) and account for 87.2% of the variation (Table D.13). In addition, these functions were relatively well correlated with group membership (canonical correlations of over 88.0%; Table D.13). The first function had large negative coefficients for (in order of decreasing magnitude) petal width, leaf length, petal to sepal ratio, sepal length, petal shape, and anther length to ovary length ratio; and large positive coefficients for petal length, leaf width, style length, pedicel length, stamen to pistil ratio, and leaf shape (Table D.14). The second function had large negative coefficients for stigma length, sepal width, petal length, sepal shape, and leaf length; and large positive coefficients for petal width, stigma to anther length, petal shape, leaf width, and style length. The structure matrix (Table D.15) indicates the correlations between the characters and the functions providing information about the nature of the functions. The first function, therefore, was primarily one of leaf width and the ovary to stigma ratio; the second function was one of sepal shape. The individual specimens and the taxon centroids are shown in discriminant space in Fig. 22.

3.4.5 The individual species.— Standard descriptive statistics of the variables showed some species-specific trends (see also Tables 20, 23, and 27). The mean pedicel length for *Trillium carolinianum* was

Table 27. Means for the standard morphometric characters for members of the Virginianum Cluster of the Pusillum Complex. Shape is a ratio calculated based on length/width. **Bold numbers** are more than one standard deviation from the global mean of the Pusillum Complex.

taxon	carolinianum	monticola	palustris	virginianum	cluster	Std. Dev.
stem	129.13	130.95	145.92	137.78	138.57	31.48
leaf length	38.44	39.05	45.98	38.22	40.62	10.51
leaf width	13.44	17.75	14.28	11.09	13.08	3.75
leaf shape	2.90	2.22	3.36	3.50	3.24	0.91
pedicel	4.47	0.70	1.98	1.78	2.06	1.84
sepal length	16.94	19.56	20.48	16.96	18.29	4.10
sepal width	5.06	6.44	5.73	4.74	5.25	1.48
sepal shape	3.28	3.12	3.66	3.74	3.60	0.76
petal length	17.75	20.50	19.87	18.57	19.16	3.26
petal width	4.92	6.13	6.11	5.37	5.65	1.65
petal shape	3.88	3.59	3.44	3.56	3.55	0.74
filament	3.80	5.50	4.45	4.23	4.38	0.99
anther	3.77	5.73	5.15	4.23	4.61	1.17
stamen	7.57	11.23	9.60	8.46	8.99	1.95
ovary	2.93	3.91	3.48	2.82	3.13	0.87
style	2.69	4.94	4.18	3.35	3.68	1.46
stigma	1.74	1.81	2.66	2.36	2.33	0.85
pistil	7.36	10.66	10.32	8.53	9.14	2.24
stem: leaf	3.53	3.40	3.25	3.71	3.52	0.80
stem: pedicel	54.54	210.29	113.24	108.58	113.68	81.63
pedicel: sepal	0.25	0.04	0.09	0.11	0.11	0.10
sepal: petal	0.93	0.96	1.04	0.92	0.96	0.16
anther: filament	1.02	1.03	1.19	1.02	1.07	0.24
filament: ovary	1.34	1.43	1.34	1.54	1.45	0.36
ovary: stigma	1.83	3.23	1.45	1.31	1.60	1.15
stigma: anther	0.53	0.35	0.54	0.56	0.53	0.21
anther: ovary	1.28	1.47	1.57	1.55	1.52	0.39
stigma: style	0.77	0.45	0.76	0.74	0.72	0.35
stamen: pistil	1.04	1.06	0.96	1.00	1.00	0.15

Table 28. Discriminant model classification results for the Virginianum Cluster of the Pusillum Complex. 93.2 % of original grouped cases correctly classified.

taxon	Predicted Group Membership			
	7	8	9	10
7. <i>T. monticola</i>	100.0 % 5 samples	0	0	0
8. <i>T. palustris</i>	0	88.9 % 16 samples	0	11.1 % 2 samples
9. <i>T. carolinianum</i>	0	0	100.0 % 5 samples	0
10. <i>T. virginianum</i>	0	6.5 % 2 samples	0	93.5 % 29 samples

Table 29. Casewise Statistics for misclassified cases in the analysis of the Virginianum Cluster of the Pusillum Complex.

Case Number	Specimen	Actual Group	Predicted Group	Discriminant Scores		
				Fxn 1	Fxn 2	Fxn 3
160	paMDwo_1227-5	8	10	-1.082	0.245	-0.082
168	paNCga_0116-4	8	10	-0.507	-1.176	0.752
197	vaVAgs_1244-1	10	8	-1.156	-1.003	1.788
208	vaVAyo_1237-1	10	8	-0.992	-0.530	1.234

Table 30. Centroids of the taxa of the Virginianum Cluster of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.

taxon	Function		
	1	2	3
<i>T. monticola</i>	1.592	-0.041	-0.430
<i>T. palustris</i>	-3.441	-0.279	-0.327
<i>T. carolinianum</i>	-2.949	-0.769	-0.184
<i>T. virginianum</i>	-3.611	-0.313	-0.450

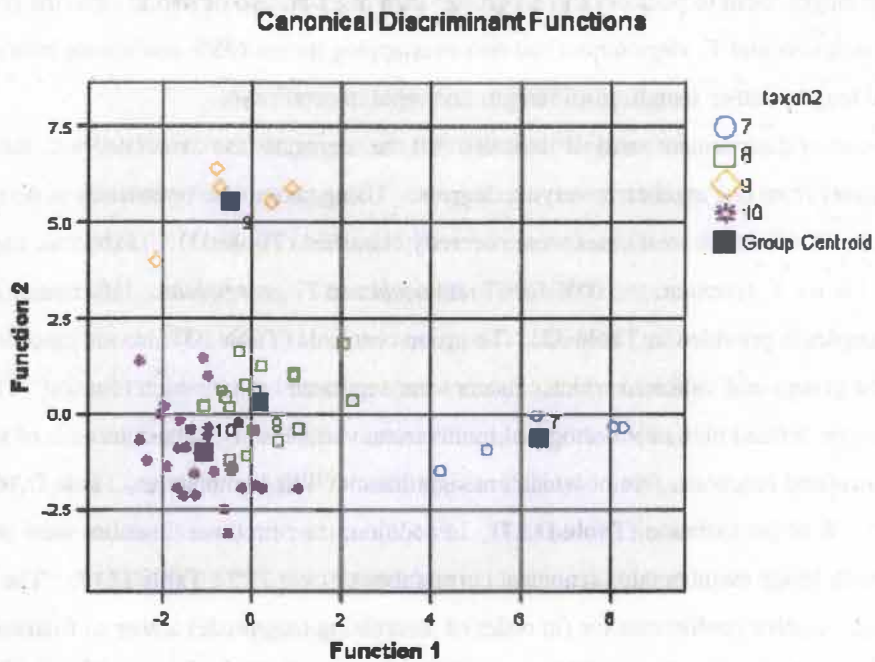


Figure 22. Two-dimensional plot of function scores of the individual specimens and taxon centroids from the discriminant analysis of the Virginianum Cluster of the Pusillum Complex. Legend: taxon2=7 is *Trillium monticola*, taxon2=8 is *T. palustris*, taxon2=9 is *T. carolinianum*, and taxon2=10 is *T. virginianum*.

more than one standard deviation from the group mean. *Trillium monticola* had the widest leaves, the largest leaf length to width ratio indicating the shortest and fattest leaves, the longest filament, the longest stamens, and the longest stem to pedicel ratio all greater than one standard deviation from the group mean. *Trillium palustris* and *T. virginianum* had non-overlapping means (95% confidence interval) for leaf width, sepal length, anther length, pistil length, and sepal to petal ratio.

The results of discriminant analysis indicated that the segregate taxa associated with the Pusillum Complex are distinct from one another in varying degrees. Using taxonomic boundaries as defined by the DNA analysis, 72.4% of the total cases were correctly classified (Table 31). Taxonomic membership ranged from 57.1% for *T. texanum*, to 100% for *T. telmacola* and *T. georgianum*. Information on the mis-classified samples is provided in Table 32. The group centroids (Table 33) also indicated the distinctness of the groups and indicated which clusters were separated out by which function. The discriminate analysis defined nine new orthogonal multivariate variables or dimensions each of which define new uncorrelated functions, five of which are significant (Wilks Lambda test, Table D.16) and accounted for 93.7% of the variation (Table D.17). In addition, the first three functions were relatively well correlated with group membership (canonical correlations of over 70%; Table D.17). The first function had large negative coefficients for (in order of decreasing magnitude) anther to filament ratio, and pedicel length; and large positive coefficients for anther length, pedicel to sepal ratio, and sepal length (Table D.18). The second function had large negative coefficients for stamen length, leaf shape and leaf width; with large positive coefficients for anther length and filament length. The third function had large negative coefficients for anther length, leaf length, and filament length; and large positive coefficients for stamen length, leaf width, and sepal length. The structure matrix (Table D.19) indicates the correlations between the characters and the functions providing information about the nature of the functions. The first function, therefore, was primarily one of pedicel characters and petal shape; the second function was concerned with leaf and petal characters, sepal length, stamen, stem, style, pistil, and filament lengths; the third function, which only accounts for 8.4% of the variation, was primarily one of ovary and stigma characters. The individual specimens and the taxon centroids are shown in two-dimensional discriminant space in Fig. 23; the group centroids are shown in three dimensional space in Fig. 24.

Table 31. Discriminant model classification results for the taxa of the Pusillum Complex. 72.4% of original grouped cases correctly classified.

taxon	Predicted Group Membership									
	1	2	3	4	5	6	7	8	9	10
1. <i>T. alabamicum</i>	63.9 23 samples	25 9 samples	0	11.1 4 samples	0	0	0	0	0	0
2. <i>T. ozarkanum</i>	17.3 9 samples	71.2 37 samples	0	1.9 1 sample	0	9.6 5 samples	0	0	0	0
3. <i>T. georgianum</i>	0	0	100 7 samples	0	0	0	0	0	0	0
4. <i>T. pusillum</i>	12.5 1 sample	12.5 1 sample	0	75 6 samples	0	0	0	0	0	0
5. <i>T. telmacola</i>	0	0	0	0	100 1 sample	0	0	0	0	0
6. <i>T. texanum</i>	0	28.6 2 samples	14.3 1 samples	0	0	57.1 4 samples	0	0	0	0
7. <i>T. monticola</i>	0	0	0	0	0	0	80 4 samples	0	0	20 1 sample
8. <i>T. palustris</i>	0	0	0	0	0	0	0	83.3 15 samples	0	16.7 3 samples
9. <i>T. carolinianum</i>	0	0	0	0	0	0	0	20 1 sample	80 4 samples	0
10. <i>T. virginianum</i>	3.2 1 sample	0	0	0	0	0	0	22.6 7 samples	3.2 1 sample	71 22 samples

Table 32. Casewise statistics for misclassified cases in the analysis of the taxa of the Pusillum Complex.

Case Number	Specimen	Actual Group	Predicted Group	Discriminant Scores				
				Fxn 1	Fxn 2	Fxn 3	Fxn 4	Fxn 5
14	alALmd_0072-4	1	2	3.453	1.615	1.034	-0.428	0.087
15	alALmd_0072-5	1	4	0.315	0.225	-1.063	0.465	1.031
16	alALmd_0072-6	1	2	2.001	1.359	-1.339	-0.490	0.805
21	alALmo_0986-2	1	2	2.377	0.936	-0.594	0.114	-0.280
24	alALmo_0986-5	1	4	0.529	-1.262	-1.582	1.759	-0.934
25	alALja_0067-1	1	2	2.615	1.605	-0.083	0.847	0.195
27	alALja_0067-3	1	4	1.342	0.304	-0.683	-0.370	0.942
28	alALja_0067-4	1	2	2.291	-0.025	0.010	0.037	0.722
32	alTNpu_0299-1	1	2	2.693	0.740	0.058	0.402	-0.083
33	alTNpu_0299-2	1	4	1.734	-2.299	0.036	0.192	1.284
34	alTNpu_0299-3	1	2	2.046	0.608	-0.604	0.326	-0.833
35	alTNli_1646-1	1	2	1.851	0.413	0.549	-0.498	-1.418
37	alTNli_1646-3	1	2	1.797	0.614	-0.555	0.275	-0.084
42	ozTNsu_1610-2	2	6	0.776	-0.576	0.798	0.277	-1.564
43	ozKYwa_1143-1	2	1	1.635	0.886	-0.328	0.776	0.600
46	ozKYwa_1143-4	2	1	3.069	-1.060	-0.365	0.452	0.256
57	ozARpo_1575-1	2	6	2.650	-0.254	0.380	1.750	-1.958
69	ozARbo_1156-3	2	6	2.588	1.006	0.914	-0.870	-0.898
71	ozARne_1161-1	2	1	2.713	0.663	-0.437	0.060	1.897
72	ozARne_1161-2	2	1	2.104	1.403	-0.724	0.967	-0.443
85	ozARma_0100-1	2	6	2.063	0.485	1.424	-0.453	-0.786
86	ozARma_0100-2	2	1	1.968	-0.336	-1.643	0.981	0.810
92	ozMOla_1165-4	2	6	2.767	0.619	0.328	0.552	-1.115
98	ozNCcl_1149-1	2	1	2.811	-1.180	0.455	0.189	0.649
102	ozNCcl_1149-5	2	1	0.427	-0.954	-0.068	-0.300	-0.635
104	ozMSjo_1147-1	2	1	2.110	0.535	-0.137	0.845	-0.725
105	ozMSjo_1147-2	2	4	-0.017	-1.025	-1.258	-0.288	0.327
106	ozMSjo_1147-3	2	1	0.870	0.320	-0.529	0.529	0.867
118	puNCpe_1166-4	4	1	2.675	0.240	0.566	0.000	2.011
122	puSCca_0114-1	4	2	-0.143	0.270	-0.205	-0.279	-1.491
136	txTXsm_0251-1	6	2	1.742	1.334	0.165	0.468	-1.393
140	txTXca_1591-2	6	3	4.503	-3.224	2.166	-1.227	0.916
145	txLAca_0247-1	6	2	2.565	0.009	0.122	-0.192	-0.824
147	moVAro_1272-1	7	10	-4.417	1.240	-0.002	-0.059	0.998
159	paMDwo_1227-4	8	10	-2.274	0.340	-0.422	0.031	0.325
168	paNCga_0116-4	8	10	-3.337	-0.905	0.381	-0.689	0.213
174	paVAsf_1247-5	8	10	-1.742	0.169	-1.844	-0.750	-0.845
181	caNCwa_1989-1	9	8	-1.100	-0.626	-0.523	-0.080	-1.144
191	vaVAhe_1233-2	10	1	-0.868	-0.103	-1.097	-1.054	-0.144
197	vaVAgs_1244-1	10	8	-2.953	-1.228	-0.379	0.373	-0.618
201	vaVAch_1232-3	10	8	-3.164	-0.304	-1.086	0.469	0.976
204	vaVAss_1269-3	10	8	-3.528	0.132	0.265	0.351	1.593
215	vaVAcc_1231-6	10	8	-4.918	-1.194	1.319	0.753	2.343
217	vaVAsr_0984-2	10	9	-2.785	-0.135	0.206	-0.695	-1.077
221	vaVAsr_0984-6	10	8	-5.266	-0.931	-0.201	1.053	0.903
225	vaVAjc_1239-1	10	8	-4.576	-1.003	1.757	0.570	-0.222
229	vaVAjc_1239-5	10	8	-3.712	0.744	-1.261	2.405	-0.008

Table 33. Centroids of the taxa of the Pusillum Complex using unstandardized canonical discriminant functions evaluated at group means.

taxon	Function				
	1	2	3	4	5
<i>T. alabamicum</i>	1.592	-0.041	-0.43	0.357	0.092
<i>T. ozarkanum</i>	1.985	0.967	-0.072	-0.283	0.095
<i>T. georgianum</i>	3.704	-3.732	1.737	-0.555	0.914
<i>T. pusillum</i>	0.361	-1.006	-0.608	-0.045	0.545
<i>T. telmacola</i>	3.115	-1.645	-0.407	6.388	-4.333
<i>T. texanum</i>	3.049	-0.5	1.473	0.219	-1.478
<i>T. monticola</i>	-5.036	1.813	4.553	0.46	0.325
<i>T. palustris</i>	-3.441	-0.279	-0.327	0.909	0.191
<i>T. carolinianum</i>	-2.949	-0.769	-0.184	-2.249	-2.793
<i>T. virginianum</i>	-3.611	-0.313	-0.45	-0.298	0.147

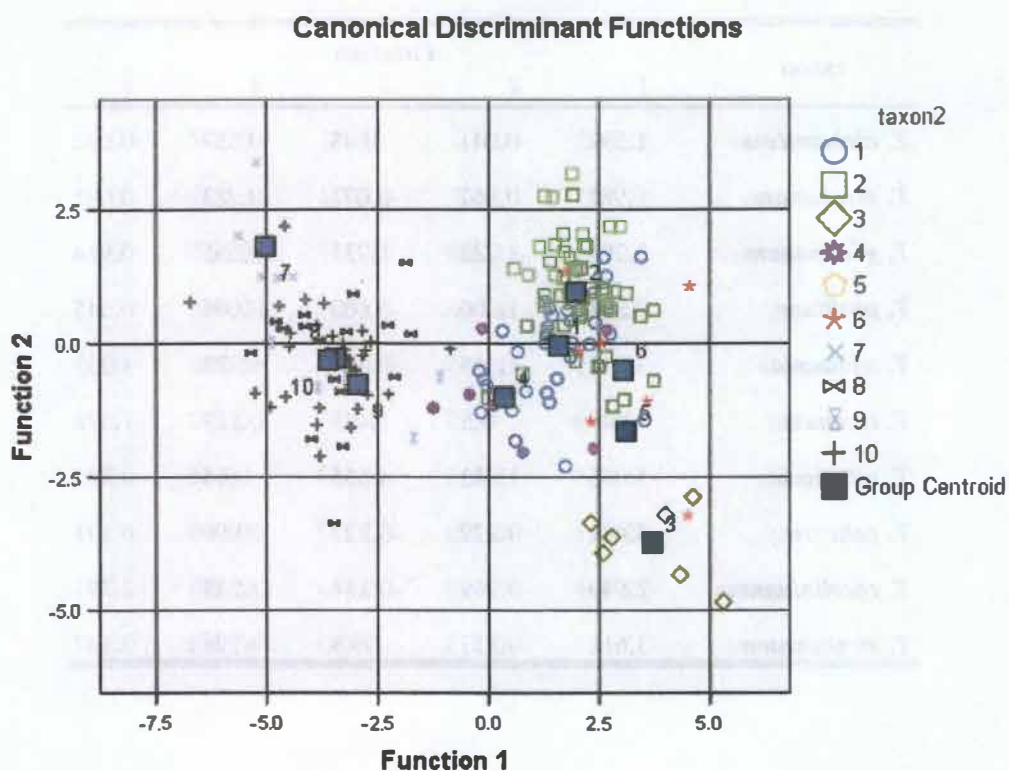


Figure 23. Two-dimensional plot of function scores of the individual specimens and taxon centroids from the discriminant analysis of the taxa of the Pusillum Complex. Legend: taxon2=1 is *Trillium alabamicum*, taxon2=2 is *T. ozarkanum*, taxon2=3 is *T. georgianum*, taxon2=4 is *T. texanum*, taxon2=5 is *T. telmacola*, taxon2=6 is *T. pusillum*, taxon2=7 is *Trillium monticola*, taxon2=8 is *T. palustris*, taxon2=9 is *T. carolinianum*, and taxon2=10 is *T. virginianum*.

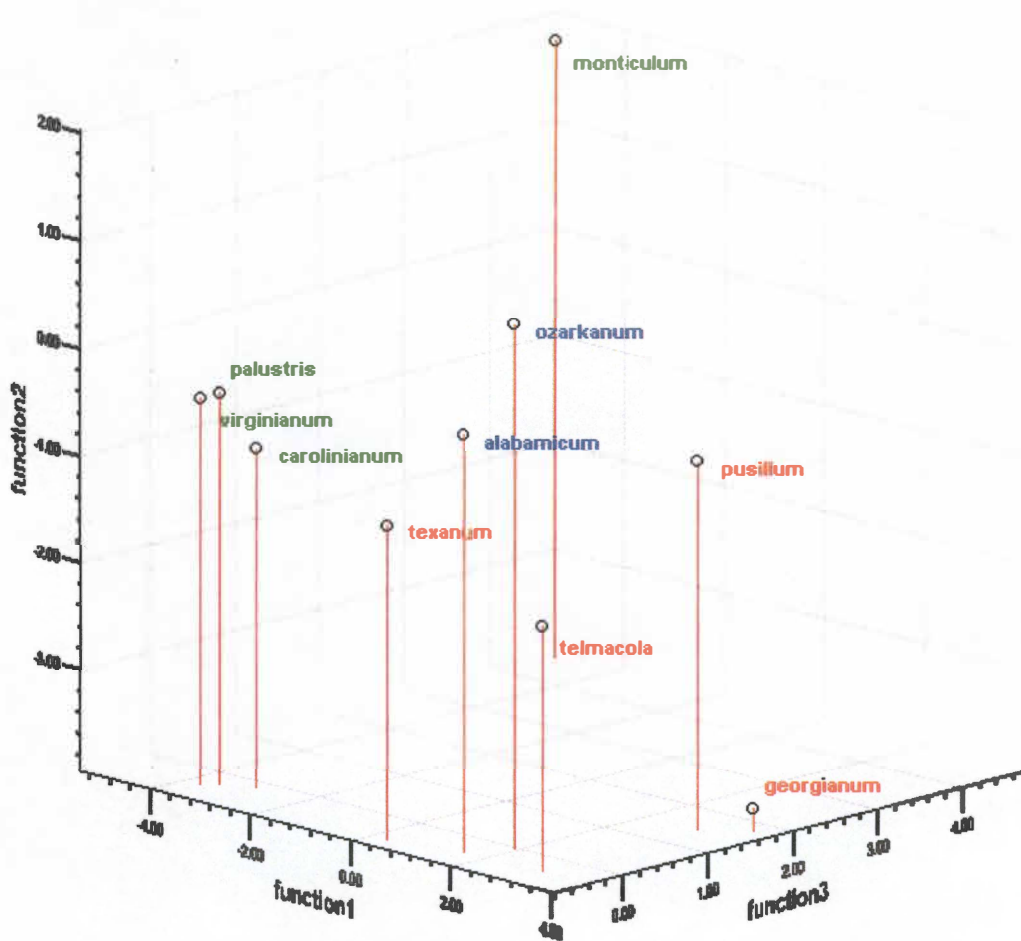


Figure 24. Three-dimensional plot of taxon centroids from the discriminant analysis of the *Pusillum* Complex. Taxa names in red comprise the *Pusillum* Cluster; blue the *Ozarkanum* Cluster; and green the *Virginianum* Cluster.

4.0 DISCUSSION

Using a variety of analysis techniques and sources of data, I was able to answer the questions that prompted this study. *Delostylis* lies within *Trillium* rather than in the basal position suggested by an earlier analysis (Farmer 2000; Farmer & Schilling 2002). *Delostylis* as it was presumed to be circumscribed was not monophyletic, but when additional morphological characters were considered, a recircumscribed, monophyletic *Delostylis* was recognized. The taxonomy of the Pusillum Complex was able to be resolved by splitting it into 10 species.

4.1 *Delostylis*: Composition and Placement

The results of the phylogenetic analysis using data from ITS, *matK*, and *psbA-trnH* sequences (see also Figs. 10, 15) suggested that the circumscription of *Delostylis* be narrowed to consist of *T. persistens*, *T. catesbaei*, and the Pusillum Complex. Molecular and phylogenetic evidence suggested that the character of a common style is homoplasious (Fig. 25) placing *T. nivale* in a sister relationship with *T. grandiflorum* (albeit with relatively long branches indicating a more distant phylogenetic relationship). A revised *Delostylis* was characterized by the presence of a style with three slender stigmatic branches in those plants whose flowers are either white-fading-to-pink or pink. The anthocyanin pigmentation in the petals excluded *T. nivale* on morphological grounds. Because of the sister relationship with *T. grandiflorum* (albeit morphologically unsupported), the inclusion of *T. nivale* would make *Delostylis* paraphyletic.

The emended *Delostylis* was well supported as a group in the analyses of the combined data sets with a bootstrap value of 99% and a posterior probability of 1.0 (Table 34). In addition there are molecular markers that delineate *Delostylis* that are not present in *T. nivale*. Analysis of the relationships of *T. nivale* and *T. grandiflorum* are beyond the scope of this project; however, because of the branch lengths involved, it is likely that long branch attraction or possibly lineage sorting is responsible.

Results of the phylogenetic analyses showed *Delostylis* to be one of four innermost clades in *Trillium* (the other three being *T. ovatum* [100%; 1.0], *T.* subgen. *Phyllantherum* [100%; 1.0], and sister taxa *T. grandiflorum* and *T. nivale* [86%; 1.0]) (see also Figs. 10, 15). The innermost position was in contrast to the basal position indicated by the phylogenetic analysis of morphological data (Farmer 2000; Farmer & Schilling 2002). Prior to this analysis, phylogenetic placement based on molecular data was not able to be determined with confidence because sequence data were available only for "*T. pusillum*" (Farmer 2000; Farmer & Schilling 2002; see also Fig. 6), now known to be *T. ozarkanum* (Fig. 17). More complete sampling of *Delostylis* helps to minimize artifacts such as long branch attraction (Felsenstein 1978; Huelsenbeck 1997; Graybeal 1998).

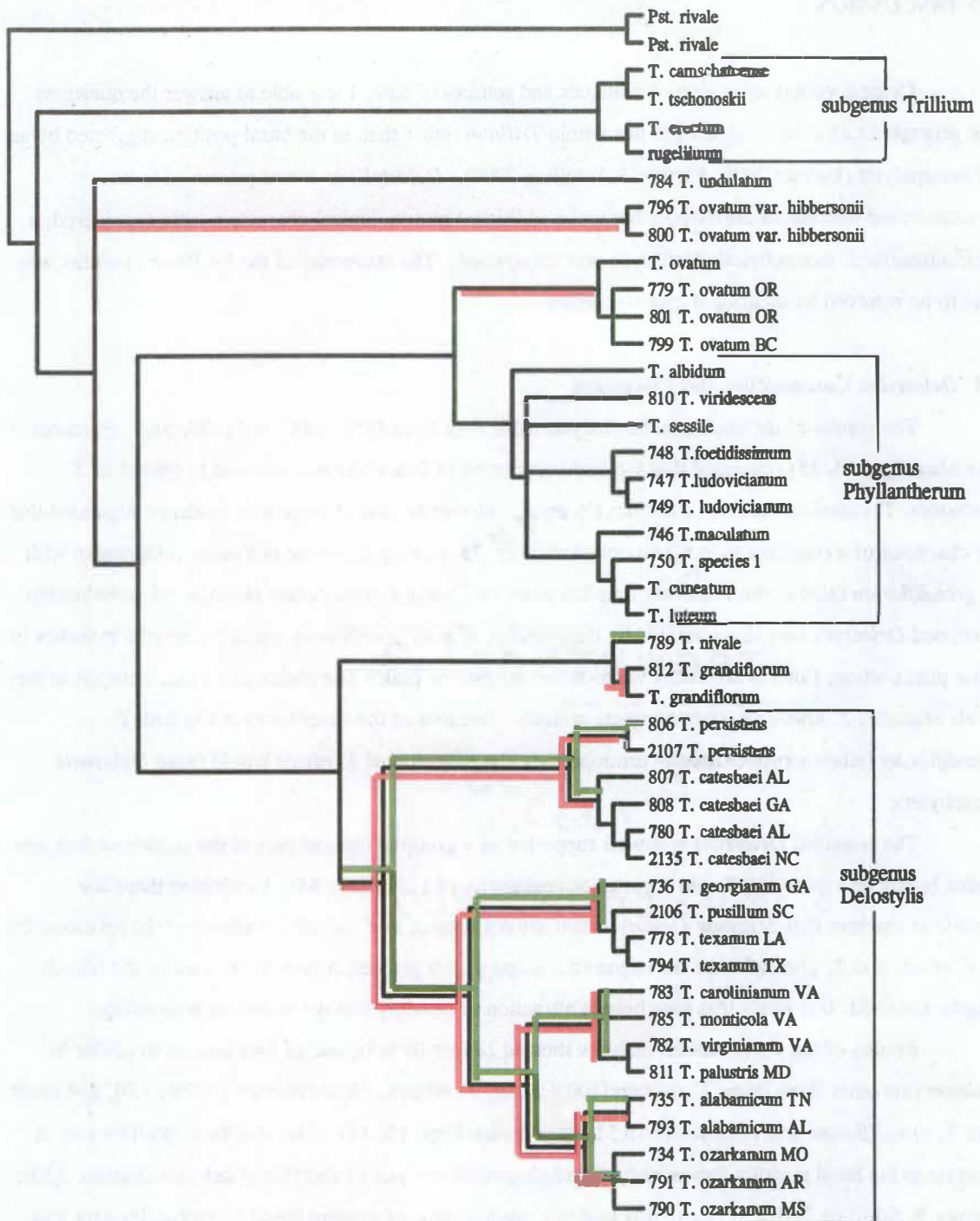


Figure 25. Tree from the parsimony analysis of the combined sequence data of *Trillium* with morphological characters defining *Delostylis* mapped onto the tree. Pink branches indicate plants that have pink flowers or white flowers aging to pink; green branches indicate a common style.

Table 34. Comparisons of clade support between DNA sequences and analysis techniques.

Bootstrap values from the parsimony analyses are given as percentage values; posterior probabilities from bayesian analyses are given as decimal fractions. "Single" indicates a single sample.

	ITS	<i>matK</i>	<i>psbA-trnH</i>	combo
Delostylis	99% 1.0	55% 0.96	n/a 0.86	99% 1.0
Catesbaei Clade	99% 1.0	74% 0.96	63% 0.99	99% 1.0
Pusillum Complex	73% 0.94	monophyletic 0.72	paraphyletic 0.95	89% 1.0
Pusillum Cluster	n/a (<i>telmacola</i> basal) ditto	n/a n/a	100% 1.0	100% 1.0
<i>T. pusillum</i>	single single	single single	86% 0.95	single single
<i>T. texanum</i>	n/a n/a polytomy	52% .71	97% 1.0	95% 1.0
<i>T. georgianum</i>	63% 0.97	single single	single single	single single
<i>T. telmacola</i>	basal to <i>pusillum</i> ditto	-- --	single single	-- --
Ozarkanum Cluster	n/a (Oz + Va) ditto	52% n/a	100% 1.0	99% 1.0
<i>T. ozarkanum</i>	n/a n/a	n/a 0.78	n/a polytomy n/a polytomy	polytomy 0.72
<i>T. alabamicum</i>	n/a n/a	59% 0.89	63% 0.75	86% 0.94
Virginianum Cluster	n/a (Oz + Va) ditto	n/a n/a	92% 0.89	84% 0.99
<i>T. virginianum</i>	single (+ <i>palustris</i> 63%) single (+ <i>palustris</i> 1.0)	single single	single single (+ <i>palustris</i> 0.72)	single single
<i>T. monticola</i>	single single	single single	single single	single single
<i>T. carolinianum</i>	single single	single single	polytomy polytomy	single single
<i>T. palustris</i>	single (+ <i>virginianum</i> 63%) single (+ <i>virginianum</i> 1.0)	single single	single single (+ <i>palustris</i> 0.72)	-- --
other	Ozarkanum + Virginianum		Delostylis paraphyletic; Va + Oz 96%	

Within a revised *Delostylis*, there were still some problems with species delimitation, only some of which were resolved. *Delostylis* was composed of sister clades in the consensus trees from all analyses: the Catesbaei Clade (containing *T. catesbaei* and *T. persistens*) and the Pusillum Clade (containing the Pusillum Cluster, the Ozarkanum Cluster, and the Virginianum Cluster). The monophyletic Catesbaei Clade was well supported with most markers (Table 34) (bootstrap of 63%-99%; posterior probability of 0.96-1.0), but the Pusillum Clade did not always receive bootstrap support of over 50%, nor was it always monophyletic.

Molecular evidence suggested that the narrowly endemic *Trillium persistens* was monophyletic, but *T. catesbaei* was not. *Trillium persistens* was represented by 2 samples, one of which was collected from cultivated material at the University of Georgia Botanic Gardens; additional work is desired to examine the genetic variability in this endangered species. Before this species was described, vegetative herbarium material had been labeled as *T. catesbaei*, indicating a possible close relationship which was supported by the molecular phylogenetic analysis. Phylogenetic evidence indicated that *T. catesbaei*, as currently circumscribed, may not be a monophyletic species (Figs. 6-10, 12-15). When Elliot (1817) described *T. catesbaei* based in part on Catesby's figure (Fig. 26), he also described *T. nervosum* followed shortly by Nuttall's publication of *T. stylosum* (1818). In the time since Catesby published "*Solanum triphyllon flore hexapetalo carneo*" in 1730, discussion has ensued about Catesby's *Trillium* beginning with Smith in 1791 and Pursh in 1814. Elliot noted differences between petal shape (lanceolate vs. oblong lanceolate), leaves ("obovate and oval, acuminate, tapering at the base" vs. "lanceolate and ovate, acute at each end, membranaceous, nerved") between *T. catesbaei* (former) and *T. nervosum*. Nuttall's *T. nervosum* had leaves that were "elliptic-lanceolate, acute at both extremities" and "oblong, obtuse" petals. Other taxonomists, including Elliott (1817), Rendle (1901), and Mitchell (1992) state that *T. cernuum* was designated upon Catesby's figure causing further confusion; however, *T. cernuum* has been lectotypified on a Kalm specimen at LINN (Gleason 1906) effectively ending that debate. Because sampling of *T. catesbaei* was not carried out in a systematic or thorough fashion, the significance of variation within the DNA sequence data cannot be ascertained at this point. There are three distinct morphs: the more western plants (GA and AL specimens) are smaller in stature with white flowers turning pink with age, the Franklin Co., NC sample is a much taller plant with pink flowers, and the Pickens Co., SC sample is shorter with white flowers like the western plants with very short stigmatic branches and an erect pedicel. Further work on this putative species complex is needed to answer questions raised by this analysis.

Based on molecular and morphological analyses of a broad sampling of populations, *Trillium pusillum*, as previously circumscribed and now referred to as the Pusillum Complex, was shown to be an amalgam of different species that will be discussed in detail in Section 4.2. The Pusillum Complex was composed of three distinct clusters of species (the Ozarkanum Cluster, the Pusillum Cluster and the

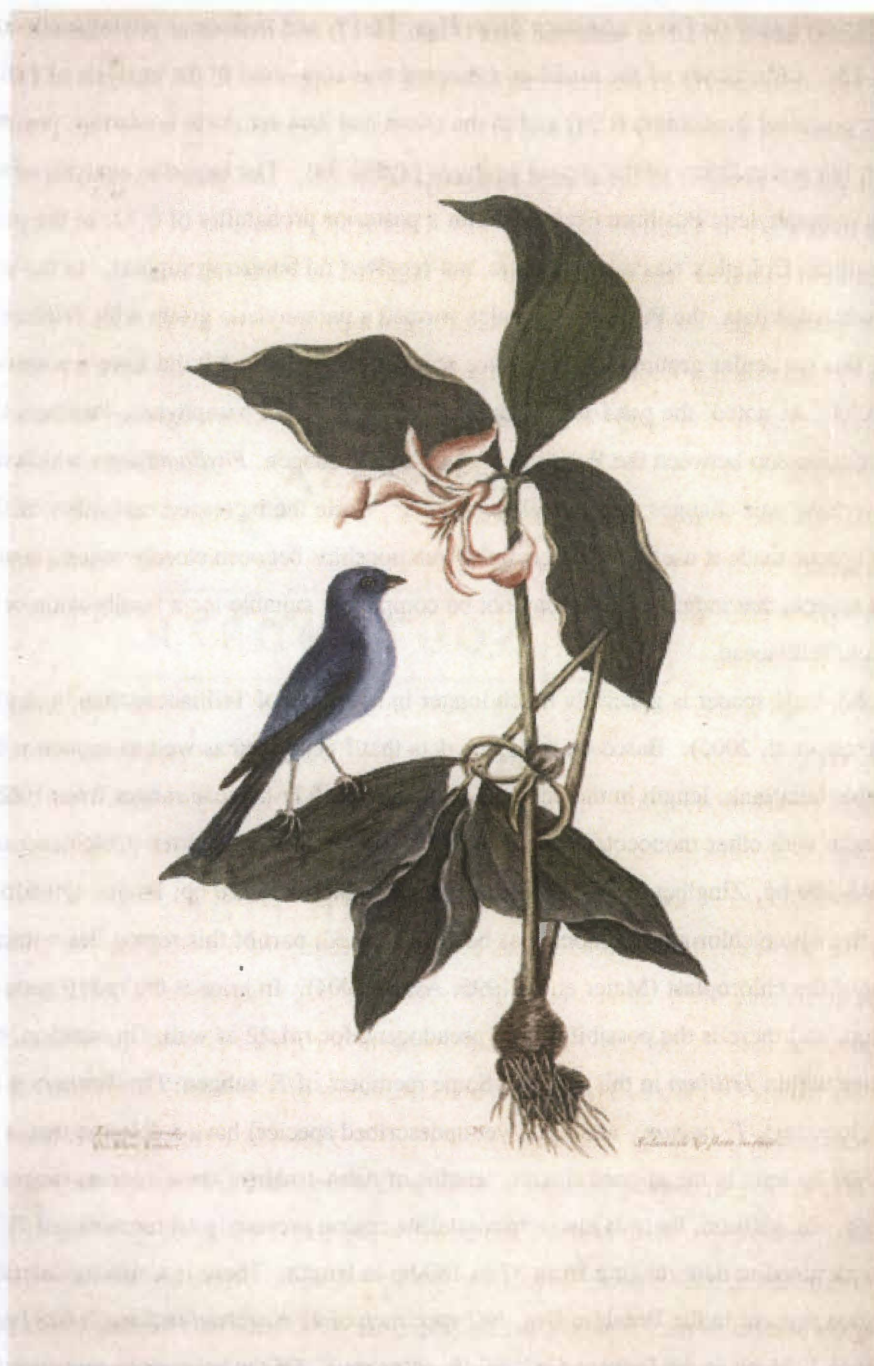


Figure 26. *Linaria Cærulea*. The Blew Linnet. *Solanum &c. flore carnoe* (Catesby 1771).

Virginianum Cluster) based on DNA sequence data (Figs. 16-17) and molecular phylogenetic analysis (Figs. 6-10, 12-15). Monophyly of the Pusillum Complex was supported in the analysis of ITS data (73% bootstrap; posterior probability 0.94) and in the combined data set (89% bootstrap; posterior probability 1.0), but not in either of the plastid analyses (Table 34). The bayesian analysis of the *marK* data produced a monophyletic Pusillum Complex with a posterior probability of 0.72; in the parsimony analysis, the Pusillum Complex was monophyletic, but received no bootstrap support. In the analyses of the *psbA-trnH* sequence data, the Pusillum Complex formed a paraphyletic group with *Trillium* subgen. *Phyllantherum*; this particular grouping did not have bootstrap support, but it did have a posterior probability of 0.95. As noted, the *psbA-trnH* sequence data indicated a paraphyletic Pusillum Complex due to a sister relationship between the Pusillum Cluster and *T.* subgen. *Phyllantherum* which was supported by two base pair changes and 2 indel characters. While the increased variability of the *psbA-trnH* intergenic spacer made it useful for determining relationships between closely related taxa, there are several unusual aspects that indicate that it may not be completely suitable for a family-wide or genus-wide study within Trilliaceae.

The *psbA-trnH* spacer is generally much longer in members of Trilliaceae than in any other angiosperms (Kress et al. 2005). Based on sequence data that I generated as well as sequence data currently available GenBank, length in the pedicellate members of Trilliaceae ranges from 1068 to 1281 base pairs in length with other monocots being at least 300-600 base-pairs shorter (Colchicaceae 390-450 bp, Liliaceae 348-390 bp, Zingiberales 492-752 bp, Commelinales 612-770 bp, Poales 479-615 bp). In grasses, where the whole chloroplast genome has been sequenced, part of this region lies within the Inverted Repeat of the chloroplast (Maier et al. 1995; Asano 2004). In grasses the *rpS19* gene also lies within this region, and there is the possibility of a pseudogene for *rpL22* as well. In addition, there are some peculiarities within *Trillium* in this region. Some members of *T.* subgen. *Phyllantherum* (e.g., *T. maculatum*, *T. cuneatum*, *T. luteum*, and an as yet undescribed species) have a deletion that is approximately 800 bp long in the aligned matrix; lengths of *psbA-trnH* for these species ranges from 626 bp to 800 bp long. In addition, there is also a minisatellite region present in all members of *T.* subgen. *Phyllantherum* examined to date ranging from 37 to 189 bp in length. There is a similar deletion and a minisatellite region present in the Franklin Co., NC specimen of *T. catesbaei* making it 628 base pairs long (in contrast to 1281 bp in the Pickens Co., SC *T. catesbaei*). Of the accessions sequenced to date, this specimen is the only pedicellate species of *Trillium* examined to exhibit any such oddities.

4.2 The Pusillum Complex

Results of molecular analyses in combination with morphology and biogeography supported dividing *Trillium pusillum* into ten distinct species. Although other researchers (Garrett 1982; Cabe 1995; Cabe & Werth 1995; Case & Case 1997; Case 2002; Timmerman-Erskine et al. 2002a) have

suggested that the Pusillum Complex includes more than one taxon, a comprehensive solution based on both molecular and morphological evidence has not yet been proposed. Other studies began with *a priori* taxon boundaries and examined the complex in light of those boundaries (Garrett 1982; Cabe 1995; Cabe & Werth 1995; and Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a, 2002b, 2003). This study began with a comprehensive molecular analysis to search for the presence of potential taxon boundaries. Based on the taxon boundaries as indicated by DNA sequence data, morphology was then tested. Note that this type of approach worked well with a group of poorly known beetles (Pons et al. 2006). As a result of both the DNA analyses and the morphological analyses, the picture that emerges of the Pusillum Complex is one of multiple species each of which has relatively discrete physiographic, and ecological aspects (Fig. 5).

The amount of DNA sequence variation within the Pusillum Complex was the first indicator that this was a complex of more than one species. Not only were there high levels of variability, but this variability was partitionable into discrete entities. In most cases, these partitions corresponded with previously proposed taxonomic boundaries. In other cases, taxonomic boundaries were reassessed on the basis of these partitions using other data such as physiography, ecology, and morphology. The amount of sequence variation between the members of the Pusillum Complex was comparable to that between other species of *Trillium* including *T.* subgen. *Phyllantherum*; using *matK* data, there is more variation between the different species within the Pusillum Complex than there is between the different species within *T.* subgen. *Phyllantherum*. Furthermore, the Erectum Complex, another group of closely related *Trillium* species, shows little discernable variation in DNA sequence data using the same gene regions (Millam 2006). Zomlefer et al. found similar levels of molecular variability between species within both *Veratrum* (Zomlefer et al. 2003) and in *Schoenocaulon* (Zomlefer et al. 2006).

Despite the invariance in the *psbA-trnH* sequence data within an individual species (i.e., *T. ozarkanum*), isozyme data shows genetic variation (Cabe & Werth 1995; Timmerman-Erskine 1999; Timmerman-Erskine et al. 2003) indicating breeding populations. Many species of *Trillium*, including members of the Pusillum Complex, propagate vegetatively by means of offsets on the rhizome. Evidence has indicated that many species of *T.* subgen. *Phyllantherum* occurring in flood plain habitats primarily propagate vegetatively (Ohara & Utech 1986; Ohara 1989; Ohara et al. 1990); the clump-forming nature and branching rhizomes of species like *T. texanum* indicates that there is significant vegetative propagation occurring in this and other wetland species of *Delostylis* as well. The isozyme variation strongly indicates that these species are not just replicates of a single clonal individual. Most isozyme studies in *Trillium*, however, have been used to study population structure and genetic diversity (Bayer et al. 1987; Whitkus et al. 1987; Kalisz et al. 1999, 2001; Timmerman-Erskine et al. 2003; Griffin & Barrett 2004a, 2004b; Gonzales & Hamrick 2005). Very little variation was detected in populations of *T. nivale* (Bayer et al. 1987), but other studies found high levels of genetic variation in other species (e.g.,

Timmerman-Erskine et al. 2003; Griffin & Barrett 2004a, 2004b; Gonzales & Hamrick 2005). However, even where high levels of variation were found, there was little evidence of gene flow among populations. Clonal reproduction, small population size, unstable habitat, and limited gene flow between populations all combine to increase the tendency for genetic drift to lead to speciation.

A novel result based on DNA sequence data (Figs. 16-17) and molecular phylogenetic analysis (Figs. 7-10, 12-15) was the separation of the Pusillum Complex into three distinct clusters (the Ozarkanum Cluster, the Pusillum Cluster and the Virginianum Cluster [Fig. 27]); these clusters appeared to be relatively well defined geographically (i.e., Pusillum Cluster-southeastern coastal plain except for *T. georgianum*; Ozarkanum Cluster-Interior of the southeastern U.S.; and Virginianum Cluster-tidewater DelMarVa peninsula except for *T. monticola*). This split into three clusters is not anticipated in any published treatment of the species (Freeman undated, 1994, 1995; Freeman et al. 1979; Garrett 1982; Case & Case 1997; Jacobs & Jacobs 1997; Timmerman-Erskine 1997, 1999; Case 2002; Timmerman-Erskine et al. 2002a, 2002b, 2003). This unanticipated division prompted closer examination of the clusters to see if they were indeed valid subdivisions for *T. pusillum* s.l.

It should be noticed that the clusters in the Pusillum Complex were not morphologically well defined (Figure 18). A distinctive character in the Pusillum Cluster is the presence of stomates on the upper surface of the leaves, but this trait is not exhibited by all members of the Cluster; overlap between the Ozarkanum cluster and the Pusillum Cluster (Appendix D.1) involves robust plants that would otherwise be differentiated by this character. The Virginianum Cluster with its very short to absent pedicels is the most morphologically distinct of the three and the only grouping to have been previously recognized (see also Case 2002) or anticipated in previous studies with a broadly defined *T. pusillum* var. *virginianum* (Cabe 1995; Cabe & Werth 1995; Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a, 2002b, 2003), but the distribution of the Virginianum Cluster was different than what had been accepted in prior studies (Freeman et al. 1979; Garrett 1982; Freeman undated, 1994, 1995; Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a, 2002b, 2003).

The species comprising the Pusillum Complex are morphologically very similar to one another and not separable on the basis of single characters, which has complicated their recognition (see also Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a). Like members of *T. subgen. Phyllanthum* and *Erectum*, the Pusillum Complex is composed of entities (Fig. 28), separation of which requires a suite of characters. As a result of her analyses, Timmerman-Erskine made several observations about the taxa of the Pusillum Complex such as "... significant differences among varieties. These exist, however, as arrays of characters rather than as single characters," "no one character at the population level distinguished any variety," and "if qualitative characteristics, geographic and ecological affinities are also used to delimit taxa, varietal distinction is accorded to the elements of the *T. pusillum* complex" (Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a); all of these statements

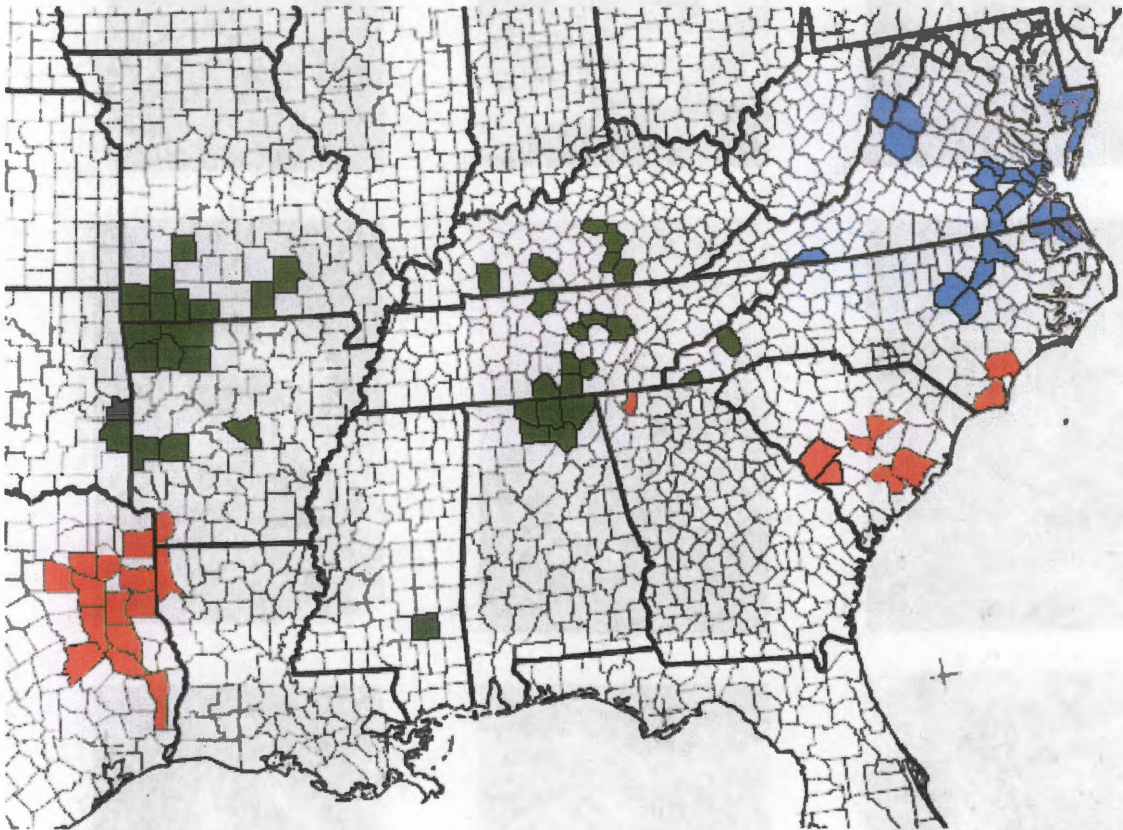


Figure 27. County-level distribution map for clusters of the *Pusillum* Complex. Green is the *Ozarkanum* Cluster, Blue the *Pusillum* Cluster, and red the *Virginianum* Cluster.



Figure 28. Sister species complexes within *Trillium*. a-c: members of the Pusillum Complex (a. *T. alabamicum*, b. *T. ozarkanum*, c. *T. monticola*); d-f: members of *T. subg. Erectum* (d. *T. erectum*, e. *T. flexipes*, f. *T. sulcatum*); g-i: members of *T. subg. Phyllantherum* (g. *T. sessile*, h. *T. cuneatum*, i. *T. luteum*).

are true of most species of *Trillium* especially groups or complexes of similar species (e.g., the Erectum Complex, *T.* subgen. *Phyllantherum*). Even within subgenera, very few species of *Trillium* can be separated from sister species on the basis of one single character; in fact, there are perhaps five or six species in *Trillium* that can be diagnosed on the basis of a single character. This is probably in part because of the simple morphology (best summarized by Elliott, “3 leaves at the summit of a stem, supporting one solitary terminal flower”), but also because of apparent recent radiation as seen evidenced by the relatively short branch lengths in phylogenetic analyses of closely related taxa. Any group of *Trillium*, in fact any species of *Trillium*, which exhibits a high degree of phenotypic plasticity especially when coupled with disjunct distributions should be examined closely because it probably “conceals many species” (Elliott 1817).

4.2.1 The Pusillum Cluster. – The Pusillum Cluster was determined to be composed of four distinct species: *Trillium pusillum*, *T. telmacola*, *T. texanum*, and *T. georgianum*. The Pusillum Cluster was not monophyletic using the ITS data set; *Trillium telmacola* was placed in basal position relative to all other members of the Pusillum Complex (Figs. 7, 12). Nor was it a monophyletic group using the *matK* data (Figs. 8, 13). However, it was monophyletic in analyses of both the *psbA-trnH* data set (Figs. 9, 14) and the combined data set (Figs. 10, 15) with bootstrap support of 100% and posterior probability of 1.0. The *matK* data identified the Pusillum Cluster on the basis of two indel characters both representing 6-base pair deletions (one of which is shared with *T.* subgen. *Phyllantherum*); characters present in the *psbA-trnH* dataset included seven unique SNPs and three indel characters (representing two insertions and one deletion) of five, six, and seven base pairs. The taxa of the Pusillum Cluster have the shortest stems on average of the three clusters (Table 16). The Pusillum Cluster was separated from the Ozarkanum Cluster by the second discriminant function (Table 19) which has the greatest loadings are for stamen characters: negative loading for the composite stamen length, and positive loadings for the filament and anther length (Table 22).

The division of the Pusillum Cluster into four distinct species was supported by both DNA sequence data (Figs. 16-17) and morphology (Fig. 19). *Trillium texanum* and *T. georgianum* can be separated from the other members of the cluster based on the presence of stomates on the upper surface of the leaves. *Trillium georgianum* also has the longest, narrowest leaves of any member of the Pusillum Complex (mean leaf/width ratio is 4.89, over one SD greater than the cluster mean). *Trillium telmacola*, an extremely rare taxon from the sand hills of the Savannah River Reservation on the Aiken and Barnwell Co. line in SC, is the most morphologically distinct with many traits either larger or smaller than one SD from the cluster mean including leaves (both the largest and with the smallest length/width

ratio), sepals (both the widest and the smallest length/width ratio), the shortest petals, shortest style, longest stigma, and shortest stem/leaf ratio; in addition two ratio characters are more than 2 SD away from the cluster mean sepal/petal ratio and stigma/style ratio (Table 23). *Trillium pusillum* has the largest petal length/width ratio (greater than one SD from the cluster mean); while within one SD of the cluster mean, it also has the shortest pedicels. Ecologically, *T. texanum* is found in relatively large clumps on hummocks around the bases of trees in wet swampy places, *T. georgianum* is found scattered in spring-wet flood plains; *T. pusillum* is found in small clusters in low, relatively dry, pine woods, and *T. telmacola* is found in a seepage area within an alluvial swamp forest.

4.2.2 The Ozarkanum Cluster. – The Ozarkanum Cluster was determined to be composed of two species: *Trillium ozarkanum* and *T. alabamicum*. In both the parsimony analysis and the bayesian analysis of ITS data, the Ozarkanum Cluster was not clearly definable as a separate entity, but was combined with the Virginianum Cluster (Figs. 7, 12). The analysis of the *matK* data showed minimal support in the parsimony analysis (52%) but the cluster as a whole was not present in the bayesian analysis; instead, each taxon was present in its own clade with support of 0.89 for *T. alabamicum* and 0.78 for *T. ozarkanum* (Figs. 8, 13). Using *psbA-trnH*, there was 100% bootstrap support and a posterior probability of 1.0 for the Ozarkanum Cluster (Figs. 9, 14); the combined data set produced similar results with 99% bootstrap support and a posterior probability of 1.0 (Figs. 10, 15). The Ozarkanum Cluster can be identified by an indel character representing a six base pair deletion as well as an SNP producing an amino acid change in the *matK* data set; characters present in the *psbA-trnH* data set that characterize the Ozarkanum Cluster were seven unique and three shared SNPs (two of which are shared with *T.* subgen. *Phyllantherum*). The taxa in the Ozarkanum Cluster have the most robust plants of any of the three clusters (Table 16) having the longest stems, largest leaves, and largest flowers. It is easily separable from the Virginianum Cluster on the basis of pedicel length. It is separated from the Pusillum Cluster by the second discriminant function (Table 19) which has the greatest loadings are for stamen characters: negative loading for the composite stamen length, and positive loadings for the filament and anther length (Table 22).

Trillium alabamicum and *T. ozarkanum* are two of the most similar species in the Pusillum Complex. The leaves and petals of *T. ozarkanum* are generally longer and wider than those of *T. alabamicum*, the mean leaf length/width ratio of 3.23 indicates that *T. alabamicum* leaves have a longer, narrower, and more elliptical shape. In addition, the leaves of *T. ozarkanum* are more ovate in shape with an acute apex than the apically rounded, elliptical leaves of *T. alabamicum*. There are habitat differences as well; *T. alabamicum* is found predominantly in swamps or spring-wet floodplains, whereas

T. ozarkanum is predominantly found on upland, dry, rocky slopes. *Trillium alabamicum* is found predominantly in the interior southern highland rim virtually surrounded by populations of *T. ozarkanum*.

4.2.3 The Virginianum Cluster. – The Virginianum Cluster was determined to be composed of four species: *Trillium virginianum*, *T. monticola*, *T. carolinianum*, and *T. palustris*. In both the parsimony analysis and the bayesian analysis of ITS data, the Virginianum Cluster was not clearly definable as a separate entity, but was combined with the Ozarkanum Cluster (Figs 7, 12); it was also not supported using the *matK* data (Figs. 8, 13). However, using *psbA-trnH*, there was 92% bootstrap support and a posterior probability of 0.89 for the Virginianum Cluster (Figs. 9, 14); the combined data set produced similar results with 84% bootstrap support and a posterior probability of 0.99 (Figs. 10, 15). Unlike the Pusillum Cluster and the Ozarkanum Cluster, the Virginianum Cluster could not be characterized by changes in the *matK* DNA sequence. There was, however, more information content in the *psbA-trnH* data set including an indel character representing an eight-base pair insertion and four unique SNPs that could be used to identify members of the Virginianum Cluster. Morphologically, the pedicel length in the Virginianum Cluster was the shortest of all members of the Pusillum Complex (Table 16); the mean was more than one standard deviation shorter than that of either of the other means.

The unexpected division of the Virginianum Cluster into four distinct species was supported by DNA sequence data (Figs. 16-17), and morphology (Fig. 21); other studies based on isozyme data (Cabe & Werth 1995) as well as morphological data (Cabe 1995) had concluded that there was only one species. Populations in central North Carolina that had previously been considered to be *T. pusillum* because of their longer pedicels were clearly shown to be members of the Virginianum Cluster based on DNA sequence data and phylogenetic evidence. The significance of the variation in pedicel length was not recognized because apparent increase in pedicel length from north to south was believed to be clinal in nature rather than morphologically significant (Timmerman-Erskine et al. 2002a). Morphologically, *Trillium monticola* has not only the broadest leaves, but the smallest leaf length/width ratio (means more than one SD smaller than the cluster mean) and the longest filament (and stamen), stem/pedicel ratio, and ovary/stigma ratio (all more than one SD larger than the cluster mean). *Trillium carolinianum* has the longest pedicel (more than one SD from the cluster mean). Variation was first seen between populations of the upper and lower coastal plain in the DNA sequence data; when the morphological analysis was done, I checked to see if this was a valid distinction supported by morphology. *Trillium palustris* (lower coastal plain) is typically a more robust plant than *T. virginianum* (upper coastal plain); it has larger, non-overlapping means (95% confidence interval) for leaf width, sepal length, anther length, pistil length, and sepal to petal ratio, but smaller length/width ratios for leaves, sepals and petals.

4.3 The Synthetic View

Having obtained a refined view of *Delostylis* based on morphology and molecular phylogenetic data, it is possible to consider other aspects of the group such as its distribution, variability, and conservation biology. Restricted to the unglaciated southeastern US and found in scattered populations, it is possible that these species are range limited by lack of long-distance seed dispersers. It is this restricted distribution that makes many members of *Delostylis* of conservation interest.

Unlike any of the other groups of *Trillium*, *Delostylis* is the only one restricted to the unglaciated southeastern US (Estill & Cruzan 2001; see also Mayewski et al. 1981). Members of the Erectum Group (e.g., *T. subgen. Trillium*) exhibit the well known eastern North America-eastern Asian disjunct distribution, while members of *T. subgen. Phyllantherum* exhibit an eastern-western North American disjunct distribution (Fernald 1931; Wood 1971, 1972; Raven 1972; Thorne 1972; Xiang et al. 1998; Wen 1999). Most individual species in *T. subgen. Phyllantherum* are, however, restricted to the unglaciated Southeast as well. It is possible that lack of long-distance dispersers is responsible for this restricted pattern of distribution.

Consideration of the detailed distribution of individual *Delostylis* species provides a further indication that dispersal may be a limiting factor for the group. With the exception of *T. ozarkanum* and *T. catesbaei s.l.*, these are not widely distributed taxa (Figure 5); this is true of most of the southern species of *T. subgen. Phyllantherum* as well. In fact half of the species (*T. persistens*, *T. georgianum*, *T. telmacola*, *T. carolinianum*, *T. monticola* and *T. pusillum*) are currently known from five counties or less. In addition, most of these species do not show wide disjunctions in locations. Even though the patterns of disjunction within *T. ozarkanum* are similar to those of other Ozark species (Steyermark 1934; see also Fernald 1931), there are gaps in the distribution that may be due to undercollection (Freeman, pers. com.) as well as lack of suitable habitat or dispersal agents.

One thing that has long puzzled taxonomists is the distribution; how did *Trillium* get to where they are? Griffin and Barrett (2004b) discuss the "rare long-distance dispersal event" responsible for the post-glaciation distribution of *T. grandiflorum* without specifics. It is known that ants (Gates 1940, 1941; Lanza et al. 1992; Kalisz et al. 1999), arachnids (Gunther & Lanza 1989), and yellow jackets (Jules 1996; Zettler & Spira 2001; Bale et al. 2003) are the primary dispersers of *Trillium* seeds; there is an aril or eliasome (Berg 1958) on the seeds composed of lipids and carbohydrates (Lanza et al. 1992) that attracts the insects and arachnids. In addition, deer (Vellend et al. 2003), which are known for predation of *Trillium* plants, may also act as a dispersal agent. For example, deer predation is a problem at Francis Beidler National Forest (pers. comm.) and the Great Smoky Mountains National Park (Webster et al. 2005). There are also reports of birds removing the red fruits characteristic of *T. undulatum* (McClements pers. comm.) and wild turkey removing the green fruits that are more typical in *Trillium*

(Vagner pers. comm.; Africa pers. comm.). It is known that the seeds will survive a trip through a deer gut albeit with reduced viability (Vellend et al. 2003), there are no such studies about seeds surviving a trip through a birds crop.

In the Coastal Plain species, there is a high correlation between chloroplast DNA sequence data and physiography which is not seen in the more interior species (e.g., *T. ozarkanum*, *T. alabamicum*, *T. carolinianum*, *T. georgianum*, and *T. monticola*). The sequence data for *T. ozarkanum* is uniform throughout its range across several different physiographic provinces. *Trillium texanum* are all found on the tertiary uplands of the south central plain (Griffith et al. 2004) or the east Texas forest region (Singhurst et al. 2002; MacRoberts & MacRoberts 2005) in three different watersheds, and the sequence data are the same. There were differences in the DNA sequence data between the Aiken Co., SC and the Dorchester Co., SC populations which were supported by morphology; investigation showed these populations to be different physiographically: sand hills (Aiken Co. SC; *T. telmacola*) and the coastal plain (Dorchester Co., SC and Pender Co., NC; *T. pusillum*). Similarly, there were DNA sequence differences between the Maryland populations of *T. virginianum*, the James City Co. VA populations, and the central North Carolina populations; investigation again showed physiographic differences: lower coastal plain (Maryland; *T. palustris*) and the upper coastal plain (James City Co.; *T. virginianum*). One potentially significant difference between the coastal plain populations and the more interior populations is habit instability due to storm disturbance in the more coastal populations.

With the adaptation of species within *Delostylis* to such disparate habitats as dry, upland rocky slopes to swamps, adaptation to habitat is apparently an important aspect of speciation. In the Pusillum Complex, habitats range from upland rocky slopes, spring-wet flood plains, to swamps subject to repeated inundation. Other than *Trillium texanum*, there have been few formal studies of the ecology of the members of the Pusillum Complex (MacRoberts & MacRoberts 2005). *Trillium texanum* plants are found on hummocks around the bases of trees in wetlands; but some *T. alabamicum* plants are found in open in areas in wetlands subject to frequent and complete inundation during the spring rains. Jacobs & Jacobs (1997) noted that wetland plants typically have narrower, elongate, branching rhizomes whereas the plants of drier habitats tend to have the thicker, stouter rhizomes. Presumably, the smaller rhizome is better adapted to the wet soil than the thicker rhizomes are. Unfortunately, no other characters have been noticed that differ between upland and wetland populations.

In addition to adaption to edaphic conditions, differences in flowering times of wetland and dryland plants could be another factor driving speciation. Wetland species typically flower approximately two weeks earlier (March to April) than "dryland" species (April-May) contributing to reproductive isolation. The upland plants are geographically the more northern plants which could account for the flowering-time lag, but this phenomenon is observed in more southern plants as well;

flowering of populations of *T. texanum* under drought conditions was delayed by two-four weeks (Singhurst, pers. comm.); in addition, upland populations of *T. alabamicum* flower later than wetland populations.

The members of the Pusillum Complex flower earlier than most pedicellate *Trillium*. This early flowering could play a part in the potential under-collection particularly across MS, AL and GA; Freeman (pers. comm.) believed under-collection accounted for "gap" in the distribution across those states. While it might be assumed that early flowering might be a limiting factor in distribution due to lack of pollinators, in other species such as *T. grandiflorum*, flowering time occurs later in the year the further north the population occurs.

4.3.1 Conservation issues. - With its fragmented and frequently small populations, member of the Pusillum Complex were readily recognized at the state level (e.g., KY, MD, NC, and TN) for needing protection as imperiled, threatened, or endangered. As noted earlier, five of the 10 species that are included within the Pusillum Complex are known from five counties or less (*T. telmacola*, *T. carolinianum*, *T. georgianum*, *T. monticola* and *T. pusillum*); in addition, more widespread species may be known from one to few counties in some states (e.g., *T. texanum* in Caddo Parish, LA or *T. ozarkanum* in MS or OK). New populations continue to be discovered; since the beginning of this study, new county record populations have been discovered in Russell Co., KY, Lancaster Co., VA, and Brunswick Co., NC. Continued surveys are needed to establish distributions of these species.

In addition to being a potential seed disperser, deer pose the largest non-human threat to *Trillium* populations (see Russell et al. 2001 and Rooney & Waller 2003 for reviews). Deer populations are on the rise nationwide (Anderson 1997; Knox 1997; Alverson et al. 1998; Rooney 2001), and some locales have instituted controlled deer hunts in attempts to control population sizes (McCullough 1997). Deer predation is believed responsible for complete or partial extirpation of several species of rare plants (including several species of *Trillium*) in the Cades Cove area of Great Smoky Mountains National Park (Webster et al. 2005). Once a population of *Trillium* has been negatively impacted, recovery to pre-impact levels may not be seen even 30 years after the initial disturbance (Jules 1998). If the disturbance is short term, recovery or rebound can be measured in as little as three years (Frankland & Nelson 2003). Long term recovery, on the other hand, can be impossible without "active restoration" (Webster et al. 2005). Even after eight years of deer exclusion, recovery in Cades Cove consists primarily of increasing numbers of survivors rather than recolonization by suppressed species (Webster et al. 2005) which is in keeping with results found by Jules (1998).

Populations of the Pusillum Complex appear to be sensitive to the amount of cover. Most populations occur in deciduous forests flowering well before the tree canopy leafs out in the spring; but

T. monticola and *T. pusillum* both occur in dry pine woods. *Trillium monticola* is found on the forested side at the edge of the forest-clearing interface, but this is not true of the populations of *T. pusillum* in the Francis Beidler Forest. The population appears to be dwindling in part because of increased evergreen sub-canopy due to tree-fall after Hurricane Hugo (Porcher unpublished data); deer predation on this population may be compounding the problem. Like *T. monticola*, the Grayson Co., VA population of *T. carolinianum* also occurs at the forest-clearing interface, but these plants tend to be found on the clearing side. The Cumberland Co., TN population of *T. alabamicum* is found on a cleared upland site. While some species of *Trillium* can be adversely affected long-term by clear-cutting (e.g., *T. ovatum* [Jules 1998]), with its propensity for surviving in open woods or at the forest-clearing interface, this would not be true of the members of the Pusillum Complex.

Based on rediscovered populations (see also Bluebell Island, Elk River, Franklin/ Grundy Cos., TN), *Trillium* rhizomes can apparently lie dormant for an indeterminate period of time, viable seed banks exist, or plants are simply overlooked so sites of "not found" populations should continue to be monitored (e.g., Calhoun Co., SC, Johnston Co., NC, etc.). The Bluebell Island site was first seen in 1973 and vouchered only by a photograph, was "not found" during the intervening years, but was rediscovered in 1998 (Priestly pers. comm.). In addition, reports believed to be erroneous (Freeman, undated, 1994) should be thoroughly checked out; Freeman believed reports of *T. pusillum* from Georgia to be erroneous (Freeman, 1994; see also Reed 1982) as well as the reports from Russell Co., KY (Freeman 1994; see also Reed 1962). There are sites known to be extirpated: the type locality for *T. pusillum* is believed to be at the bottom of a reservoir in Berkeley Co., SC, and no populations of *T. texanum* in Panola Co., TX have ever been relocated.

Human impact on populations of *Trillium* can be just as devastating if not more so than that by deer predation. Human impact can include wild digging for the nursery trade, and negative impacts on habitat from clear-cutting, habitat fragmentation, to outright habitat destruction. As Jules (1998) and Webster et al. (2005) indicate, recovery after severe impact can take many years if ever without an active restoration plan. However, because of the propensity of populations of members of the Pusillum Complex to be found on the forest-clearing interface, habitat fragmentation may not be a critical issue as with other species. Habitat destruction, however, is not as easily overcome as habitat fragmentation. *Trillium georgianum* is known from few populations in an industrial park in Whitfield Co., GA the largest population of which was subjected to site preparation prior to construction this past year (Patrick, pers. comm.; Hollis, pers. comm.). Many populations of members of the Pusillum Complex, however, are on protected land. *Trillium telmacola*, for instance, occurs on DOE property in Aiken and Barnwell Cos. in South Carolina. Other populations are on National Forest or National Park land or other protected property like the Francis Beidler forest which is owned by the National Audubon Society.

Many species within *Delostylis* are in need of protection both at the state and federal level. As noted earlier, many populations of the Pusillum Complex were readily recognized at the state level for needing protection as imperiled, threatened, or endangered. Unfortunately, state regulations frequently lack the necessary power for adequate protection of rare species. Some species have some protection already as they occur on state, national or other protected lands (e.g., *T. telmacola* on DOE property at the SRL in Aiken SC; *T. pusillum* in the National Audubon Society Francis Beidler Forest in Four Holes Swamp). However, even public lands can change hands. With the new, narrower circumscription of species, there are several potential candidates for listing under the Federal Endangered Species Act. As noted earlier, five of the 10 species that are included within the Pusillum Complex are known from five counties or less (*T. telmacola*, *T. carolinianum*, *T. georgianum*, *T. monticola* and *T. pusillum*) and should certainly be considered for protection.

5.0 TAXONOMIC TREATMENT

5.1 Subgeneric Classification of *Trillium*.

Despite its history of species and subspecific nomenclature, the subgeneric classification of *Trillium* has needed study. *Delostylis*, proposed by Rafinesque (1819) as a genus whose members exhibited the common style with three slender stigmas, was previously placed in synonymy with *Trillium*. *Phyllantherum* (Rafinesque 1820) and, by extension, the autonym *Trillium*, are the only currently recognized subgeneric, supraspecific names. A full formal hierarchy was proposed by Murray (1983, 1984) for *T.* subgen. *Phyllantherum* from section to subseries. Formal subgroups have not been proposed for *Trillium* subgen. *Trillium*, but two subgroups are often mentioned (e.g., Patrick 1984) based on the anthocyanin chemistry exhibited in aging petals: the Erectum Group (where the white petals just turn brown) and the Grandiflorum Group (where the petals turn a vivid pink). If formal subgeneric names are to be proposed for the autonymic *T.* subgen. *Trillium*, the epithet *Trillium* must stay with the Erectum Group because it includes the type species for *Trillium*, *T. cernuum* (Britton and Brown 1913; see also Gleason 1906; Freeman 1975; Reveal 1992).

There are three possible approaches regarding subgeneric names: use the PhyloCode and only name clades, retain the informal names, or erect a formal structure under the ICBN. Because the PhyloCode is still in draft stage and under revision, it is premature to erect formal names with this nomenclatural system. Inherent in the continued use of informal names is the potential for ambiguity (see also Table 1); in addition, there is little phylogenetic content in informal names. Therefore, I have elected to erect a formal subgeneric framework for the pedicellate species of *Trillium*. There are two full subgeneric ranks available for use (ICBN, Article 21; Greuter et al. 2000): Section and Series with allowable subdivisions subsection and subseries. I prefer to use full ranks to allow for possible future expansion using either subdivisions or supradivisions.

Because the current autonymic *Trillium* subgen. *Trillium* is not monophyletic (Figs. 7-10, 12-15), it should be replaced with a series of monophyletic groups. Using the principle of exhaustive subsidiary taxa (Simpson 1961; de Queiroz and Gauthier 1992; Cantino et al. 1997), a full framework is described. All but one of the monophyletic clades based on molecular analysis are definable on morphological grounds and are proposed as subgenera. The exception is the clade containing *T. grandiflorum* and *T. nivale*. These species will be designated *incertae sedis* (Wiley 1979) and not placed in any subgenus (see also Cantino et al. 1997). The subgenera for *Trillium* are: *Phyllantherum* (Freeman 1975), *Undulatum*, *Hibbersonii*, *Ovatum*, *Delostylis* and the autonymic *Trillium*. Subgenera *Undulatum* and *Hibbersonii* are each mono-specific containing *T. undulatum* and *T. ovatum* var. *hibbersonii* (elevation to species in preparation by O'Neill and Farmer), respectively. Subgenus *Ovatum* contains 2-4

species; *Trillium* subgen. *Ovatum* is shown in these analyses to need additional sampling and analysis to clarify the status of these species; the elevation of *T. ovatum* ssp. *oettingeri* to species is in preparation by Renner and Farmer. The subgenera *Delostylis* and *Trillium* would each contain two sections: *T.* subgen. *Delostylis* with sections *Catesbaei* containing the species now included in *T. catesbaei*, and *T. persistens*; and *Pusillum* containing the Pusillum Complex; and *T.* subgen. *Trillium* with sections *Orientale* containing the Asian species and the autonymic *T.* sect. *Trillium* containing the Erectum Complex.

Key to the Subgenera of *Trillium*

1. Stigmatic branches united into a common style, 0.5-10 mm in length 2
 2. Flowers white, pink, or aging to pink *T.* subgen. *Delostylis*
 2. Flowers remaining white *T. nivale*
1. Stigmatic branches not united into a common style, sessile and separate upon the ovary 3
 3. Flower sessile upon the leaves *T.* subgen. *Phyllantherum*
 3. Flower pedicellate 4
 4. Leaves petiolate; flower petals white with prominent red "V" at base of petals (rarely albino) *T.* subgen. *Undulatum*
 4. Leaves sessile; flower petals not so marked 5
 5. Plants < 10 cm tall *T.* subgen. *Hibbersonii*
 5. Plants ≥ 10 cm tall 6
 6. Petals red, maroon, or white (rarely pink), if white, aging to brown; ovary red, white, purple or black *T.* subgen. *Trillium*
 6. Petals white fading to pink or maroon; ovary green 7
 7. Corolla funnel-form, concealing ovary *T. grandiflorum*
 7. Corolla not funnel-form, ovary evident *T.* subgen. *Ovatum*

5.2 *Trillium* subgen. *Delostylis* (Raf.) stat. nov.

Delostylis Raf.

Delostylis Raf. J. Phys. Chim. Hist. Nat. Arts 89: 96-107.

Type species: *Trillium catesbaei* Ell.

Petals white or pink, if white turning pink with age. Stigmatic branches united at the base into a common style.

As in all groups of *Trillium* species, individual plants can be difficult to key out. To echo what John Freeman said (1975), "Due to the simple morphology of *Trillium* plants, the variety of characteristics suitable for key-making is limited. The notorious variability of *Trillium* greatly limits the usefulness of many easily observed features ... intra-and interspecific variation makes simple, effective, natural, dichotomous keys based on morphology alone an impossibility in this genus." There can be significant overlap between individuals within the same population. It is essential, whenever possible, that populations are keyed rather than individuals. This key was prepared based primarily on measurements of dried specimens.

Key to *Trillium* subgenus *Delostylis*

1. Leaves with stomates on the upper surface, appearing farinose 2
 2. Leaves linear, 4x longer than wide 5. *T. georgianum*
 2. Leaves ovate or elliptic, not linear, less than 4x longer than wide 11. *T. texanum*
1. Leaves without stomates on the upper surface, appearing glabrous 3
 3. Pedicel $\leq$ 10 mm long (rarely to 12 mm) 4
 4. Pedicel (0-) 0.5-1 mm long; leaves (1.5-) 2.0-2.4 (-2.9) x as long as broad; leaves (14-) 15-20 (-22.5) mm wide; filament (4.5-) 4.6-5.8 (-8) mm; plants of upland woods ... 6. *T. monticola*
 4. Pedicel (0.5-) 1-6.5 (-11) mm long; leaves (2.1-) 2.5-3.8 (-6) x as long as broad; leaves (6-) 10-17 (-25) mm wide; filament (2.5-) 3-5 (6.5) mm; plants of wetland woods 5
 5. Anthers (3.2-) 4.5-6 (-7) mm; stamens (5.7-) 8.4-10.9 (-12.5) mm; stigmas (1-) 2.1-3 (-5) mm; leaves elliptic; plants of the lower coastal plain 8. *T. palustris*
 5. Anthers (2.5-) 2.7-4.8 (-6.8) mm; stamens (5.7-) 6-9.2 (-13.2) mm; stigmas (1-) 1.2-2.5 (-4.7) mm; leaves ovate; plants of the upper coastal plain and piedmont 6
 6. Pedicel (1.2-) 1.8-6.6 (-11) mm; stamens (5.7-) 6-7.6 (-13.2) mm; stigmas (1-) 1.2-2 (-2.5) mm; plants of piedmont to edge of upper coastal plain 4. *T. carolinianum*
 6. Pedicel (0.5-) 1-2.1 (-7) mm; stamens (6-) 7.5-9.2 (-11) mm; stigmas (1-) 2-2.5 (-4.7) mm; plants of upper coastal plain 12. *T. virginianum*
 3. Pedicel 10-56 mm long 7
 7. Pedicel horizontal or recurved, rarely erect; sepals reflexed or curved; petals arcuate-recurved; anthers strongly curved outwards 1. *T. catesbaei*
 7. Pedicel erect or leaning; sepals spreading or in the same plane with petals; petals erect to widely spreading; anthers erect or spreading, straight or gently curved 8
 8. Ovary sharply angled; sepals $\frac{1}{2}$ to $\frac{3}{4}$ length of petals 2. *T. persistens*

- 8. Ovary weakly angled to rounded; sepals $\pm$ equal to petals (0.9-1.5) 9
- 9. Leaves ascending; sepals 1.4 times as long as petals; plants of sphagnum bogs in the Carolina sand hills 10. *T. telmacola*
- 9. Leaves horizontal to declining; sepals and petals $\pm$ equal in length (0.9-1.1 times long as wide); 10
- 10. Leaves narrower, (1.9-) 3-4.1 (-5.6) times as long as wide; pedicel (23-) 25-33 (-56) mm; plants of dry pine woods 9. *T. pusillum*
- 10. Leaves broader, (2-) 2.7-3.4 (-5.5) times as long as wide; pedicel (7-) 13-30 (-45) mm 11
- 11. Leaves ovate, acute; sepals (14-) 22-30 (-41) x (4-) 6-11 (-17) mm, (2-) 3-4.3 (-5.6) times as long as wide; plants generally of upland rocky slopes 7. *T. ozarkanum*
- 11. Leaves elliptic, rounded; sepals (13-) 18-25 (-30) x (4-) 6-8 (-12) mm, (1.8-) 2.7-3.1 (-4) times as long as wide; plants generally of swamps and flood plains 3. *T. alabamicum*

The species descriptions of *Trillium persistens* and *T. catesbaei* are compiled from Gleason (1906), Barksdale (1938), Gates (1917), Garrett (1982), Samejima & Samejima (1987), Mitchell (1990), Case & Case (1997), Jacobs & Jacobs (1997), Case (2002), and species protologs. The list of representative specimens examined for the Pusillum Complex is given in **Appendix A**.

5.2.1 *Trillium* subgen. *Delostylis* sect. *Catesbaei* nom. nov.

Plants with flowers that are either white fading to pink with age, or pink, and possessing a common style. Different from sect. *Pusillum* in that the sepals are reflexed, recurved, or spreading instead of being in the same plane with the petals. Ovary deeply or sharply 6-angled.

1. *Trillium catesbaei* (Figs. 1c, 4)

Trillium catesbaei Elliott, Sketch Bot. S. Carolina 1: 429, 1817.

Trillium affine Rendle; *T. nervosum* Elliott; *T. stylosum* Nuttall.

TYPE: United States: SC: Pendleton Co.; *Baker & Perry s.n.*, sin date. GH.

Rhizomes short, tapered to a point distally. Stems 1-2, round in cross section, 20-45 cm, slender, glabrous. Leaves usually raised somewhat, exposing flower; blade green, often with underlying purplish maroon in well-lighted plants, major veins on adaxial surface deeply engraved, elliptic-ovate,

6.5-15 x 4-8 cm, including elongated petiolelike base, proximal 1/3 of leaf gradually tapered to base, margins often slightly raised, apex blunt-acute to acuminate. Pedicel deflexed, recurved, or erect (Picket Co., SC), angled, 2-4(-5) cm. Flower opening at or recurved below bracts, rarely erect; sepals falcate-recurved, green or streaked with purple, linear-lanceolate, 20-45 x 7-8 mm, margins $\pm$ flat, apex acuminate; petals falcate-recurved distally, white, pink, or rose, darkening to or remaining pink with age, veins visible but not appearing engraved, elliptic-oblong to oblong-lanceolate, 3.5-5 x 1-2+ cm, thin-textured, bases somewhat imbricated, forming funnel-like tube, margins undulate, apex acuminate; stamens prominent, recurved, 16-25 mm; filaments white, slightly longer and narrower than anthers; anthers recurving, bright yellow. 5-14 mm, thick, dehiscence introrse; ovary inconspicuous, white, angular-ovoid, deeply 6-angled, 4-12 x 3-7 mm, slender, base narrow; style 2-6 mm; stigmas prominent (except in Picket Co., SC populations), strongly curled (circinate) to erect, connate basally, pale green or white, not lobed, 4-10 mm, uniformly thin. Fruits baccate, greenish or whitish, ovoid-globose, angles less prominent when mature, 1-1.5 cm diam., pulpy but not juicy.

Flowering spring-summer (late Mar – early June). Acid soils, open dry or rich woods, laurel and rhododendron thickets, cove forests; 50-800 m; AL, GA, NC, SC, TN.

The epithet *catesbaei* was chosen in honor of Mark Catesby.

Trillium catesbaei, like *T. pusillum*, appears to have some problematic nomenclatural history of its own. In addition to Elliott himself describing *T. catesbaei* and one of its synonymous taxa (*T. nervosum*), Linnaeus (1753) referenced Catesby's specimen in his description of *T. cernuum* in addition to a Kalm specimen later lectotypified as *T. cernuum*. In 1901, Rendle affixed the name to the Catesby drawing (Fig. 26) having neither Catesby's nor Elliott's specimens available to him. Fernald (1944) suggested that based on the descriptions and the specimens, Elliott confused the specimens of *T. catesbaei* and *T. nervosum* when he was describing the two species. Mitchell (1992) suggested that the problem was further compounded when *T. nervosum* was dropped in favor of the later name *T. stylosum* by such authors as Watson (1879) and Small (1913).

There are at least three different floral morphs that are currently identified as *T. catesbaei*. There is a small plant with initially white flowers (aging to pink as all members of subgen. *Delostylis*), there is a larger plant with vivid pink flowers, and there is another plant with an erect pedicel and minimal stigmatic branches. DNA sequence data of individual samples showed that these three morphs have divergent sequences; a more thorough survey should clarify both taxonomic and geographic boundaries for potential additional species in this group.

2. *Trillium persistens* (Figs. 1b, 3)

Trillium persistens W.H. Duncan, Rhodora 73: 244. 1971.

TYPE: United States: GA, Rabun Co.; *Duncan 23548*, 23 March 1971. US!

Rhizomes horizontal to erect, short, praemorse. Stems 1-2, round in cross section, 20-30 cm, slender, glabrous. Leaves horizontal to drooping distally, sessile; blade 3-5-veined, ovate-lanceolate to lanceolate, 3-8.5+ x 1.5-3.5 cm, adaxial surface faintly glossy, rarely with ± 2 mm, winged petiolelike base, apex acuminate. Pedicel erect or slightly leaning, 1-3 cm, $\frac{1}{4}$ - $\frac{1}{2}$ bract length at anthesis. Flower opening above leaves; sepals spreading, green, elliptic to narrowly ovate, 11-22 x 5-6 mm, thin-textured, margins entire, apex acute; petals erect proximally, spreading distally, white, fading to deep pink with inverted C-shape basal portion remaining white, veins not engraved, linear-elliptic to occasionally linear, 2-3.5 x 0.5-1 cm, thin-textured, margins undulate at least in distal portion, apex acute; stamens prominent, erect to divergent, straight, 9-14 mm; filaments $\pm$ equaling anthers; anthers straight, yellow or white, dehiscence introrse; connective barely longer than anther sacs; ovary white or greenish white, obovate, very sharply 6-angled, 2.5-6 mm; style 2-6 mm; stigmas erect, slightly divergent at tip, delicate, not lobed, shortly connate basally, uniformly thin. Fruits baccate, greenish white, 6-angled, pulpy, not juicy.

Flowering spring (early Mar – mid Apr.). Humus soils in mixed deciduous-pine woodlands, along stream flats and at edges of *Rhododendron* thickets, occasionally in open *Vaccinium*-filled clearings; of conservation concern; 50 m; GA, SC.

The epithet *persistens* was based on the fact that the foliage persists well into summer unlike other species.

Trillium persistens is the only pedicellate species of *Trillium* on the Federal Endangered Species List. It is known from 7 populations in and around the Tallulah Gorge in Habersham and Rabun Cos. in GA and Oconee and Stephens Cos. in SC (Patrick, pers comm.).

5.2.2 *Trillium* subgen. *Delostylis* sect. *Pusillum* nom. nov.

Plants with flowers that are either white fading to pink with age, or pink, and possessing a common style. Different from sect. *Catesbaei* in that the sepals are in the same plane with the petals instead of being recurved, reflexed or spreading. Ovary obscurely 6-angled, conspicuous.

3. *Trillium alabamicum* – stat. nov.

Trillium alabamicum sp. nov. J.G. Garrett ex S.B. Farmer nom ined.

T. pusillum var. *alabamicum* J.G. Garrett, Taxonomic relationships among *Trillium* taxa allied with *Trillium pusillum* p. 47, 1982.

TYPE: United States, AL: Madison Co., *Freeman* 739, 20 March 1972, AUA!

Stems (64-) 75-211 (-213) mm. **Leaves** (26-) 27-62 (-63) mm x (7-) 8-21 (-25) mm, (2-) 2.5-4.5 (-5.1) times longer than wide, usually declining, blade dark green often with maroon undertones especially when young, not mottled, 3 primary veins, lanceolate, rounded-acute. **Pedicel** 7-35.8 (-37) mm, erect to ascending. **Flower** above leaves; **sepals** 13-30 mm x 4-11 (-12) mm, (1.8-) 2.3-3.7 (-4) times longer than wide, conspicuous, spreading to same plane as petals, dark green with maroon undertones when young; **petals** 15-27 (-30) mm x (4-) 5-12 (-13) mm, (1.9-) 2.1-3.8 (-4) times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations, apex; **stamens** (6-) 7-13 (-13.7) mm, erect-spreading; **filaments** (2.5-) 2.7-4.8 (-5) mm, whitish-green, generally shorter than anthers, slender; **anthers** (3-) 3.5-9.5 (-9.7) mm, ± straight, yellow, thicker than filaments, dehiscence introrse; **connectives** not extended beyond anther sacs; **ovary** (2-) 2.2-4.5 mm tall, conspicuous, green, ovoid, obscurely 6-angled; **style** (1.2-) 1.3-5 (-5.5) mm, green; **stigmas** 3, (1.3-) 1.7-5 (-6.5) mm, confluent with style, green, linear, long-spreading, uniformly thin and threadlike. **Fruits** white or pale greenish, ovate.

Flowering: early March to late April. **Habitat and distribution:** predominantly flood plains and swamps of south central Tennessee and north central Alabama although scattered populations occur outside this area, particularly in Tennessee (e.g., Putnam Co. and Cumberland Co.).

The epithet *alabamicum* was based on the fact that the type locality and primary populations for this taxon were in Alabama.

Specimens that were measured for the morphological analysis are Jackson Co., AL, *Freeman & Jones s.n.*, 23 March 1995, TROY, ref. #0067; Limestone Co., AL, *Diamond, Gunn & Freeman s.n.*, 11 March 1993, TROY, ref. #0068; Madison Co., AL, *Diamond* 8095, 11 Mar 1993, TROY, ref. #0072; Marshall Co., AL, *Freeman & Jones s.n.*, 23 March 1995, TROY, ref. #0074; Morgan Co., AL, *Freeman & Keener s.n.*, 16 March 1996, TENN, ref. #0986; Coffee Co., TN, *Smith* 112, 13 April 1982, VDB, ref. #1626; Cumberland Co., TN, *Farmer & Rhinehart s.n.* 10 April 2005, TENN, ref. #2499; Lincoln Co., TN, *Horn* 109, 2 April 1983, VDB, ref. #1646; Putnam Co., TN, *Patrick* 4802, 14 April 1983, TENN, ref. #0299.

Trillium pusillum var. *alabamicum* was published in a thesis in 1982 which is not considered valid publication under the ICBN; formal publication of the name is in preparation (Garrett, pers. comm.). Despite its invalid status nomenclaturally, this name has been used by Freeman (undated, 1994, 1995), Case and Case (1997), and Jacobs and Jacobs (1997). This species differs in habitat (wetland

versus upland) and leaf morphology (3 versus 5 primary veins; apex rounded-acute versus apex pointed-acute) from its sister species *T. ozarkanum*.

Based on the ITS sequence data, it is possible that chloroplast transfer has taken place in the Cumberland Co., TN population, between *T. alabamicum* and *ozarkanum*. The habitat of this population is certainly more like *T. ozarkanum* than *T. alabamicum*, but the psbA-trnH chloroplast DNA sequences are clearly that of *T. alabamicum* while the nuclear data indicate a closer relationship to *T. ozarkanum*. The morphology does not appear to be significantly different from the typical *T. alabamicum* in these plants.

4. *Trillium carolinianum* – nom. nov.

Trillium carolinianum sp. nov. S.B. Farmer nom. ined.

TYPE: To be designated upon publication.

Different from the other species in the Virginianum Cluster (*T. monticola*, *T. virginianum* and *T. palustris*) by its longer pedicel combined with shorted stamens and stigmas.

Stems 60-163.2 (-180) mm. Leaves 23-45 (-53) mm x 7-16 (-17) mm, 2.1-3.2 (-3.5) times longer than wide, spreading horizontally to declining, blade dark green often with maroon undertones especially when young, not mottled, 3 primary veins; ovate to broadly ovate, apex rounded to obtuse. Pedicel 1.2-6.5 (-11) mm. Flower above leaves; sepals 9-22.5 (-29) mm x 3.5-6.8 (-7) mm, 2.2-3.9 (-4) times longer than wide, conspicuous, spreading to same plane as petals, dark green with maroon undertones when young; petals 14-21 (-24) mm x 3-6.5 (-8) mm, 2.5-4.9 (-5.3) times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, shape, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; stamens 5.7-7.6 (-13.2) mm, erect-spreading; filaments 2.8-4.5 (-6.4) mm, green, ± equaling or slightly shorter than anthers, slender; anthers 2.5-4.8 mm, ± straight, yellow, thicker than filaments, dehiscence introrse; connectives not extended beyond anther sacs; ovary 1.8-3.8 (-4.0) mm, conspicuous, green, ovoid, obscurely 6-angled; style 1.2-4 (-4.5) mm, green; stigmas 3, 1.0-2 (-2.5) mm, confluent with style, green, linear, long-spreading, uniformly thin and threadlike. Fruits white or pale greenish, ovate.

Flowering: March to April. Habitat and distribution: moist woods and flood plains of central North Carolina and south-central Virginia.

The epithet *carolinianum* was chosen because the bulk of the populations are in North Carolina.

Herbarium sheets that were measured for the morphological analysis are Nash Co., NC, *Farmer*, *Franklin*, & *Vasquez* s.n., 3 Mar 2005, TENN, ref. #2500; Wake Co., NC, *Fox & Adams* 3561, 20 April

1950, MO, ref. #1989; Grayson Co., VA, Wieboldt, Rawinski & Hobbs 7566, 1 May 1991, VPI, ref. #1243.

These plants were formerly considered part of *T. pusillum* because of the longer pedicel length. DNA sequence data, however, shows that they are distinct from any members of the Pusillum Cluster and most closely related to the Virginianum Cluster. While longer pedicels can be found in the coastal plain species (*T. virginianum* and *T. palustris*), the average pedicel length of 4.98 is significantly longer than either of the other average pedicel lengths (1.78 and 1.98 respectively).

5. *Trillium georgianum* –

Trillium georgianum sp. nov. S.B. Farmer nom. ined.

T. pusillum var. *georgianum* var. nov. Jacobs & Jacobs, Trilliums in woodland and garden p. 54, 1997. nom. ined.

TYPE: To be designated upon publication.

Similar to *T. texanum* in that there are stomates on the upper leaf surface; different from all other members of the Pusillum Complex with its long narrow leaf.

Stems 98-116 (-137) mm. Leaves 35-48.5 (-56) mm x 7-10 (-11) mm, 3.9-5.9 (-6) times longer than wide, numerous stomata evenly distributed in upper surface, blade dark green with maroon undertones when especially when young, not mottled, 3 primary veins, linear to linear-lanceolate. Pedicel 13-24 (-29) mm. Flower above leaves, ascending to erect; sepals 15-17 (-19) mm x 5-7 mm, 2.4-3 (-3.8) times longer than wide, conspicuous, spreading to same plane as petals, dark green with maroon undertones when young; petals 15-19 mm x 5-9 mm, 1.7-2.7 (-3.2) times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; stamens 7-8 (-8.2) mm, erect-spreading; filaments 3-3.5 (-4) mm, shorter than anthers, greenish-white, slender; anthers 4-4.5 (-5.2) mm, ± straight, yellow, thicker than filaments, dehiscence introrse; connectives not extended beyond anther sacs, pinkish-purple; ovary 2.7-3.5 mm tall, conspicuous, green, ovoid, obscurely 6-angled; style 1.5-3 (-3.7) mm, green; stigmas 3, 1.5-2.2 mm, confluent with style, green, long-spreading, uniformly thin and threadlike. Fruits white or pale greenish, ovate.

Flowering: late March to early April. Habitat and distribution: spring wet flood-plains in Whitfield Co., Georgia.

The epithet *georgianum* was chosen because the population is in the state of Georgia.

The herbarium sheet that was measured for the morphological analysis is Whitfield Co., GA, Allison and Dickman 9096, 26 March 1996, GA, ref. #1120.

Trillium georgianum shares the rare trait of adaxial stomatal openings on the leaves with *T. texanum*. These give the leaves a farinose or spotted appearance. In addition, the leaves of *T. georgianum* are very narrow, much more so than any of the other species in the Pusillum Complex. These two characters make this an easily identifiable species.

6. *Trillium monticola* – stat. nov.

Trillium monticola (Bodkin & Reveal) S.B. Farmer stat. nov., nom ined.

Trillium pusillum var. *monticola* Bodkin & Reveal, Brittonia 34(2): 141, 1982.

TYPE: United States, VA: Rockingham Co.; James L. Reveal, Norlyn L. Bodkin #5586, 18 April 1981, MARY!

Stems 86-188.6 (-192) mm. Leaves 26-54.5 (-55) mm x 14-22.4 (-22.5) mm, 1.3-2.9 times longer than wide, spreading horizontally to declining, blade dark green frequently with maroon undertones especially when young, not mottled, 3 main veins, broadly ovate to elliptical; apices rounded. Pedicel (0-) 0.4-1 mm. Flower above leaves; sepals 16-22 (-26) mm x 4-8 (-9) mm, 2.3-3.4 (-4) times longer than wide, conspicuous, spreading to same plane as petals, dark green with maroon undertones when young; petals 16-23 (-27) mm x 4-8.2 (-9) mm, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, shape, size, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; stamens 8.8-11.8 (-17.5) mm, erect-spreading; filaments 4.5-5.9 (-8) mm, whitish-green, slender; anthers 3.8-6 (-9.5) mm, ± straight, yellow, thicker than filaments, dehiscence introrse; connectives not extended beyond anther sacs; ovary 3-4.5 (-5.5) mm, conspicuous, green, ovoid, obscurely 6-angled; style 3-5.9 (-9.5) mm, green; stigmas 3, 0.5-2.5 (-3.5) mm, confluent with style, green, linear, long-spreading, uniformly thin and threadlike. Fruits white or pale greenish, ovate.

Flowering: April to May. Habitat and distribution: upland rocky slopes in dry pine woods in the mountains on the Virginia/ West Virginia border.

The epithet *monticola* indicates "mountain-dweller" because the type locality was in the Virginia and West Virginia mountains.

Herbarium sheets that were measured for the morphological analysis are Augusta Co., VA, Cabe, Day and Garvin 129, 20 April 1983, WILLI, ref. #1295; Rockingham Co., VA, Cabe & Gavin 128, 29 April 1983, WILLI, ref. #1272; Pendleton Co., WV, Freeman & Stanley s.n., 3 May 1986, AUA, ref. #1155.

Trillium monticola has been shown to be distinct not only in habitat (upland pine woods versus swamps and flood plains), pedicel to at most 1 mm or more frequently absent, and leaf morphology (broadly ovate versus lanceolate or elliptic) from the other members of the Virginianum Complex.

7. *Trillium ozarkanum* –

Trillium ozarkanum E.J. Palmer & Steyermark., Ann. Missouri Bot. Gard. 22:504, 1935.

T. pusillum var. *ozarkanum* (E.J. Palmer & Steyermark.) Steyermark., Rhodora 62:130, 1960.

T. pusillum var. *kentuckianum* Jacobs & Jacobs, Trilliums in woodland and garden p.

54, 1997. *nom. ined.*

TYPE: United States: MO: Barry Co.; *Julian A. Steyermark* #18628, 20 April 1935, MO!

Stems (90-) 107-273.5 (-285) mm. **Leaves** (23-) 28.8-76.8 (-80) mm x (8-) 10.3-24 (-30), (2.08-) 2.3-4.2 (-4.9) times longer than wide, spreading horizontally-declining, blade dark green often with maroon undertones especially when young, not mottled, 5 primary veins, ovate to elliptic-ovate, acute. **Pedicel** (9-) 11.3-35.4 (-45) mm. **Flower** above leaves, erect to ascending; **sepals** (14-) 16.2-38.2 (-41) mm x (4-) 5-14.6 (-17) mm, (1.3-) 1.8-4.13 (-6) times longer than wide, conspicuous, spreading to same plane as petals, dark green with maroon undertones when young, shape, apex; **petals** (14-) 18-32.8 (33) mm x 6-15 (-16) mm, (1.7-) 1.8-3.8 (-4.2) times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, shape, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; **stamens** 7-13.5 (-16), erect-spreading; **filaments** 3-5.5 (-5.7) mm, whitish-green, slender; **anthers** 2.5-9.5 (-11) mm, ± straight, yellow, thicker than filaments, dehiscence introrse; **connectives** not extended beyond anther sacs; **ovary** (1-) 2.4-5 (-6) mm tall, conspicuous, green, ovoid, obscurely 6-angled, **style** (1.2-) 2-6.5 (-9.5) mm, green; **stigmas** 3, (0.5-) 1.5-4.5 (-6.5) mm, confluent with style, green, linear, long-spreading, uniformly thin and threadlike. **Fruits** white or pale greenish, ovate.

Flowering: late March to April. **Habitat and distribution:** upland rocky slopes (rarely seeps) in Missouri, Arkansas, Kentucky, Tennessee, North Carolina, and Mississippi.

The epithet *ozarkanum* refers to the fact that the type locality was in the Ozark Mountains.

Specimens that were measured for the morphological analysis are Benton Co., AR, *Kral & Carter* 63225, 31 March 1979, VDB, ref. #1607; Boone Co., AR, *Diamond* 8092, 12 April 1992, AUA, ref. #1156; Carroll Co., AR, *Diamond & Freeman* 8088, 11 April 1992, AUA, ref. #1157; Madison Co., AR, *Redfearn* 29313, 11 April 1974, NCU, ref. #0100; Montgomery Co., AR, *Barr* 66, 2 April 1980, GA, ref. #1115; Newton Co., AR, *Redfearn* 16953, 3 April 1965, AUA, ref. #1161; Polk Co., AR, *Orzell & Pell*, 00094, 19 April 1984, BRIT, ref. #1575; Pulaski Co., AR, *Shepherd & Rettig s.n.*, 17 April 1908, ASTC, ref. #2502; Washington Co., AR, *Farmer & Whitsell s.n.*, 22 Mar 2002, TENN, ref. #0250; Casey Co., KY, *Murphy* 92, 28 April 1961, KY, ref. #1252; Christian Co., KY, *Chester & Noel* 12965, 2 April 1993, AUA, ref. #1142; Marion Co., KY, *Keith s.n.*, 17 April 1965, TENN, ref. #0301; Russell Co., KY, *Farmer & Estes s.n.*, 31 May 2005, TENN, ref. #2501; Wayne Co., KY, *Hannan, Phillippe, Cladwall s.n.*, 3 April 1979, AUA, ref. #1143; Barry Co., MO, *Redfearn & Timme*

32619, 10 April 1981, UMO, ref. #1261; Barry Co., MO, *Redfearn* 32613, 3 April 1981, UMO, ref. #1262; Howell Co., MO, *Summers, Skinner, & Dugger* 10015, 20 April 2003, MO, ref. #1992; Lawrence Co., MO, *Diamond* 8085, 11 April 1992, AUA, ref. #1165; Clay Co., NC, *Freeman, Patrick & Diamond s.n.*, 16 April 1988, AUA, ref. #1149.

Trillium ozarkanum is the most robust of the members of the Pusillum Complex. In addition, its habitat of dry cherty soil on wooded slopes of deciduous upland woods is quite different from that of most other species; although *T. monticola* is also found in upland woods. The two can be easily separated by the lack of a pedicel in *T. monticola*.

8. *Trillium palustris* – nom. nov.

Trillium palustris sp. nov. S.B. Farmer nom. ined.

TYPE: To be designated upon publication.

Different physiographically from the other members of the Virginianum Complex (lower coastal plain versus upper coastal plain to the mountains), and from *T. virginianum* by leaf shape (ovate versus elliptic).

Stems (113-) 113.6-228.7 (-235) mm. **Leaves** (33-) 33.3-77.2 (-82.5) mm x (8-) 8.6-24.1 (-25) mm, 2.4-5.5 (-5.7) times longer than wide, spreading horizontally to declining, blade dark green usually with maroon undertones especially when young, not mottled, 3 primary veins, elliptic, apices rounded. **Pedicel** 0.5-6.6 (-7) mm. **Flower** above leaves; **sepals** (13-) 13.5-30.8 (-31) mm x 4-9.8 (-10) mm, 2.6-4.9 (-5) times longer than wide, conspicuous, spreading to same plane as petals, dark green with maroon undertones when young, shape, apex; **petals** (13-) 13.3-27.6 (-28) mm x 3.5-11.6 (-12.0) mm, 2-4.9 (-5.1) times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, shape, size, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; **stamens** (5.7-) 5.9-12.4 (-12.5) mm, erect-spreading; **filaments** (2.5-) 2.6-5.9 (-6.0) mm, green, slightly shorter than anthers, slender; **anthers** 3.2-7.0 mm, ± straight, yellow, thicker than filaments, dehiscence introrse; **connectives** not extended beyond anther sacs; **ovary** (1.7-) 1.7-6.8 (-7) mm, conspicuous, green, ovoid, obscurely 6-angled; **style** (2.0-) 2.-9.2 (-9.5) mm, green; **stigmas** 3, (1.0-) 1.1-4.9 (-5.0), confluent with style, green, linear, long-spreading, uniformly thin and threadlike. **Fruits** white or pale greenish.

Flowering: April. **Habitat and distribution:** wetlands and swamps of the lower coastal plain of Virginia, Maryland, and North Carolina.

The epithet *palustris* was chosen because the populations are found in swampy areas on the lower coastal plain.

Herbarium sheets that were measured for the morphological analysis are Worcester Co., MD, *Cabe & Day 126*, 23 April 1983, VPI, ref. #1227, *Reveal 5117*, 20 April 1980, NY, ref #2262; Gates Co., NC, *Williams & Dunn s.n.*, 2 April 1982, NCU, ref. #0116; Suffolk/ Nansemond Co., VA, *Fleming 10115*, 30 March 1995, VPI, ref. #1247.

The DNA sequence data provided the first indication that the Maryland and other lower coastal plain populations (e.g., the Great Dismal Swamp of Virginia and North Carolina) might be different from *T. virginianum*. In light of this information, other factors such as physiography were examined. When I realized that there were two different physiographic provinces involved, I examined the morphology to see if the differences in DNA sequence data and physiography were supported by morphology. This plant is generally larger and more robust than its sister species *T. virginianum*; but the sepals, leaves, and petals have a narrower shape. In addition, the leaves are generally ovate or lanceolate rather than elliptic as are the leaves in *T. virginianum*.

9. *Trillium pusillum* –

Trillium pusillum Michx., Fl. Bor.-Amer. (Michaux) 1: 215, 1803.

T. pusillum Pursh

TYPE: United States: SC: Berkeley Co.; *Michaux s.n.*, sin date, P!-digital.

Stems 140-202.6 (-208) mm. Leaves (42-) 45-65.2 (-70) mm x 9-24.2 (-27.5) mm, 1.9-5.1 (5.7) times longer than wide, spreading horizontally to declining, lanceolate-elliptic to ovate-elliptic, blunt, blade dark green often with maroon undertones especially when young, typically 3 major veins. Pedicel 23-45.8 (-56) mm. Flower above leaves, erect, facing upwards; sepals 15-288 (-300) mm x 3-10.3 (-12) mm, 2.1-5.3 (-5.7) times longer than wide, conspicuous, spreading to same plane as petals, dark green with maroon undertones when young; petals 15-24.8 (-25) mm x 7-9.9 (-12) mm, 1.8-2.9 (-3.1) times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; stamens 6.5-12.8 (-13) mm, erect-spreading; filaments 2.5-5.4 (-5.5) mm, greenish-white, shorter than the anthers, slender; anthers 4.0-7.5 mm, ± straight, yellow, thicker than filaments, dehiscence introrse; connectives not extended beyond anther sacs, pinkish-lavender; ovary 2.7-6.8 (-7) mm, conspicuous, pale green, ovoid, obscurely 6-angled; style 2.5-4.9 (-5.0) mm, green; stigmas 3, 1.0-3.9 (-4.0) mm, confluent with style, green, long-spreading, uniformly thin and threadlike. Fruits white or pale greenish, ovate.

Flowering: late March to early April. Habitat and distribution: dry pine woods of the coastal plain of North Carolina and South Carolina.

The epithet *pusillum* is indicative of the diminutive size of the plants.

Herbarium sheets that were measured for the morphological analysis are Pender Co., NC, *Garrett s.n.* 23 March 1979, AUA, ref #1166; Berkeley Co., SC, *Mara T. Ravenel s.n.*, May 1895, GH, ref. #1340; Calhoun Co., SC, *Ahles and Haesloop 21700*, 31 Mar. 1957, NCU, ref #0114; and Dorchester Co., SC, *Ahles and Haesloop 21890*, 1 Apr. 1957, NCU, ref. #0115.

Trillium pusillum was first described in 1803 by Michaux who recognized it as distinct because of its small stature. The range of *T. pusillum* as here defined is much smaller than previously considered (Garrett 1982; Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a, 2002b, 2003; see also Fig. 5). This species is found in dry pine woods of the coastal plain of North and South Carolina and can be separated from *T. telmacola* on the basis of its smaller, narrower leaves and larger petals. In addition, the leaves are horizontal to declined whereas in *T. telmacola* they are ascending.

10. *Trillium telmacola* – nom. nov.

Trillium telmacola sp. nov. S.B. Farmer nom ined.

TYPE: To be designated upon publication.

Similar to *T. texanum* with the ascending leaves, but different because of the lack of stomates on the upper leaf surface.

Stem 150 mm. **Leaves** 65x22 mm, 2.9 times longer than wide, ascending, blade dark green often with maroon undertones especially when young, not mottled, 3 primary veins, ovate, acute. **Pedicle** 30 mm. **Flower** above leaves, erect to ascending; **sepals** 21x9 mm, 2.3 times longer than wide, conspicuous, spreading to same plane as petals, dark green often with maroon undertones especially when young, apex; **petals** 15x6 mm, 2.5 times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; **stamens** 9.5 mm, erect-spreading; **filaments** 4 mm, greenish-white, shorter than anthers, slender; **anthers** 5.5 mm, ± straight, yellow, thicker than filaments, dehiscence introrse; **connectives** not extended beyond anther sacs; **ovary** 4 mm, conspicuous, white, ovoid, obscurely 6-angled; **style** 1.5 mm, white; **stigmas** 3, 3.0 mm, confluent with style, white, linear, long-spreading, uniformly thin and threadlike. **Fruits** not seen.

Flowering: late March to early April. **Habitat and distribution:** sphagnum bogs in Aiken and Barnwell Cos. South Carolina.

The epithet *telmacola*, from the Greek telma (bog) + -cola, was chosen because the populations occurs in sphagnum bogs.

The herbarium sheet that was measured for the morphological analysis is Aiken Co., SC, *McMillan & Porcher 2241*, 12 April 1997, USCH, ref. #0057.

This species shares the unusual trait with *T. texanum* of ascending rather than horizontal leaves. In addition, this species appears to have developed a unique strategy for insuring deposition of the fruits away from the parent plant; mature plants prostrate themselves upon the soil. There are other species with unique strategies for insuring contact of the fruit with the soil: *T. nivale* bends over and the pedicel of *Pst. rivale* continues to elongate. This is the first instance in *Trillium* that I am aware of where the plant prostrates itself once pollination has occurred.

11. *Trillium texanum* –

Trillium texanum Buckley, Proc. Acad. Nat. Sci. Philadelphia 1860: 443, 1860.

T. pusillum var. *texanum* (Buckley) Reveal & C.R. Broome, Castanea, 46(1): 56, 1981.

T. pusillum var. *texanum* (Buckley) C.F. Reed, Phytologia 50(4): 279, 283, 1982.

TYPE: Here designated. United States: TX: Cass Co., *Correll #36935*, 9 April 1969, GH!

Stems 65-212.8 (-214) mm. Leaves 29-58.5 (-60) mm x 7.5-17.1 (-18) mm, 2.6-4.6 (-4.8)

times longer than wide, ascending, numerous stomata evenly distributed in upper surface, sub-petiolate to petiolate, blade dark green often with maroon undertones especially when young, not mottled, 3 main veins, elliptic, obtuse. Pedicel 6-21.8 (-23) mm. Flower above leaves, erect to slightly leaning; sepals 13-23.8 (-24) mm x 4.5-8 mm, 2.5-2.9 (-4) times longer than wide, conspicuous, spreading to same plane as petals, dark green often with maroon undertones especially when young; petals 13- 25.6 (-26) x 4.5-7 (-8) mm, 2.8-3.8 (-4.3) times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; stamens 6.7-9.8 (-10) mm, erect-spreading; filaments 3-4.7 (-5) mm, whitish-green, slightly shorter than anthers, slender; anthers 3.2-6.4 (-6.5) mm, ± straight, yellow, thicker than filaments, dehiscence introrse; connectives not extended beyond anther sacs, pinkish-purple; ovary 2-3.4 (-3.5) mm, conspicuous, green, ovoid, obscurely 6-angled; style 1.5-3.5 mm; stigmas 3, 1.0-3.5 mm, confluent with style, green, long-spreading, uniformly thin and threadlike. Fruits white or pale greenish, ovate.

Flowering: late February to late March. Habitat and distribution: found in hummocks around the bases of trees in swamps in east Texas and Caddo Parish, Louisiana.

The epithet *texanum* is indicative of the fact that the type locality was in Texas.

Herbarium sheets that were measured for the morphological analysis are Caddo Parish, LA, *Raymond LRR1950*, 13 April 2002, TENN, ref. #0247; Angelina Co., TX, *Schultz 0434*, 17 April 1993, TEX, ref. #1596; Cass Co., TX, *Orzell & Bridges 4927*, 27 March 1987, TEX, ref. #1591; Houston Co., TX, *Palmer 13179*, 26 March 1918, MO, ref. #1967; Nacogdoches Co., TX, *Orzell 5946*, 17

March 1988, BRIT, ref. #1581; and Smith Co., TX, *Farmer, Singhurst, Loeper & Loeper s.n.*, 22 March 2002, TENN, ref #0251.

Trillium texanum was first described in 1861; Buckley does not indicate if it is habitat, size or just disjunct location that prompted his description. *Trillium texanum* is now known to exhibit very distinctive character traits in *Trillium*. This species is one of two pedicellate *Trillium*, both members of the Pusillum Complex (*T. georgianum* being the other one), that have stomatal openings on the adaxial surface of the leaves. In addition, the leaves of *T. texanum* ascend rather than being horizontal to slightly declining as in most other species in the Pusillum Complex. Because these characters were not continuous, they did not lend themselves to being included in the discriminant analysis. In addition, while most other taxa within the Pusillum Complex are also found in wetlands, *T. texanum* tends to be found on elevated hummocks around the bases of trees within swampy areas rather than being within the swamp itself.

12. *Trillium virginianum* –

Trillium pusillum var. *virginianum* Fernald, *Rhodora* 45:397, 1943.

T. virginianum (Fernald) C.F. Reed, *Phytologia* 50: 279,283, 1982.

TYPE: United States: VA: Dinwiddie Co.; *Laura H. Lippitt s.n.*, 9 May 1943, GH!

Stems (60-) 74.4-181.6 (-187.0) mm. Leaves (24-) 25.2-56.8 (-63) mm x (6-) 8-15 mm, (2.3-) 2.4-5.5 (-6.1) times longer than wide, spreading horizontally to declining, blade dark green often with maroon undertones especially when young, not mottled, 3 primary veins; elliptic to elliptic- or linear-lanceolate, apices rounded-acute. Pedicel 0.5-3.7 (-7) mm. Flower above leaves, erect; sepals 11.5-21.9 (-22) mm x 3-6.5 (-7.5) mm, 2.5-3.9 (-4) times longer than wide, conspicuous, spreading to same plane as petals, dark green with maroon undertones when young, shape, apex; petals (12.5-) 13.6-23 mm x 3.5-7.0 mm, 2.8-3.8 (-4.3) times longer than wide, of short duration, spreading-ascending, exposing stamens and ovary, weakly recurved in distal ½, white, aging to deep rosy pink, veins not engraved but major petal veins clearly visible, shape, thin-textured, widest above base, margins strongly undulate, quite variable in petal width and degree of undulation between individuals and populations; stamens (6-) 6.5-11 mm, erect-spreading; filaments 3-6 (-6.5) mm, whitish-green, longer than anthers, slender; anthers (2.6-) 3-5 (-5.7) mm, ± straight, yellow; length, thicker than filaments, dehiscence introrse; connectives not extended beyond anther sacs; ovary (1.7-) 2-3.8 (-4.0) mm, conspicuous, green, ovoid, obscurely 6-angled; style (2.2-) 2.3-5 mm, green; stigmas 3, (1.0-) 1.2-4.2 (-4.7) mm, confluent with style, green, linear, long-spreading, uniformly thin and threadlike. Fruits white or pale greenish.

Flowering: March to April. Habitat and distribution: swamps of the upper coastal plain of Virginia.

The epithet *virginianum* is indicative of the fact that the type locality was in Virginia.

Specimens that were measured for the morphological analysis are Charles City Co., VA, *Weiss s.n.*, 15 April 1958, VPI, ref. #1231; Gloucester Co., VA, *Cabe & Day 122*, 2 April 1983, VPI, ref. #1238; Greensville Co., VA, *Wieboldt 2954*, 17 April 1984, VPI, ref. #1244; Henrico Co., VA, *Lusby s.n.*, 20 April 1943, VPI, ref. #1233; James City, Co., VA, *Cabe 119*, 9 April 1983, VPI, ref. #1239; Richmond/Chesterfield Co., VA, *Lusby s.n.*, 31 March 1943, VPI, ref. #1232; Surry Co., VA, *Freeman 499*, 25 April 1966, TENN, ref. #0984; Sussex Co., VA, *Cabe & Garvin 120*, 15 March 1983, WILLI, ref. #1269; York Co., VA, *Cabe 123*, 20 April 1983, VPI, ref. #1237.

Trillium virginianum was first described as distinct from *T. pusillum* in 1943 based on its lack of a pedicel. Early specimens were often identified as *T. lanceolatum*. It is probable that Rafinesque included this species in his list of taxa within *Phyllantherum*; but *T. virginianum* exhibits a style, in contrast to sessile-flowered *Trillium* which lack this feature. In addition, the pedicel is not always completely absent in this species. Once DNA evidence showed this taxon to have a much narrower geographic range than earlier hypothesized (Garrett 1982; Timmerman-Erskine 1997, 1999; Timmerman-Erskine et al. 2002a, 2002b, 2003; see also Fig. 5) being confined to the upper coastal plain, morphological characters that defined *T. virginianum* became more evident. These characters include a shorter pedicel than that found in *T. carolinianum*. The plant in many respects is smaller than its sister species, *T. palustris*; but the sepals, leaves, and petals have a broader shape. In addition, the leaves are generally elliptic whereas *T. palustris* has more ovate or lanceolate leaves.

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Appendix A

Representative Specimens of the Pusillum Complex Examined

Table A.1 Herbarium specimens of the *Pusillum* Complex examined for this study.

herbarium	date collected	taxon	location	collection number	accession number	collectors
AUA	March 21, 1981	T. alabamicum	AL: Jackson Co.	004	36363	J. Garrett
AUA	March 23, 1995	T. alabamicum	AL: Jackson Co.	s.n.	63664	J.D. Freeman & B. Jones
AUA	April 08, 1979	T. alabamicum	AL: Jackson Co.	1029	37737	Thomas S. Patrick
TROY	March 23, 1995	T. alabamicum	AL: Jackson Co.	s.n.	16087	J.D. Freeman & B. Jones
VDB	April 04, 1982	T. alabamicum	AL: Jackson Co.	68111		R. Kral
VDB	April 01, 1984	T. alabamicum	AL: Jackson Co.	71143		R. Kral
TENN	March 15, 1996	T. alabamicum	AL: Jackson Co.	s.n.		J. Freeman & B. Keener
TENN	March 15, 1996	T. alabamicum	AL: Jackson Co.	s.n.		J. Freeman & B. Keener
AUA	March 11, 1993	T. alabamicum	AL: Limestone Co.	s.n.	63657	A.R. Diamond with S.C. Gunn and J.D. Freeman
AUA	March 22, 1995	T. alabamicum	AL: Limestone Co.	s.n.	63661	J.D. Freeman and B. Jones
JSU	March 1996	T. alabamicum	AL: Limestone Co.	00165	JSU00009395	T. Hoffman, S. Threlkeld
TENN	April 18, 1987	T. alabamicum	AL: Limestone Co.	5274		David H. Webb
TENN	March 13, 2002	T. alabamicum	AL: Limestone Co.	s.n.		S.B. Farmer and M/M Walker
TROY	March 11, 1993	T. alabamicum	AL: Limestone Co.	s.n.	3337	A.R. Diamond with S.C. Gunn and J.D. Freeman
TROY	March 22, 1995	T. alabamicum	AL: Limestone Co.	s.n.	16084	J.D. Freeman & B. Jones
VDB	April 18, 1987	T. alabamicum	AL: Limestone Co.	5274		David H. Webb
VDB	March 27, 1993	T. alabamicum	AL: Limestone Co.	s.n.		J. D. Freeman
AUA	March 11, 1993	T. alabamicum	AL: Madison Co.	8095	58077	A.R. Diamond
AUA	March 11, 1993	T. alabamicum	AL: Madison Co.	8544	53719	A.R. Diamond
AUA	March 12, 1993	T. alabamicum	AL: Madison Co.	s.n.	63656	A.R. Diamond with J. D. Freeman
AUA	April 02, 1978	T. alabamicum	AL: Madison Co.	13	46112	Charles Drost
AUA	March 20, 1972	T. alabamicum	AL: Madison Co.	739	24092	J. D. Freeman
AUA	March 20, 1981	T. alabamicum	AL: Madison Co.	001	36362	J. Garrett
AUA	March 21, 1981	T. alabamicum	AL: Madison Co.	002	36365	J. Garrett
AUA	March 23, 1995	T. alabamicum	AL: Madison Co.	s.n.	63663	J.D. Freeman & B. Jones
GA	March 20, 1972	T. alabamicum	AL: Madison Co.	739	104467	J.D. Freeman
GH	March 20, 1972	T. alabamicum	AL: Madison Co.	739	00030169	J. D. Freeman
JSU	March 23, 1995	T. alabamicum	AL: Madison Co.	s.n.	JSU00008957	J.D. Freeman & B. Jones
JSU	March 23, 1995	T. alabamicum	AL: Madison Co.	s.n.	JSU00008956	J.D. Freeman & B. Jones
JSU	March 23, 1996	T. alabamicum	AL: Madison Co.	130	JSU00009396	S.J. Threlkeld, J.F. Duke
JSU	March 23, 1996	T. alabamicum	AL: Madison Co.	130	JSU00001230	S.J. Threlkeld, J.F. Duke
MO	March 20, 1972	T. alabamicum	AL: Madison Co.	739	2587272	J.D. Freeman
NY	March 20, 1972	T. alabamicum	AL: Madison Co.	739	00505419	J.D. Freeman
PH	March 20, 1972	T. alabamicum	AL: Madison Co.	739	01030409	J.D. Freeman
SMU	March 20, 1972	T. alabamicum	AL: Madison Co.	739		J.D. Freeman
TROY	March 11, 1993	T. alabamicum	AL: Madison Co.	8095	3338	A.R. Diamond

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
TROY	March 12, 1993	T. alabamicum	AL: Madison Co.	s.n.	3339	A.R. Diamond with J.D. Freeman
TROY	March 23, 1995	T. alabamicum	AL: Madison Co.	s.n.	16086	J.D. Freeman & B. Jones
VDB	March 20, 1972	T. alabamicum	AL: Madison Co.	739		J.D. Freeman
VDB	March 28, 1973	T. alabamicum	AL: Madison Co.	49410		R. Kral
VDB	June 21, 1985	T. alabamicum	AL: Madison Co.	s.n.		Susan Weber
VDB	March 30, 1978	T. alabamicum	AL: Madison Co.	s.n.	159247	W. Michael Dennis, Terry Goldsby
AUA	March 23, 1995	T. alabamicum	AL: Marshall Co.	s.n.	63665	J.D. Freeman & B. Jones
AUA	April 02, 1980	T. alabamicum	AL: Marshall Co.	s.n.	58463	John E. Butler
JSU	March 23, 1995	T. alabamicum	AL: Marshall Co.	s.n.	JSU00008955	J.D. Freeman & B. Jones
TROY	March 23, 1995	T. alabamicum	AL: Marshall Co.	s.n.	16088	J.D. Freeman and B. Jones
VDB	April 13, 1981	T. alabamicum	AL: Marshall Co.	66884		R. and M. Kral
VDB	June 08, 1981	T. alabamicum	AL: Marshall Co.	67378		R. Kral
VDB	April 22, 1990	T. alabamicum	AL: Marshall Co.	77343		R. Kral
TROY	March 06, 1993	T. alabamicum	AL: Morgan Co.	s.n.	9043	J.D. Freeman
VDB	March 11, 1992	T. alabamicum	AL: Morgan Co.	s.n.		J.D. Freeman, A.R. Diamond, S.C. Gunn
VDB	March 26, 1986	T. alabamicum	AL: Morgan Co.	73136		R. Kral
TENN	March 16, 1996	T. alabamicum	AL: Morgan Co.	s.n.		J. Freeman & B. Keener
TENN	March 16, 1996	T. alabamicum	AL: Morgan Co.	s.n.		J. Freeman & B. Keener
VDB	April 13, 1982	T. alabamicum	TN: Coffee Co.	112		Tom Smith
AUA	May 08, 1986	T. alabamicum	TN: Cumberland Co.	s.n.	58602	J.D. Freeman with T.S. Patrick et al.
AUA	April 21, 1985	T. alabamicum	TN: Cumberland Co.	5512	58600	Thomas S. Patrick & Jan Garrett
AUA	April 21, 1985	T. alabamicum	TN: Cumberland Co.	5512	63654	Thomas S. Patrick & Jan Garrett
NY	May 08, 1986	T. alabamicum	TN: Cumberland Co.	86-44	00505408	B. Eugene Wofford with Victor Ma
TENN	May 08, 1986	T. alabamicum	TN: Cumberland Co.	86-44		B. Eugene Wofford with Victor Ma
TENN	April 25, 1984	T. alabamicum	TN: Cumberland Co.	s.n.		Margaret Rhinehart
TENN	April 10, 2005	T. alabamicum	TN: Cumberland Co.	s.n.		S.B. Farmer & M. Rhinehart
VDB	May 20, 1994	T. alabamicum	TN: Cumberland Co.	83549		R. Kral with Bob Brown
TENN	April 08, 1973	T. alabamicum	TN: Franklin Co.	s.n.		Dr. Harry C. Yeatman
AUA	March 30, 1983	T. alabamicum	TN: Lincoln Co.	s.n.	37961	C.J. Garrett
TENN	April 29, 1980	T. alabamicum	TN: Lincoln Co.	1113		Thomas S. Patrick with Catherine Simmons

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
VDB	April 04, 1983	T. alabamicum	TN: Lincoln Co.	110		D. & S. Horn
VDB	April 02, 1983	T. alabamicum	TN: Lincoln Co.	109		D. Horn
VDB	April 29, 1980	T. alabamicum	TN: Lincoln Co.	1113		Thomas S. Patrick with Catherine Simmons
VDB	March 27, 1982	T. alabamicum	TN: Lincoln Co.	s.n.		Tom Smith
TENN	April 14, 1983	T. alabamicum	TN: Putnam Co.	4802		Thomas S. Patrick
TENN	March 03, 2005	T. carolinianum	NC: Nash Co.	s.n.		S.B. Farmer, M. Franklin, D. Vasquez
GA	April 20, 1950	T. carolinianum	NC: Wake Co.	3561	186683	William B. Fox & Dave Adams
GH	April 20, 1970	T. carolinianum	NC: Wake Co.	83		Sandra K. Ittenback
MO	April 20, 1950	T. carolinianum	NC: Wake Co.	3561	4057358	W.B. Fox & D. Adams
AUA	1992	T. carolinianum	VA: Grayson Co.	s.n.	63676	J.D. Freeman
TENN	May 01, 1995	T. carolinianum	VA: Grayson Co.	s.n.		J. Freeman
TENN	May 16, 2002	T. carolinianum	VA: Grayson Co.	s.n.		S.B. Farmer and T. Wieboldt
TENN	May 16, 2002	T. carolinianum	VA: Grayson Co.	s.n.		S.B. Farmer and T. Wieboldt
VPI	May 01, 1991	T. carolinianum	VA: Grayson Co.	7566	89133	T.F. Wieboldt, with T.J. Rawinski & S. Hobbs
GA	March 26, 1996	T. georgianum	GA: Whitfield Co.	9096	218782	James R. Allison w/ Brian Dickman
GA	March 26, 1996	T. georgianum	GA: Whitfield Co.	9096	222753	James R. Allison w/ Brian Dickman
TENN	April 05, 2002	T. georgianum	GA: Whitfield Co.	s.n.		S.B. Farmer
TENN	April 11, 2001	T. georgianum	GA: Whitfield Co.	s.n.		S.B. Farmer and S.M. Noble
USCH	March 26, 1996	T. georgianum	GA: Whitfield Co.	9096	70718	James R. Allison w/ Brian Dickman
JMU	June 01, 1982	T. monticola	VA: Augusta Co.	59		H.R. Haynes
VPI	April 25, 1982	T. monticola	VA: Augusta Co.	17979	85837	Charles E. Stevens
WILLI	May 24, 1983	T. monticola	VA: Augusta Co.	180	44601	Paul R. Cabe
WILLI	April 20, 1983	T. monticola	VA: Augusta Co.	129	44606	Paul R. Cabe with Fenton Day & Joe Garvin
GH	April 18, 1981	T. monticola	VA: Rockingham Co.	5586	00030170	James L. Reveal, Norlyn L. Bodkin
JMU	April 18, 1981	T. monticola	VA: Rockingham Co.	5586		James L. Reveal, Norlyn L. Bodkin
JMU	April 04, 1981	T. monticola	VA: Rockingham Co.	81110		N. Bodkin
TENN	April 27, 2002	T. monticola	VA: Rockingham Co.	s.n.		S.B. Farmer & C. Cleveenger

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
TENN	April 27, 2002	T. monticola	VA: Rockingham Co.	s.n.		S.B. Farmer & C. Clevenger
VPI	May 20, 1980	T. monticola	VA: Rockingham Co.	442	68764	S.Q. Croy
WILLI	April 30, 1976	T. monticola	VA: Rockingham Co.	1271	24732	Gerald F. Roe
WILLI	May 24, 1983	T. monticola	VA: Rockingham Co.	181	44602	Paul R. Cabe
WILLI	May 24, 1983	T. monticola	VA: Rockingham Co.	182	44604	Paul R. Cabe
WILLI	May 24, 1983	T. monticola	VA: Rockingham Co.	183	44600	Paul R. Cabe
WILLI	May 24, 1983	T. monticola	VA: Rockingham Co.	186	44605	Paul R. Cabe
WILLI	May 24, 1983	T. monticola	VA: Rockingham Co.	187	44621	Paul R. Cabe
WILLI	April 29, 1983	T. monticola	VA: Rockingham Co.	128	44614	Paul R. Cabe with Joe Garvin
AUA	May 22, 1993	T. monticola	WV: Pendleton Co.	s.n.	63696	J.D. Freeman & B. Rush
AUA	May 03, 1986	T. monticola	WV: Pendleton Co.	s.n.	56722	J.D. Freeman with Mark Stanley
JMU	May 10, 1984	T. monticola	WV: Pendleton Co.	84/105		Walter Heeb and Cathy Gunter
TENN	May 22, 1993	T. monticola	WV: Pendleton Co.	s.n.		J. Freeman & B. Bash
WILLI	May 14, 1975	T. monticola	WV: Pendleton Co.	1067	24708	Gerald F. Roe
WILLI	April 30, 1976	T. monticola	WV: Pendleton Co.	1270	24719	Gerald F. Roe
SMU	sin date	T. ozarkanum	AR	21786	SMU 163	J.T. Bucholz
NY	1921	T. ozarkanum	AR: Benton Co.	s.n.	00505413	J.G. Buckholz
TEX	March 27, 1927	T. ozarkanum	AR: Benton Co.	s.n.	31053	J. Frank Normand
VDB	March 31, 1979	T. ozarkanum	AR: Benton Co.	63225	168629	R. Kral and R. Carter
VDB	March 31, 1909	T. ozarkanum	AR: Benton Co.	63224	168621	R. Kral, R. Carter
VDB	March 31, 1979	T. ozarkanum	AR: Benton Co.	63224	168622	R. Kral, R. Carter
VPI	April 16, 1928	T. ozarkanum	AR: Benton Co.	s.n.	34420	J.E. Benedict, Jr.
AUA	April 12, 1992	T. ozarkanum	AR: Boone Co.	8092	52027	A.R. Diamond
AUA	April 11, 1992	T. ozarkanum	AR: Carroll Co.	8088	57437	A.R. Diamond with J.D. Freeman
GH	April 06, 1958	T. ozarkanum	AR: Madison Co.	643		Dan L. Marsh
MO	April 15, 1928	T. ozarkanum	AR: Madison Co.	4803	960346	Moore and Demaree
NCU	April 11, 1974	T. ozarkanum	AR: Madison Co.	29313	NCU00006369	Paul L. Redfearn, Jr.
NY	April 15, 1928	T. ozarkanum	AR: Madison Co.	4803	00505415	D. Demaree
NY	April 06, 1958	T. ozarkanum	AR: Madison Co.	643	00505404	Dan L. Marsh
VDB	March 30, 1979	T. ozarkanum	AR: Madison Co.	63193	168620	R. Kral, R. Carter
VDB	March 30, 1979	T. ozarkanum	AR: Madison Co.	63193	168619	R. Kral, R. Carter
GA	April 02, 1980	T. ozarkanum	AR: Montgomery Co.	66	154732	Cheryl B. Barr
TENN	March 22, 2002	T. ozarkanum	AR: Montgomery Co.	s.n.		S.B. Farmer and T. Whitsell
AUA	April 12, 1992	T. ozarkanum	AR: Newton Co.	8095	58044	A.R. Diamond with J.D. Freeman
AUA	April 04, 1964	T. ozarkanum	AR: Newton Co.	5	57440	Jan Eshbaugh
AUA	April 03, 1965	T. ozarkanum	AR: Newton Co.	16953	57441	P.L. Redfearn, Jr.

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
AUA	April 04, 1964	T. ozarkanum	AR: Newton Co.	1	57439	Pam Cornwell
AUA	April 03, 1965	T. ozarkanum	AR: Newton Co.	16953	56721	Paul L. Redfearn, Jr.
GA	April 04, 1964	T. ozarkanum	AR: Newton Co.	14356	83790	Paul L. Redfearn, Jr.
NCU	April 29, 1967	T. ozarkanum	AR: Newton Co.	21355	NCU00006370	Paul L. Redfearn, Jr.
RSA	April 04, 1964	T. ozarkanum	AR: Newton Co.	14356	179369	Paul L. Redfearn, Jr.
SMU	April 04, 1964	T. ozarkanum	AR: Newton Co.	14356		Paul L. Redfearn, Jr.
TENN	April 04, 1964	T. ozarkanum	AR: Newton Co.	14356		Paul L. Redfearn, Jr.
TROY	April 12, 1992	T. ozarkanum	AR: Newton Co.	8095	3336	A.R. Diamond
UMO	early April, 1971	T. ozarkanum	AR: Newton Co.	360	109674	Jeffrey H. Huber & W.E. Elder
UMO	April 04, 1964	T. ozarkanum	AR: Newton Co.	14356	61042	Paul L. Redfearn, Jr.
UMO	March 15, 1977	T. ozarkanum	AR: Newton Co.	12	99346	S. Emme
AUA	June 02, 1976	T. ozarkanum	AR: Polk Co.	s.n.	30534	John C. Lambert
BRIT	April 19, 1984	T. ozarkanum	AR: Polk Co.	1303	00094	Steve L. Orzell and Bill Pell
ASTC	March 21, 1976	T. ozarkanum	AR: Pulaski Co.	s.n.		Bill Shepherd
ASTC	March 21, 1976	T. ozarkanum	AR: Pulaski Co.	s.n.		Bill Shepherd
ASTC	April 12, 1908	T. ozarkanum	AR: Pulaski Co.	016144		Bill Shepherd & J.H. Rettig
MO	June 01, 1923	T. ozarkanum	AR: Washington Co.	4803?	899687	John T. Buchholz
NY	June 05, 1923	T. ozarkanum	AR: Washington Co.	s.n.	00505410	J.J. Buckholz
NY	April, 1923	T. ozarkanum	AR: Washington Co.	s.n.	00505412	J.T. Buckholz
NY	June 05, 1923	T. ozarkanum	AR: Washington Co.	s.n.	00505403	J.T. Buckholz
NY	April 05, 1923	T. ozarkanum	AR: Washington Co.	s.n.	00505411	J.T. Buckholz
NY	May 05, 1925	T. ozarkanum	AR: Washington Co.	12307	00505418	John K. Small and E.T. Wherry
SMU	April 03, 1940	T. ozarkanum	AR: Washington Co.	40009		D.M. Moore
VDB	May 05, 1925	T. ozarkanum	AR: Washington Co.	26998	142055	E.J. Palmer
GH	April 20, 1922	T. ozarkanum	AR: Washington Co. ?	s.n.		J.T. Buchholz
KY	May 13, 1961	T. ozarkanum	KY: Casey Co.	4089	22638	Elizabeth M. Browne, Edward T. Browne, Jr., with the Glenn Murphys
KY	April 28, 1961	T. ozarkanum	KY: Casey Co.	92	21378	Glenn W. Murphy
KY	April 29, 1962	T. ozarkanum	KY: Casey Co.	372	30593	Glenn W. Murphy
NCU	April 29, 1962	T. ozarkanum	KY: Casey Co.	372	NCU00006371	Glenn W. Murphy
AUA	April 02, 1993	T. ozarkanum	KY: Christian Co.	12965	57117	Edward W. Chester, Sallie Noel
VDB	April 02, 1993	T. ozarkanum	KY: Christian Co.	12965		Edward W. Chester, Sallie Noel
TENN	April 17, 1965	T. ozarkanum	KY: Marion Co.	s.n.		John Keith
TENN	May 31, 2005	T. ozarkanum	KY: Russell Co.	s.n.		S.B. Farner and D. Estes

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
AUA	April 03, 1979	T. ozarkanum	KY: Wayne Co.	s.n. ?	40215	Rich Hannan, Loy R. Phillippe, ron Caldwall
TENN	April 03, 1979	T. ozarkanum	KY: Wayne Co.	s.n.		Rich Hannan, Loy R. Phillippe, ron Caldwall
AUA	April 13, 1983	T. ozarkanum	MO: Barry Co.	s.n.	37964	S.W. Morgan
GH	April 20, 1935	T. ozarkanum	MO: Barry Co.	18628	00030167	Julian A. Steyermark
MO	April 15, 1994	T. ozarkanum	MO: Barry Co.	s.n.	04581903	G. Yatskievych, T. Smith, P. McKenzie
MO	April 17, 1994	T. ozarkanum	MO: Barry Co.	94-39	04577454	G. Yatskievych, T. Smith, P. McKenzie
MO	April 06, 1981	T. ozarkanum	MO: Barry Co.	348	3347308	Gordon T. Maupin & Sharon Morgan
MO	April 06, 1981	T. ozarkanum	MO: Barry Co.	348	3347307	Gordon T. Maupin & Sharon Morgan
MO	April 06, 1981	T. ozarkanum	MO: Barry Co.	349	3347306	Gordon T. Maupin & Sharon Morgan
MO	April 20, 1935	T. ozarkanum	MO: Barry Co.	18628	1137068	Julian A. Steyermark
MO	April 20, 1935	T. ozarkanum	MO: Barry Co.	18628	1137067	Julian A. Steyermark
MO	April 03, 1981	T. ozarkanum	MO: Barry Co.	32613	3140005	Paul L. Redfearn, Jr.
MO	April 15, 1988	T. ozarkanum	MO: Barry Co.	2668	3525709	T.E. Smith
TENN	June 27, 2001	T. ozarkanum	MO: Barry Co.	s.n.		Sue Hollis
TENN	June 27, 2001	T. ozarkanum	MO: Barry Co.	s.n.		Sue Hollis
UMO	April 13, 1930	T. ozarkanum	MO: Barry Co.	s.n.	8561	Cora Shoop
UMO	April 20, 1935	T. ozarkanum	MO: Barry Co.	18628	19128	Julian A. Steyermark
UMO	April, 1930	T. ozarkanum	MO: Barry Co.	s.n.	1283	Mrs. H.H. Leake
UMO	April 03, 1981	T. ozarkanum	MO: Barry Co.	32613	166754	Paul L. Redfearn, Jr.
UMO	April 10, 1981	T. ozarkanum	MO: Barry Co.	32619	166753	Paul L. Redfearn, Jr. & Steve Timme
VPI	April 13, 1983	T. ozarkanum	MO: Barry Co.	s.n.	97583	S.W. Morgan
MO	April 08, 1991	T. ozarkanum	MO: Howell Co.	4108	4235079	B. Summers
MO	June 16, 2001	T. ozarkanum	MO: Howell Co.	9798	5551626	B. Summers
MO	April 20, 2003	T. ozarkanum	MO: Howell Co.	10015	5767661	B. Summers, P.L. Skinner, C. Duggar
MO	April 14, 1997	T. ozarkanum	MO: Howell Co.	s.n.	05098340	Doug Newman
AUA	April 11, 1992	T. ozarkanum	MO: Lawrence Co.	8085	52028	A.R. Diamond
SMU	April 21, 1953	T. ozarkanum	MO: Lawrence Co.	55359		E.J. Palmer
UMO	March 30, 1967	T. ozarkanum	MO: Lawrence Co.	5	90940	David C. Hamm
AUA	April 08, 1990	T. ozarkanum	MS: Jones Co.	6805	52025	A.R. Diamond
AUA	March 19, 1989	T. ozarkanum	MS: Jones Co.	5737	49927	A.R. Diamond with J.D. Freeman

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
AUA	February 26, 1995	<i>T. ozarkanum</i>	MS: Jones Co.	s.n.	63655	A.R. Diamond with J.D. Freeman
AUA	February 25, 1996	<i>T. ozarkanum</i>	MS: Jones Co.	10135	57160	A.R. Diamond with J.D. Freeman
MNHP	May 09, 1978	<i>T. ozarkanum</i>	MS: Jones Co.	468	9673	David Morgan
AUA	April 16, 1988	<i>T. ozarkanum</i>	NC: Clay Co.	s.n.	58624	J.D. Freeman with T. Patrick & A.R. Diamond
AUA	May 17, 1986	<i>T. ozarkanum</i>	NC: Clay Co.	s.n.	57501	J.S. Freeman with L.L. Gaddy
NCU	May 02, 1898	<i>T. ozarkanum</i>	NC: Haywood Co.	s.n.	NCU00006360	T.G. Harbison
NCU	sin date	<i>T. ozarkanum</i>	NC: Haywood Co.	s.n.	NCU00011663	T.G. Harbison
VDB	April 11, 1975	<i>T. ozarkanum</i>	TN: Sumner Co.	125	145218	J. Alcorn
VDB	May 05, 1975	<i>T. ozarkanum</i>	TN: Sumner Co.	302	145219	J. Alcorn
VDB	March 28, 1976	<i>T. ozarkanum</i>	TN: Sumner Co.	1113	145220	J. Alcorn
VDB	April 21, 1975	<i>T. ozarkanum</i>	TN: Sumner Co.	56444	158420	R. Kral
VDB	April 21, 1975	<i>T. ozarkanum</i>	TN: Sumner Co.	56444	158419	R. Kral
VDB	April 07, 1980	<i>T. ozarkanum</i>	TN: Sumner Co.	63252	168651	R. Kral
VDB	April 07, 1980	<i>T. ozarkanum</i>	TN: Sumner Co.	63252	168653	R. Kral
VDB	May 06, 1979	<i>T. ozarkanum</i>	TN: Sumner Co.	1753	166208	Richard Carter
BRIT	April 23, 1983	<i>T. palustris</i>	MD: Worcester Co.	126	02124	Paul R. Cabe with Fenton Day
GH	April 25, 1982	<i>T. palustris</i>	MD: Worcester Co.	11120		Steven R. Hill
JMU	May 02, 1981	<i>T. palustris</i>	MD: Worcester Co.	5587		James L. Reveal, Norlyn L. Bodkin
JMU	May 02, 1981	<i>T. palustris</i>	MD: Worcester Co.	5588		James L. Reveal, Norlyn L. Bodkin
NCU	April 06, 1980	<i>T. palustris</i>	MD: Worcester Co.	s.n.	NCU00006372	A.O. Tucker
NCU	May 02, 1981	<i>T. palustris</i>	MD: Worcester Co.	5587	NCU00006373	James L. Reveal, Norlyn L. Bodkin
NCU	May 02, 1981	<i>T. palustris</i>	MD: Worcester Co.	5588	NCU00006374	James L. Reveal, Norlyn L. Bodkin
NY	April 20, 1980	<i>T. palustris</i>	MD: Worcester Co.	5115		James L. Reveal
NY	April 20, 1980	<i>T. palustris</i>	MD: Worcester Co.	5117		James L. Reveal
NY	April 20, 1980	<i>T. palustris</i>	MD: Worcester Co.	5122		James L. Reveal
NY	May 02, 1981	<i>T. palustris</i>	MD: Worcester Co.	5587	00505405	James L. Reveal, Norlyn L. Bodkin
NY	May 02, 1981	<i>T. palustris</i>	MD: Worcester Co.	5588	00505406	James L. Reveal, Norlyn L. Bodkin
NY	April 25, 1982	<i>T. palustris</i>	MD: Worcester Co.	11120	00505407	Steven R. Hill
PH	May 04, 1947	<i>T. palustris</i>	MD: Worcester Co.	s.n.	01030406	Edgar T. Wherry
PH	April 22, 1973	<i>T. palustris</i>	MD: Worcester Co.	s.n.	01030407	Frank Hirst
VPI	April 23, 1983	<i>T. palustris</i>	MD: Worcester Co.	125	80163	Paul R. Cabe with Fenton Day
VPI	April 23, 1983	<i>T. palustris</i>	MD: Worcester Co.	126	80162	Paul R. Cabe with Fenton Day
NY	sin date	<i>T. palustris</i>	NC: Curtis Co.	s.n.	00505414	Chapman
NCU	April 02, 1982	<i>T. palustris</i>	NC: Gates Co.	s.n.	NCU00006363	F. Williams, M.L. Dunn

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
BRIT	March 29, 1984	<i>T. palustris</i>	VA: Gloucester Co.	8936	02125	Donna M. Eggers Ware with Paul Reid Cabe
BRIT	April 02, 1983	<i>T. palustris</i>	VA: Gloucester Co.	122	02119	Paul R. Cabe with Fenton Day
VDB	March 29, 1984	<i>T. palustris</i>	VA: Gloucester Co.	8936		Donna M. Eggers Ware with Paul R. Cabe
VDB	April 02, 1983	<i>T. palustris</i>	VA: Gloucester Co.	122		Paul R. Cabe with Fenton Day
VPI	April 02, 1983	<i>T. palustris</i>	VA: Gloucester Co.	122	80159	Paul R. Cabe with Fenton Day
WILLI	March 29, 1984	<i>T. palustris</i>	VA: Gloucester Co.	8936	44817	Donna M. Eggers Ware with Paul Reid Cabe
WILLI	April 02, 1983	<i>T. palustris</i>	VA: Gloucester Co.	122	44613	Paul R. Cabe with Fenton Day
VPI	March 30, 1995	<i>T. palustris</i>	VA: Suffolk	10115	90529	Gary P. Fleming
VPI	March 05, 1975	<i>T. palustris</i>	VA: Suffolk	4835	79439	Lytton J. Musselman
WILLI	March 05, 1975	<i>T. palustris</i>	VA: Suffolk	4835	22568	Lytton J. Musselman
TENN	April 04, 2005	<i>T. pusillum</i>	NC: Brunswick Co.	s.n.		Frank Galloway
AUA	March 23, 1979	<i>T. pusillum</i>	NC: Pender Co.	s.n.	37740	J. Garrett
NCU	June 29, 1963	<i>T. pusillum</i>	NC: Pender Co.	616	NCU00006362	A. McCrary
NCU	June 29, 1963	<i>T. pusillum</i>	NC: Pender Co.	58370	NCU00006361	Harry E. Ahles with Anne McCrary
UC	June 29, 1963	<i>T. pusillum</i>	NC: Pender Co.	58370	M313089	Harry E. Ahles with Anne McCrary
VDB	May 08, 1981	<i>T. pusillum</i>	NC: Pender Co.	7491		S.W. Leonard
GH	sin date	<i>T. pusillum</i>	SC	s.n.		Michaux
GH	May, 1895	<i>T. pusillum</i>	SC: Berkeley Co.	s.n.		Dr. E. Peyre
GH	April, 1897	<i>T. pusillum</i>	SC: Berkeley Co.	s.n.		Porcher
NCU	May, 1898	<i>T. pusillum</i>	SC: Berkeley Co.	s.n.	NCU00006368	Maria P. Ravenel?
NCU	May, 1898	<i>T. pusillum</i>	SC: Berkeley Co.	s.n.	NCU00006365	label says Mellichamp; but annotations say F.P. Porcher
MO	sin date	<i>T. pusillum</i>	SC: Berkeley Co. ?	s.n.	760841	Mr. Porcher
GA	March 31, 1957	<i>T. pusillum</i>	SC: Calhoun Co.	21700	67081	Porcher
GH	March 31, 1957	<i>T. pusillum</i>	SC: Calhoun Co.	21700		Harry E. Ahles with John G. Haesloop
NY	March 31, 1957	<i>T. pusillum</i>	SC: Calhoun Co.	21700	00505417	Harry e. Ahles with John G. Haesloop
VDB	March 31, 1957	<i>T. pusillum</i>	SC: Calhoun Co.	21700	21344	Harry E. Ahles with John G. Haesloop

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
NCU	March 31, 1957	<i>T. pusillum</i>	SC: Calhoun Co.	21700	NCU00006364	Harry E. Ahles with John G. Haesloop
NCU	April 01, 1957	<i>T. pusillum</i>	SC: Dorchester Co.	21890	NCU00006367	Harry E. Ahles with John G. Haesloop
NCU	March 11, 1977	<i>T. pusillum</i>	SC: Dorchester Co.	12462	NCU00006366	Richard D. Porcher
USCH	April 12, 1997	<i>T. telmacola</i>	SC: Aiken Co.	2241	73297	P.D. McMillan & R.D. Porcher
NY	April 01, 1978	<i>T. texanum</i>	LA: Caddo Parish	2595	00505401	D.T. MacRoberts
TENN	April 16, 2002	<i>T. texanum</i>	LA: Caddo Parish	LRR1950		Larry R. Raymond
TENN	April 16, 2002	<i>T. texanum</i>	LA: Caddo Parish	LRR1950		Larry R. Raymond
VDB	March 23, 1977	<i>T. texanum</i>	LA: Caddo Parish	s.n.		B.R. & M.H. MacRoberts
TEX	April 17, 1993	<i>T. texanum</i>	TX: Angelina Co.	0434	00039164	J.G. Schultz
GH	May 18, 1968	<i>T. texanum</i>	TX: Cass Co.	35746		D. S. Correll and Helen B. Correll
GH	April 09, 1969	<i>T. texanum</i>	TX: Cass Co.	36935		D.S. Correll
LL	May 18, 1968	<i>T. texanum</i>	TX: Cass Co.	35746	00324278	D.S. Correll & Helen B. Correll
LL	Spring, 1968	<i>T. texanum</i>	TX: Cass Co.	s.n.	00324277	Mrs. Cleo Barnwell
NCU	April 09, 1969	<i>T. texanum</i>	TX: Cass Co.	36935	NCU00006385	D.S. Correll
NCU	May 18, 1968	<i>T. texanum</i>	TX: Cass Co.	35746	NCU00006384	D.S. Correll & Helen B. Correll
TEX	March 27, 1987	<i>T. texanum</i>	TX: Cass Co.	4927	00039162	Steve L. Orzell and Edwin L. Bridges
UC	May 18, 1968	<i>T. texanum</i>	TX: Cass Co.	35746	1358250	D.S. Correll & Helen B. Correll
VDB	May 18, 1968	<i>T. texanum</i>	TX: Cass Co.	35746		D.S. Correll & Helen B. Correll
VDB	April 01, 1979	<i>T. texanum</i>	TX: Cass Co.	63228	168630	R. Kral, R. Carter
MO	March 26, 1918	<i>T. texanum</i>	TX: Houston Co.	13179	855045	E.J. Palmer
VDB	March 26, 1918	<i>T. texanum</i>	TX: Houston Co.	13179	142053	E.J. Palmer
BRIT	March 17, 1988	<i>T. texanum</i>	TX: Nacogdoches Co.	5946		Steve L. Orzell
LL	April 14, 1970	<i>T. texanum</i>	TX: Nacogdoches Co.	1806	00324279	E.S. Nixon
TEX	May 29, 1997	<i>T. texanum</i>	TX: Nacogdoches Co.	3342	00324280	B.R. and M.H. MacRoberts
TEX	March 17, 1988	<i>T. texanum</i>	TX: Nacogdoches Co.	5946	00039163	Steve L. Orzell
VDB	April 20, 1980	<i>T. texanum</i>	TX: Nacogdoches Co.	64795		R. Kral
VDB	April 20, 1980	<i>T. texanum</i>	TX: Nacogdoches Co.	64795		R. Kral
VDB	March 13, 1991	<i>T. texanum</i>	TX: Nacogdoches Co.	6220		S. & G. Jones
TENN	March 22, 2002	<i>T. texanum</i>	TX: Smith Co.	s.n.		S.B. Farmer, J. Singhurst, M/M Loeper
NCU	April 23, 1974	<i>T. virginianum</i>	VA: Charles City Co.	5285	NCU00006376	Donna M.E. Ware with Thomas F. Wieboldt

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
NCU	April 15, 1958	<i>T. virginianum</i>	VA: Charles City Co.	s.n.	NCU00006375	O.H. Weiss
VDB	April 15, 1958	<i>T. virginianum</i>	VA: Charles City Co.	s.n.	107814	O.H. Weiss
VPI	April 15, 1958	<i>T. virginianum</i>	VA: Charles City Co.	s.n.	37388	O.H. Weiss
WILLI	April 23, 1974	<i>T. virginianum</i>	VA: Charles City Co.	5285	18379	Donna M.E. Ware with Thomas f. Wieboldt
GH	April 24, 1926	<i>T. virginianum</i>	VA: Chesapeake	413	00030171	Paul W. Warren
NCU	April 25, 1936	<i>T. virginianum</i>	VA: Chesterfield Co.	s.n.	NCU00006377	W.L. Hunt
VPI	March 31, 1943	<i>T. virginianum</i>	VA: Chesterfield Co.	s.n.	37386	Stan Lusby
GH	May 09, 1943	<i>T. virginianum</i>	VA: Dinwiddie Co.	s.n.	00030171	Laura H. Lippitt
VPI	April 17, 1984	<i>T. virginianum</i>	VA: Greensville Co.	4954	76594	T.F. Wieboldt
VPI	April 20, 1943	<i>T. virginianum</i>	VA: Henrico Co.	s.n.	37389	Stan Lusby
AUA	April 05, 1973	<i>T. virginianum</i>	VA: James City Co.	17063	36878	J.T. Baldwin, Jr.
BRIT	June 11, 1949	<i>T. virginianum</i>	VA: James City Co.	898		Bernard Mikula
BRIT	April 06, 1976	<i>T. virginianum</i>	VA: James City Co.	5627	18285	Donna M.E. Ware
BRIT	April 05, 1973	<i>T. virginianum</i>	VA: James City Co.	17063	18438	J.T. Baldwin, Jr.
BRIT	April 09, 1983	<i>T. virginianum</i>	VA: James City Co.	118	02284	Paul R. Cabe
BRIT	April 09, 1983	<i>T. virginianum</i>	VA: James City Co.	119	02122	Paul R. Cabe
BRIT	April 21, 1983	<i>T. virginianum</i>	VA: James City Co.	124	024118	Paul R. Cabe
GA	April 08, 1979	<i>T. virginianum</i>	VA: James City Co.	7322	158188	Domma M.E. Ware with Stewart A. Ware
GA	May 05, 1951	<i>T. virginianum</i>	VA: James City Co.	s.n.	51093	F.H. Sargent
GH	May 04, 1949	<i>T. virginianum</i>	VA: James City Co.	12550		J.T. Baldwin, Jr.
GH	May 03, 1931	<i>T. virginianum</i>	VA: James City Co.	s.n.		W.G. Guy and Dr. Stetson
NCU	June 16, 1968	<i>T. virginianum</i>	VA: James City Co.	728	NCU00006378	Allene Varans
NCU	June 11, 1949	<i>T. virginianum</i>	VA: James City Co.	898	NCU00006382	Bernard Mikula
NCU	May 01, 1976	<i>T. virginianum</i>	VA: James City Co.	6535	NCU00006380	Donna M.E. Ware
NCU	April 08, 1979	<i>T. virginianum</i>	VA: James City Co.	7321	NCU00006379	Donna M.E. Ware with Douglas E. Blackman
NCU	April 05, 1973	<i>T. virginianum</i>	VA: James City Co.	17063	NCU00006381	J.T. Baldwin, Jr.
PH	May 01, 1937	<i>T. virginianum</i>	VA: James City Co.	s.n.	01030408	Harold G. Rugg (to ETW)
POM	April 21, 1951	<i>T. virginianum</i>	VA: James City Co.	33345	339470	F.R. Fosberg
SMU	May 05, 1951	<i>T. virginianum</i>	VA: James City Co.	s.n.		F.H. Sargent
VDB	June 16, 1968	<i>T. virginianum</i>	VA: James City Co.	728	76528	Allene Barans
VDB	April 09, 1983	<i>T. virginianum</i>	VA: James City Co.	119		Paul R. Cabe
VDB	April 21, 1983	<i>T. virginianum</i>	VA: James City Co.	124		Paul R. Cabe
VDB	April 12, 1979	<i>T. virginianum</i>	VA: James City Co.	64751		R. Kral
VDB	April 12, 1980	<i>T. virginianum</i>	VA: James City Co.	64753		R. Kral
VDB	April 12, 1980	<i>T. virginianum</i>	VA: James City Co.	64753		R. Kral
VDB	April 12, 1980	<i>T. virginianum</i>	VA: James City Co.	64754		R. Kral
VPI	April 03, 1982	<i>T. virginianum</i>	VA: James City Co.	7806	75840	Domma M. E. Ware with Paul Cabe, Gretchen North, and Bill Saunders
VPI	April 21, 1979	<i>T. virginianum</i>	VA: James City Co.	7327	75838	Donna M. Eggers Ware with Cynthia Long

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
VPI	April 08, 1979	<i>T. virginianum</i>	VA: James City Co.	7322	78903	Donna M.E. Ware with Stewart A. Ware
VPI	March 29, 1963	<i>T. virginianum</i>	VA: James City Co.	316	37391	O. H. Weiss
VPI	March 23, 1957	<i>T. virginianum</i>	VA: James City Co.	s.n.	37385	O.H. Weiss
VPI	March 30, 1957	<i>T. virginianum</i>	VA: James City Co.	s.n.	37387	O.H. Weiss
VPI	April 09, 1983	<i>T. virginianum</i>	VA: James City Co.	119	80160	Paul R. Cabe
VPI	April 21, 1983	<i>T. virginianum</i>	VA: James City Co.	124	80153	Paul R. Cabe
VPI	April 03, 1977	<i>T. virginianum</i>	VA: James City Co.	2734	68129	Thomas F. Wieboldt, C.E. Stevens
WILLI	June 11, 1949	<i>T. virginianum</i>	VA: James City Co.	898	21249	Bernard Mikula
WILLI	April 07, 1982	<i>T. virginianum</i>	VA: James City Co.	38	42689	Bridget Greaves with G. Hall
WILLI	May 01, 1976	<i>T. virginianum</i>	VA: James City Co.	6535	22970	Donna M. E. Ware
WILLI	April 08, 1979	<i>T. virginianum</i>	VA: James City Co.	7321	33016	Donna M. E. Ware with Douglas E. Blackman
WILLI	April 21, 1979	<i>T. virginianum</i>	VA: James City Co.	7327	42365	Donna M. Eggers Ware with Cynthia Long
WILLI	April 06, 1976	<i>T. virginianum</i>	VA: James City Co.	6527	21496	Donna M.E. Ware
WILLI	April 03, 1982	<i>T. virginianum</i>	VA: James City Co.	7806	37666	Donna M.E. Ware with Paul Cabe, Gretchen North, and Bill Saunders
WILLI	April 08, 1979	<i>T. virginianum</i>	VA: James City Co.	7322	33015	Donna M.E. Ware with Stewart A. Ware
WILLI	April 05, 1973	<i>T. virginianum</i>	VA: James City Co.	17063	13440	J. T. Baldwin, Jr.
WILLI	March 11, 1974	<i>T. virginianum</i>	VA: James City Co.	17135	50149	J. T. Baldwin, Jr.
WILLI	April 09, 1983	<i>T. virginianum</i>	VA: James City Co.	118	44619	Paul R. Cabe
WILLI	April 09, 1983	<i>T. virginianum</i>	VA: James City Co.	119	48475	Paul R. Cabe
WILLI	April 14, 1983	<i>T. virginianum</i>	VA: James City Co.	121	44617	Paul R. Cabe
WILLI	April 21, 1983	<i>T. virginianum</i>	VA: James City Co.	124	44616	Paul R. Cabe
NY	May 03, 1931	<i>T. virginianum</i>	VA: James City Co.	s.n.	00505409	Mrs. W.G. Guy and Dr. Stetson
TENN	April 08, 2005	<i>T. virginianum</i>	VA: Lancaster Co.	s.n.		Tom Teeple
VDB	March 20, 1971	<i>T. virginianum</i>	VA: Nansemond Co.	221	92365	Susan Waters
NCU	March 05, 1975	<i>T. virginianum</i>	VA: Suffolk	4835	NCU00006383	Lytton J. Musselman
AUA	April 25, 1966	<i>T. virginianum</i>	VA: Surrey Co.	499	56723	J.D. Freeman
GH	July 04, 1949	<i>T. virginianum</i>	VA: Surrey Co.	2234		Bernard Mikula
TENN	April 25, 1966	<i>T. virginianum</i>	VA: Surrey Co.	499		J.D. Freeman
TROY	April 25, 1966	<i>T. virginianum</i>	VA: Surrey Co.	499	16081	J.D. Freeman
VPI	April 15, 1983	<i>T. virginianum</i>	VA: Sussex Co.	120	80164	Paul R. Cabe with Joe Garvin
WILLI	April 15, 1983	<i>T. virginianum</i>	VA: Sussex Co.	120	44618	Paul R. Cabe with Joe Garvin
BRIT	April 20, 1983	<i>T. virginianum</i>	VA: York Co.	123	02117	Paul R. Cabe
VPI	April 20, 1983	<i>T. virginianum</i>	VA: York Co.	123	80161	Paul R. Cabe

Table A.1 continued.

herbarium	date collected	taxon	location	collection number	accession number	collectors
WILLI	April 21, 1979	<i>T. virginianum</i>	VA: York Co.	7324	42391	Domme M. Eggers Ware with Cynthia Long
WILLI	April 18, 1992	<i>T. virginianum</i>	VA: York Co.	s.n.	62114	Janis Miller
WILLI	April 30, 1983	<i>T. virginianum</i>	VA: York Co.	123	44620	Paul R. Cabe

Appendix B

Aligned DNA Sequence Data Matrices

[illegible]

735.lincolntW
736.whitfieldGA
746.maculatum
747.louisiana1
748.louisiana2
749.louisiana3
750.freemania
778.caddoLA
779.washingtonOR
780.eschambiaAL
781.eschambiaAL
782.jamesCityVA
783.graysonVA
784.undulatum
785.rockinghamVA
786.caasTX
789.nivale
790.mississippi
791.montgomeryAR
792.nivale
793.limestoneAL
794.smithTX
796.hibbersonii
799.ovatum
800.hibbersonii
801.ovatum
806.persistanes
807.catesbeiiAL
807.catesbeiiGA
809.georgis
810.viridescens
811.worcesterMD
812.grandiflorum
816.sikensSC
817.cumberlandTN
albidentum_DQ404198
camachatsenense_AB0188
camachatsenense_DQ4041
cernuum_DQ404193
chloropetalum_AB0188
cuneatum_DQ404199
decipiens_AB018810
discolor_AB018811
erectum_AB018812
erectum_DQ404196
grandiflorum_AB01881
grandiflorum_DQ40419
lanceifolium_AB018815
luteum_DQ404200
maculatum_AB018816
ovatum_AB018817
ovatum_DQ404194
petiolatum_AB018818
pusillum_AB018819
recurvatum_AB018820
reliquum_AB018821
rugelae_AB018822
riversii_AB018823
sessiliter_AB018824
sessiliter_DQ006019
tschonoskii_DQ404201
undulatum_AB018825

[illegible]

121_Pet_rivale
2106_dorcestorsC
2107_persistans
2119_humboldtCA
2120_skagiTWA
2131_scouleri
2133_fraxineaUNC
2141_jonesNS
2161_cumberrandTN
2290_nivalePA
2319_rivale
2330_nivalePA_2
2331_catesbaeiSC
734_barryMO
735_lincolntW
736_whitfieldGA
746_maculatum
747_louisiana1
748_louisiana2
749_louisiana3
750_fresenius
778_raddola
789_washingtonOR
780_scanbia1AL
781_scanbia1AL
782_jamesCityVA
783_graysonVA
784_undulatum
785_rockinghamVA
786_cassTX
789_nivale
790_mississippi
791_montgomeryAR
792_rivale
793_limestoneAL
794_smithTX
796_hibbersonii
799_ovatum
800_hibbersonii
801_ovatum
806_persistans
807_catesbaeiGA
808_catesbaeiGA
809_georgia
810_viridescens
811_worcesterMD
812_oranderFlorum

[illegible]

[illegible]

0_Pet_riverdale_mscK
2106_rochesterSC
2107_perkinsena
2135_franklinMC
734_barryMO
735_lincolnTN
736_GA
746_maculatum
747_louisianaL
748_louisiana2
749_louisiana3
750_freemanni
751_freemanni
778_caddoLA
779_washingtonOR_paba
780_escambiaLA
781_escambiaLA
782_jamesCityVA
783_grayscnVA
784_undulatum
785_rockinghamVA
786_cassTX
787_davisAL
788_davisGA
789_nivale
790_nississippi
791_montgomeryAR
792_rivale

[illegible]

[illegible]

lancifolium AB017394
ludovicianum AB017395
lutum AB017396
maculatum AB017397
niveum AB017398
ovatum AB017399
petiolatum AB017400
polyphyllad AB018828
pusillum AB017401
recurvatum AB017402
reliquum AB017403
rivalePst AB017404
rugellii AB017405
sessile AB017406
simile AB017407
smallii AB017408
stamineum AB017409
sulcatum AB017410
tetraphyllaP AB018833
thibetica AB018829
thibetica AB040199
thibeticuskii AB017411
undulwoodii AB017412
yusei AB017413
vaseyi AB017414
verticillatP AB018834
violacea AB018830
viridescens AB017416
virider AB017415

[illegible]

2106_Pst_privale_maxKC
 2106_dorchesterCS
 2107_persistens
 2135_franklinWC
 734_barryMO
 735_lincolntWN
 736_GA
 746_maculatum
 747_louisiana1
 748_louisiana2
 749_louisiana3
 750_freemanii
 751_freemanii
 778_caddoLA
 779_washingtonOR_psha
 780_escambiaAL
 781_escambiaAL
 782_jamesCityVA
 783_graysonVA
 784_undulatum
 785_rockinghamVA
 786_casaeTX
 787_daivael
 788_daivae2
 789_nivale
 790_mississippi
 791_montgomeryAR
 792_rivale
 793_limestoneAL
 794_smithTX
 796_hibbersonii
 799_ovatum
 800_hibbersonii
 801_ovatum
 802_barryMO
 805_persistens
 807_nipperisKAL
 808_jamesCityVA
 810_viridescens
 811_worcesterMD
 812_grandiflorum
 albidum_AB017376
 angustipetalum_AB017377
 apetalum_AB017378
 casmachatsense_AB017379
 caudaeblat_AB017380
 cernuum_AB017381
 chloropetalum_AB017382
 cuneatum_AB017384
 decipiens_AB017385
 decumbens_AB017386
 densatum_AB017387
 erectum_AB017388
 fargesiiID_AB018827
 flexilePst_AB017389
 foetidissimum_AB017390
 giganteum_Cf_AB017383
 gowanianum_AB017391
 grandiflorum_AB017392
 inaequalP_AB018832
 japonica_K_AB018831
 kurabayeshii_AB017393
 lancifolium_AB017394
 ludovicianum_AB017395
 luteum_AB017396
 maculatum_AB017397
 nivale_AB017398
 ovatum_AB017399
 petiolatum_AB017400
 polyphyllad_AB018828
 purpureum_AB017401
 recurvatum_AB017402
 reliquum_AB017403
 rivalePst_AB017404
 rugelii_AB017405
 sessiliter_AB017406
 similiter_AB017407
 smalliter_AB017408
 stamineum_AB017409
 sulcatum_AB017410

[illegible]

736_GA
746_maculatum
747_louisianal
748_louisiana2
749_louisiana3
750_freemani1
751_freemani1
778_caddoLA
779_washingtonOR_paba
780_escambiaAL
781_escambiaAL
782_jamesCityVA
783_graysonVA
784_undulatum
785_rockinghamNC
786_cavataTX
787_dallasTX
788_daiswa2
789_rivale
790_mississippiAR
791_montgomeryAR
792_rivale
793_limestoneAL
794_smithTX
796_hibbersonii1
799_ovatum
800_hibbersonii1
801_ovatum
802_barryMO
806_persistens
807_nippersInkAL
808_catesbaeiGA
810_viridescensTX
811_worcesterMO
812_grandiflorum
albident_ABO17376
angustipetalum_ABO17378
apetalum_ABO17378
camachatsenseAT_ABO17380
catesbaeiAT_ABO17380
cernuum_ABO17381
chloropetalum_ABO17383
cuneatum_ABO17384
decipiensAT_ABO17385
decumbensAT_ABO17386
discolorAT_ABO17387
erectum_ABO17388
fargesiiID_ABO18827
foeniculifolium_ABO17389
foeniculifolium_ABO17393
giganteumAT_ABO17383
govanianumAT_ABO17391
grandiflorum_ABO17393
incompletum_ABO18832
japonicaAT_ABO18831
kurabayashiiAT_ABO17393
lanceifolium_ABO17394
lodicuianumAT_ABO17393
luteum_ABO17396
maculatum_ABO17397
nivealeAT_ABO17398
ovatum_ABO17399
petiolatum_ABO17400
polyphyllum_ABO18828
pusilliflorum_ABO17401
rargravatum_ABO17402
rargravatum_ABO17403
rivaleAT_ABO17404
rugeliiAT_ABO17405
sessileAT_ABO17406
smillettAT_ABO17407
smalliiAT_ABO17408
stamineumAT_ABO17409
sulcatumAT_ABO17410
tetraphyllum_ABO18833
thibeticum_ABO18829
thibeticum_ABO18829
thibeticumAT_ABO17411
tchonoskiiAT_ABO17411
tuckermoodiiAT_ABO17412
undulatumAT_ABO17413
vaseyiAT_ABO17414
verticillatum_ABO18834
violaceum_ABO18830
viridescensAT_ABO17416
viridetAT_ABO17415

[illegible]

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2107_persistans
2135_franklinNC
734_barryMO
735_lincolntWN
736_GA
746_meculatum
747_louisianal
748_louisiana2
749_louisiana3
750_freemanii
751_freemanii
778_caddoLA
779_washingtonOR_paba
780_escambialA
781_escambialA
782_jamesCityVA
783_graysonVA
784_undulatum
785_rockinghamVA
786_cassTX
787_daiswal
788_daisw2

[illegible]

810_viridescens	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
811_worcesterMD	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
812_grandiflorum	-----CCAAACCTCTTGATATACATTTTGGTATGCTCCCATATTTT-----CTTT-A-----TA
816_alkenSC	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
817_cumberlandTN	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2105_viridescens	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2106_dorcestersC	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2107_persistens	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2108_wakeNC	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2109_williamsii	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2110_penderNC	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2111_recurvatum	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2112_caseyKY	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2113_lancifolium	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2114_nashNC	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2115_clayNC	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2116_hibbersonii	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2117_oettingeri	A---TAT-----AAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2119_humboldtCA	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2120_skagitWA	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2132_russellKY	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2133_montana	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2134_cumberlandTN	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2135_franklinNC	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2137_colorado	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2139_luteum	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2141_jonesMS	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2290_nivalePA_1	A---TAT-----AAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2319_rivale	A---TAT-----CAATAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2330_nivalePA_2	A---TAT-----AAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
2331_catesbaeiSC	A---TAT-----AAATAGTATATCAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
albium_DQ404232	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
camtschatcense_DQ404231	ATAGTATAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
cernuum_DQ404227	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
cuneatum_DQ404233	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
erectum_DQ404230	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
grandiflorum_DQ404229	-----CCAAACCTCTTGATATACATTTTGGTATGCTCCCATATTTT-----CTTT-A-----TA
luteum_DQ404234	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
ovatum_DQ404228	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
sessile_T_DQ00620	A---TAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
tschonoskii_DQ404235	ATAGTATAT-----CAAGAGG-----TTTGGTATGCTCCCATATTTT-----CTTTTC-----TA
O_Pet_rivale	TTTTATA-----GG-----TAATATAGCACTTATAA-----TTAT-----AAA
734_parryMO	TTTTATA-----TTTTATACCATTTATAGG-----TAATATAGCACTTATCA-----TTATAATGATCAA
735_lincolnTN	TTTTATA-----TTTTATACCATTTATAGG-----TAATATAGCACTTATCA-----TTATAATGATCAA
736_whitfieldGA	TTTTATA-----TTTTATACCATTTATAGG-----TTATAAGCACTTATCA-----TTATAATGATCAA
740_graysonVA	TTTTATA-----TTTTATACCATTTATAGG-----TAATATAGCACTTATCA-----TTATAATGATCAA
746_maculatum	TTTTATA-----TTTTATACCATTTATAGG-----TAATATAGCACTTATCA-----TTATAATGATCAA
747_louisiana1	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
748_louisiana2	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
749_louisiana3	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
750_freemanii	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
778_caddoLA	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
779_washingtonOR	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
780_escambiaAL	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
782_jamesCityVA	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
783_graysonVA	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
784_undulatum	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
785_rockinghamVA	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
789_nivale	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
790_mississippi	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
791_montgomeryAR	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
793_limestoneAL	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
794_smithTX	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
796_hibbersonii	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
799_ovatum	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
800_hibbersonii	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
801_ovatum	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
802_cuneatum	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
806_persistens	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
807_nippersinkAL	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
808_catesbaeiGA	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
810_viridescens	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
811_worcesterMD	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
812_grandiflorum	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
816_alkenSC	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
817_cumberlandTN	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2105_viridescens	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2106_dorcestersC	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2107_persistens	TTTTATATTTTATTTTATTTATACCATTTATAGG-----TAATATAGCACTTATCA-----TTATAATGATCAA
2108_wakeNC	TTTTATA-----TTTTATACCATTTATAGG-----TAATATAGCACTTATCA-----TTATAATGATCAA
2109_williamsii	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2110_penderNC	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2111_recurvatum	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2112_caseyKY	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2113_lancifolium	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2114_nashNC	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2115_clayNC	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2116_hibbersonii	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2117_oettingeri	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2119_humboldtCA	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2120_skagitWA	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2132_russellKY	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2133_montana	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2134_cumberlandTN	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2135_franklinNC	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2137_colorado	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2139_luteum	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2141_jonesMS	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2290_nivalePA_1	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2319_rivale	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2330_nivalePA_2	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
2331_catesbaeiSC	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
albium_DQ404232	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
camtschatcense_DQ404231	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
cernuum_DQ404227	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
cuneatum_DQ404233	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
erectum_DQ404230	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
grandiflorum_DQ404229	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA
luteum_DQ404234	TTTTATA-----TTTTATACCATTTATAGG-----TTATAGGTAAATATAGCACTTAGAA-----TTCT-----AAA

[illegible]

2108_wakeNC ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2109_williamsii ACAGGATCTCTTCCTTATCATAAATCTTCTCTTATCATAAAGTTATCCCCCAACAAATGAATAAAAGTGCCTTCTTTT-----
 2110_penderNC ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2111_recurvatum ACAGGATCTCTTCCTTATCATAAATCTTCTCTTATCATAAAGTTATCCCCCAACAAATGAATAAAAGTGCCTTCTTTT-----
 2112_caseyKY ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2113_lancifolium ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTTCTTTT-----
 2114_nashNC ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2115_clayNC ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2116_hibbersonii ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2117_oettingeri ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2119_humboldtCA ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2120_skagitWA ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2132_russellKY ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2133_montana ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2134_cumberlandTN ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2135_franklinNC ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2137_colorado ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2139_luteum ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2141_jonesMS ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2290_nivalePA_1 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2319_rivale ACAGGATCTCTTCCTTATCATAA-----ATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2330_nivalePA_2 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 2331_catesbaeiSC ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 albidumf_DQ404232 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTTCTTTT-----
 camschatsenset_DQ404231 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCTACTTAATCT-----
 cernuumf_DQ404227 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCTACTTAATCT-----
 cuneatumf_DQ404233 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCTACTTAATCT-----
 erectumf_DQ404230 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCTACTTAATCT-----
 grandiflorumf_DQ404229 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 luteumf_DQ404234 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 ovatumf_DQ404228 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCT-----
 sessile_T_DQ00620 ACAGGATCTCTTCCTTATCATAAAGTTATCCCCCAACAAATGAATAAAAGTGCCTTCTTTT-----
 techonoskii_T_DQ404235 ACAGGATCTCTTCCTTATCATAA-----GTTATCCCCCAACAAATGAATAAAAGTGCCTT-----CTTTATCTACTTAATCT-----
 O_Pet._rivale ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 734_barryMD ACTTATCTTTA-----TTTCTATTTAGAAATTTAGATTATATAAAATCAATTAACGACGAGATTGATTATC-----
 735_lincolntn ACTTATCTTTA-----TTTCTATTTAGAAATTTAGATTATATAAAATCAATTAACGACGAGATTGATTATC-----
 736_whitfieldGA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 740_graysynVA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 746_maculatum ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 747_louisianal AGAT-----
 748_louisiana2 AGAT-----
 749_louisiana3 AGAT-----
 750_freemanii AGAT-----
 778_caddoLA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 779_washingtonOR ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 780_escambiaAL ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 782_jamestownVA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 783_graysynVA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 784_undulatum ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 785_rockinghamVA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 789_nivale ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 790_mississippi ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 791_montgomeryAR ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 793_limestoneAL ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 794_smithTX ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 796_hibbersonii ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 799_ovatum ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 800_hibbersonii ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 801_ovatum ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 802_cuneatum ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 806_persistens ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 807_nippersinkAL ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 808_catesbaeiGA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 810_viridescens AGAT-----
 811_worcesterMD ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 812_grandiflorum ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 816_sikensC ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 817_cumberlandTN ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2105_viridescens AGAT-----
 2106_dorchesterSC ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2107_persistens ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2108_wakeNC ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2109_williamsii ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2110_penderNC ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2112_caseyKY ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2113_lancifolium ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2114_nashNC ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2115_clayNC ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2116_hibbersonii ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2117_oettingeri ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2119_humboldtCA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2120_skagitWA ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2132_russellKY ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2133_montana ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2134_cumberlandTN ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2135_franklinNC ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2137_colorado ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2139_luteum AGAT-----
 2141_jonesMS ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2290_nivalePA_1 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2319_rivale ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2330_nivalePA_2 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 2331_catesbaeiSC ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 albidumf_DQ404232 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 camschatsenset_DQ404231 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 cernuumf_DQ404227 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 cuneatumf_DQ404233 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 erectumf_DQ404230 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 grandiflorumf_DQ404229 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 luteumf_DQ404234 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 ovatumf_DQ404228 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 sessile_T_DQ00620 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 techonoskii_T_DQ404235 ACTTATCTTTA-----TTTCTATTTTGAATTTTGAATTTATATAAAATCAATTAACGACGAGATTGATTATC-----
 O_Pet._rivale -----GCATGCTCTTGAAGATTAGATTATATAAAATCAATTAACGACGAGATTGATTATC-----
 734_barryMD -----GCATGCTCTTGAAGATTAGATTATATAAAATCAATTAACGACGAGATTGATTATC-----
 735_lincolntn -----GCATGCTCTTGAAGATTAGATTATATAAAATCAATTAACGACGAGATTGATTATC-----
 736_whitfieldGA -----GCATGCTCTTGAAGATTAGATTATATAAAATCAATTAACGACGAGATTGATTATC-----

[illegible]

2116_hibbersonii AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0011000--1101101000100
 2117_oettingeri AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2119_humboldtCA AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2120_akagitWA AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2132_russellKY AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2133_montana AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2134_cumberlandTN AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2135_franklinNC AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2137_colorado AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2139_luteum AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2141_jonesMS AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2290_nivalePA_1 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2319_rivale AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2330_nivalePA_2 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 2331_catesbaeiSC AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 albidum_T_DQ404232 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
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 erectum_T_DQ404230 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 grandiflorum_T_DQ404229 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 luteum_T_DQ404234 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 ovatum_T_DQ404228 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 sessile_T_DQ00620 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100
 tschonoskii_T_DQ404235 AAGAAATAATCAATATGATGAATGAAAAAGAAAAA---GAGAAATCCTTTAGCTAGA-----TAAGG-0001000--110110100100

 0_Pst._rivale 0010010000101010-000010001
 734_barryMO 1010010000101010-000110010
 735_lincolntW 1010110000101010-000110010
 736_whitfieldGA 0000010000101010-000111030
 740_graysonVA 1010010000101010-000110010
 746_maculatum -----10-----01001010000
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 748_louisiana2 00100032110---01001010100
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 783_graysonVA 1010010000101010-000110010
 784_undulatum 0010010000100010-000010000
 785_rockinghamVA 1010011000101010-000110010
 789_nivale 0010010000100110-000010000
 790_mississippi 1010010000101010-000110010
 791_montgomeryAR 1010010000101010-000110010
 793_limestoneAL 1010110000101010-000110010
 794_smithTX 0010010000101010-000111040
 795_hibbersonii 0010010000101010-000010000
 800_hibbersonii 0010010000101010-000010000
 801_ovatum 0010010000101010-000010000
 802_cuneatum -----10-----01001010000
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 807_nipperinkAL 0010010000100010-000110000
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 2120_akagitWA 0010010000101010-000010000
 2132_russellKY 1010010000101010-000110010
 2133_montana 0010010000101010-000010000
 2134_cumberlandTN 1010110000101010-000110010
 2135_franklinNC -----110-000110000
 2137_colorado 0010010000101010-000010000
 2139_luteum -----10-----01001010000
 2141_jonesMS 1010010000101010-000110010
 2290_nivalePA_1 0010010000100110-000010000
 2319_rivale 0010010100100010-000010001
 2330_nivalePA_2 0010010000100110-000010000
 2331_catesbaeiSC 0010010000100110-000110000
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End;

734_r_rivale
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 735_r_alabanicum
 736_r_georgianum
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 749_louisiana3
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 779_washingtonOK
 785_cambialAL
 786_greeneTXA
 781_graysonOK
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 785_rockinghamVA
 789_rivale
 790_mississippi
 791_montereyAR
 793_limestoneAL
 794_lsmithTX
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 801_ovatum
 802_ovalis
 803_catesbaeIA
 809_catesbaeIA

[illegible]

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783_...graysonVA	ctctgactcaaaaatcgataccatagtcgccggtatctctcttattggatcgtgtgtctaaagctaaaatttggactatatggggatcctctattagtaa
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camshatensis	ctctcttttttcaatttccaaatggcattctatgtccaaatctcga	-tttaagtatg	-agggtgaagtaaaatcaaatatgat
cuneatum	ctctcttttttcaatttccaaatggcattctatgtccaaatctcga	-tttaagtatg	-agggtgaagtaaaatcaaatatgat
erectum	ctctcttttttcaatttccaaatggcattctatgtccaaatctcga	-tttaagtatg	-agggtgaagtaaaatcaaatatgat
grandiflorum	ctctcttttttcaatttccaaatggcattctatgtccaaatctcga	-tttaagtatg	-agggtgaagtaaaatcaaatatgat
luteum	ctctcttttttcaatttccaaatggcattctatgtccaaatctcga	-tttaagtatg	-agggtgaagtaaaatcaaatatgat
ovatum	ctctcttttttcaatttccaaatggcattctatgtccaaatctcga	-tttaagtatg	-agggtgaagtaaaatcaaatatgat
rubrum	ctctcttttttcaatttccaaatggcattctatgtccaaatctcga	-tttaagtatg	-agggtgaagtaaaatcaaatatgat
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rugosellum	ctctcttttttcaatttccaaatggcattctatgtccaaatctcga	-tttaagtatg	-agggtgaagtaaaatcaaatatgat
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ovatum         gaatggaaaagaaaaa--gagaaaatccttagctaga--tagg
rivalePst      gaatggaaaagaaaaa--gagaaaatccttagctagtagctaga--tagg
rugeliium      gaatggaaaagaaaaa--gagaaaatccttagctaga--tagggnnnnnn
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 735_T.alabamicum
 736_T.georgianum
 746_T.meculatum
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 748_louisiana2
 749_louisiana3
 750_freemanii
 778_caddoLA
 779_washingtonOR
 780_escambiaAL
 782_jamesCityVA
 783_graysonVA
 784_undulatum
 785_rockinghamVA
 789_nivale
 790_mississippi
 791_montgomeryAR
 793_limestoneAL
 794_smithTX
 796_hibbersonii
 798_ovatum
 800_hibbersonii
 801_ovatum
 806_persistens
 807_catesbaeiAL
 808_catesbaeiGA
 810_viridescens
 811_worcesterMD
 812_grandiflorum
 2106_dorchesterSC
 2107_persistens
 2135_franklinNC
 albidum
 cameschatcenseT
 cuneatum
 erectum
 grandiflorum
 luteum
 ovatum
 ovatumPst
 rivalePst
 rugellium
 sessileT
 tschonoskiiT

Pst._rivale -----
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 735_T.alabamicum -----
 736_T.georgianum -----


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806_persistens -----0001020--11011011001000010010000100010-000110000
807_catesbaeiAL -----0001020--11011011001000010010000100010-000110000
808_catesbaeiGA -----0001020--11011011001000010010000100010-000110000
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811_worcesteriED -----0101020--11011011001001010010000101010-000110010
812_grandiflorum -----0002---1111010010001000010010000100010-000010000
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2107_persistens -----0001020--11011011101000010010000100010-000110000
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grandiflorumT -----0002--1111010010001000010010000100010-000010000
luteumT -----0011000--11111011-----10---01001010000
ovatumT -----0001000--11011011001000010010000101010-000010000
rivalePst -----0001000--110110112010000100101000100010-000010001
rugelliumT -----0001000--11211010001000010010000110010-100010001
sessileT -----0011000--110110110410-00100032110---01001010000
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```

End;

Appendix C

Standard Statistics from the Morphological Characters

Table C.1. Descriptive statistics from the SPSS analysis of members of the Pusillum Complex.

	taxon	N	Mean	Std. Dev.	Variance	Min	Max	Skewness	Kurtosis
stem	alabamicum	39	147.24	38.73	1499.92	64.00	213.00	-0.16	-0.63
	ozarkanum	65	184.18	51.32	2634.03	90.00	285.00	0.29	-0.87
	georgianum	7	111.29	13.29	176.57	98.00	137.00	1.27	1.93
	pusillum	15	131.33	49.71	2470.81	65.00	214.00	0.56	-1.07
	telmacola	1	150.00	.	.	150.00	150.00	.	.
	texanum	15	175.13	21.00	441.12	140.00	208.00	-0.17	-1.12
	monticola	10	130.95	33.11	1096.03	86.00	192.00	0.25	-0.20
	palustris	25	145.92	31.79	1010.66	113.00	235.00	1.32	1.48
	carolinianum	8	129.13	39.93	1594.41	60.00	180.00	-0.55	-0.34
	virginianum	41	137.78	29.29	857.93	60.00	187.00	-0.78	0.36
leaf length	alabamicum	40	44.78	10.95	119.96	26.00	63.00	0.04	-1.14
	ozarkanum	65	52.29	12.18	148.43	23.00	80.00	0.01	0.37
	georgianum	7	42.50	7.57	57.25	35.00	56.00	0.97	0.43
	pusillum	12	45.33	9.27	85.88	29.00	60.00	-0.25	-0.72
	telmacola	1	65.00	.	.	65.00	65.00	.	.
	texanum	15	53.80	6.54	42.74	42.00	70.00	0.85	2.02
	monticola	10	39.05	9.42	88.69	26.00	55.00	0.38	-0.93
	palustris	25	45.98	11.79	138.97	33.00	82.50	1.53	2.58
	carolinianum	9	38.44	8.99	80.78	23.00	53.00	-0.02	0.07
	virginianum	41	38.22	9.32	86.93	24.00	63.00	0.79	0.16
leaf width	alabamicum	40	14.31	4.44	19.73	7.00	25.00	0.37	-0.76
	ozarkanum	65	17.29	4.54	20.58	8.00	30.00	0.24	-0.25
	georgianum	7	8.79	1.41	1.99	7.00	11.00	0.49	-0.94
	pusillum	12	12.67	2.70	7.29	7.50	18.00	0.10	0.84
	telmacola	1	22.00	.	.	22.00	22.00	.	.
	texanum	15	15.43	4.98	24.82	9.00	27.50	1.16	0.99
	monticola	10	17.75	3.14	9.85	14.00	22.50	0.35	-1.54
	palustris	25	14.28	4.29	18.40	8.00	25.00	0.73	0.11
	carolinianum	9	13.44	3.09	9.53	7.00	17.00	-0.96	1.52
	virginianum	40	11.09	1.95	3.82	6.00	15.00	0.01	0.01
pedicel	alabamicum	40	19.38	8.06	64.92	7.00	37.00	0.40	-0.65
	ozarkanum	65	23.79	7.36	54.22	9.00	45.00	0.34	0.14
	georgianum	7	20.21	6.11	37.32	13.00	29.00	0.04	-1.57
	pusillum	15	13.80	4.65	21.60	6.00	23.00	0.29	0.05
	telmacola	1	30.00	.	.	30.00	30.00	.	.
	texanum	15	31.13	8.26	68.27	23.00	56.00	2.07	5.63
	monticola	7	0.70	0.28	0.08	0.40	1.00	0.31	-2.69
	palustris	22	1.98	1.62	2.61	0.50	7.00	1.72	3.20
	carolinianum	9	4.47	3.15	9.92	1.20	11.00	1.13	1.15
	virginianum	38	1.78	1.18	1.40	0.50	7.00	2.41	9.37
sepal length	alabamicum	40	21.36	4.83	23.33	13.00	30.00	0.05	-0.74
	ozarkanum	67	26.44	6.55	42.92	14.00	41.00	0.31	-0.56
	georgianum	7	16.57	1.40	1.95	15.00	19.00	0.57	0.38
	pusillum	12	19.21	3.12	9.75	13.00	24.00	-0.23	0.10
	telmacola	1	21.00	.	.	21.00	21.00	.	.
	texanum	15	22.20	4.28	18.31	15.00	30.00	0.11	-0.58
	monticola	9	19.56	3.24	10.53	16.00	25.00	0.26	-1.09
	palustris	24	20.48	4.84	23.42	13.00	31.00	0.93	0.22
	carolinianum	8	16.94	6.69	44.75	9.00	28.00	0.42	-0.53
	virginianum	40	16.96	2.25	5.04	11.50	22.00	0.03	0.48

Table C.1. continued.

	taxon	N	Mean	Std. Dev.	Variance	Min	Max	Skewness	Kurtosis
sepal width	alabamicum	40	7.39	2.06	4.23	4.00	12.00	0.34	-0.35
	ozarkanum	67	9.04	2.93	8.58	4.00	17.00	0.53	-0.13
	georgianum	7	6.00	0.82	0.67	5.00	7.00	0.00	-1.20
	pusillum	12	6.25	1.12	1.25	4.50	8.00	0.18	-0.64
	telmacola	1	9.00			9.00	9.00		
	texanum	15	6.60	2.51	6.29	3.00	13.00	0.77	2.15
	monticola	9	6.44	1.69	2.84	4.00	9.00	0.50	-0.38
	palustris	24	5.73	1.62	2.61	4.00	10.00	1.07	1.02
	carolinianum	8	5.06	1.43	2.03	3.50	7.00	0.52	-1.68
	virginianum	40	4.74	1.11	1.23	3.00	7.50	0.15	-0.36
petal length	alabamicum	39	21.08	3.66	13.39	15.00	30.00	-0.01	-0.24
	ozarkanum	63	25.01	4.52	20.42	14.00	33.00	-0.11	-0.62
	georgianum	7	17.29	1.60	2.57	15.00	19.00	-0.31	-1.83
	pusillum	10	20.25	3.28	10.74	13.00	26.00	-0.72	3.01
	telmacola	1	15.00			15.00	15.00		
	texanum	10	20.85	3.02	9.11	15.00	25.00	-0.79	0.29
	monticola	9	20.50	3.54	12.50	16.00	27.00	0.42	0.06
	palustris	23	19.87	3.90	15.23	13.00	28.00	0.36	-0.27
	carolinianum	6	17.75	3.71	13.78	14.00	24.00	1.04	0.45
	virginianum	34	18.57	2.46	6.05	12.50	23.00	-0.48	0.07
petal width	alabamicum	39	8.00	2.12	4.50	4.00	13.00	0.34	-0.35
	ozarkanum	59	9.95	2.46	6.04	6.00	16.00	0.35	-0.36
	georgianum	7	8.00	1.53	2.33	5.00	9.00	-1.57	1.97
	pusillum	8	6.06	1.21	1.46	4.50	8.00	0.29	-1.02
	telmacola	1	6.00			6.00	6.00		
	texanum	8	8.88	1.58	2.48	7.00	12.00	1.15	1.29
	monticola	8	6.13	1.89	3.55	4.00	9.00	0.96	-0.51
	palustris	23	6.11	2.14	4.59	3.50	12.00	1.17	1.37
	carolinianum	6	4.92	1.86	3.44	3.00	8.00	0.94	0.25
	virginianum	34	5.36	1.02	1.04	3.50	7.00	-0.05	-0.70
filament	alabamicum	40	3.77	0.59	0.35	2.50	5.00	-0.04	-0.50
	ozarkanum	59	4.14	0.59	0.35	3.00	5.70	0.57	0.41
	georgianum	7	3.39	0.35	0.13	3.00	4.00	0.61	0.25
	pusillum	12	3.73	0.51	0.26	3.00	5.00	1.27	2.68
	telmacola	1	4.00			4.00	4.00		
	texanum	11	3.77	0.93	0.87	2.50	5.50	0.37	-0.08
	monticola	8	5.50	1.13	1.29	4.50	8.00	1.76	3.66
	palustris	22	4.45	0.95	0.90	2.50	6.00	-0.50	-0.55
	carolinianum	7	3.80	1.28	1.63	2.80	6.40	1.77	2.99
	virginianum	40	4.22	0.78	0.61	3.00	6.50	0.96	1.32
anther	alabamicum	40	5.77	1.59	2.52	3.00	9.70	0.39	-0.36
	ozarkanum	59	6.30	1.72	2.96	2.50	11.00	0.15	0.10
	georgianum	7	4.39	0.41	0.17	4.00	5.20	1.38	2.31
	pusillum	12	4.78	0.99	0.97	3.20	6.50	0.20	-0.84
	telmacola	1	5.50			5.50	5.50		
	texanum	11	5.95	1.17	1.37	4.00	7.50	-0.21	-0.98
	monticola	8	5.73	1.70	2.89	3.80	9.50	1.72	4.10
	palustris	22	5.15	1.10	1.22	3.20	7.00	0.15	-0.39
	carolinianum	7	3.77	1.54	2.37	2.50	6.80	1.58	2.07
	virginianum	40	4.23	0.65	0.42	2.60	5.70	-0.22	-0.04

Table C.1. continued.

	taxon	N	Mean	Std. Dev.	Variance	Min	Max	Skewness	Kurtosis
ovary	alabamicum	38	3.28	0.68	0.46	2.00	4.50	0.11	-0.93
	ozarkanum	57	3.59	0.89	0.80	1.00	6.50	0.21	1.98
	georgianum	7	3.24	0.38	0.14	2.70	3.50	-1.09	-1.03
	pusillum	12	2.64	0.50	0.25	2.00	3.50	0.74	-0.49
	telmacola	1	4.00			4.00	4.00		
	texanum	10	4.12	1.24	1.53	2.70	7.00	1.44	2.68
	monticola	8	3.91	0.84	0.70	3.00	5.50	1.03	0.29
	palustris	21	3.48	1.12	1.26	1.70	7.00	1.43	4.08
	carolinianum	7	2.93	0.81	0.66	1.80	4.00	0.00	-1.32
	virginianum	40	2.82	0.54	0.29	1.70	4.00	0.30	-0.33
style	alabamicum	38	3.39	0.99	0.99	1.20	5.50	-0.10	-0.14
	ozarkanum	57	4.28	1.42	2.00	1.20	9.50	0.54	2.30
	georgianum	7	2.59	0.68	0.46	1.50	3.70	0.09	1.14
	pusillum	12	2.68	0.66	0.44	1.50	3.50	-0.19	-0.92
	telmacola	1	1.50			1.50	1.50		
	texanum	10	3.48	0.69	0.48	2.50	5.00	1.00	1.78
	monticola	8	4.94	2.09	4.39	3.00	9.50	1.73	3.21
	palustris	21	4.18	1.86	3.45	2.00	9.50	1.40	2.15
	carolinianum	7	2.69	1.17	1.36	1.20	4.50	0.64	-0.56
	virginianum	40	3.35	0.75	0.56	2.20	5.00	0.62	-0.38
stigma	alabamicum	38	2.92	0.97	0.95	1.30	6.50	1.53	4.05
	ozarkanum	57	2.90	1.04	1.09	0.50	6.50	0.70	1.39
	georgianum	7	2.01	0.25	0.06	1.50	2.20	-1.79	3.69
	pusillum	12	2.16	0.79	0.63	1.00	3.50	0.26	-0.86
	telmacola	1	3.00			3.00	3.00		
	texanum	10	2.07	0.90	0.82	1.00	4.00	1.10	1.16
	monticola	8	1.81	1.00	1.00	0.50	3.50	0.40	-0.61
	palustris	21	2.66	0.81	0.65	1.00	5.00	0.94	2.93
	carolinianum	7	1.74	0.53	0.28	1.00	2.50	-0.16	-1.00
	virginianum	40	2.36	0.81	0.65	1.00	4.70	1.06	1.37
stamen	alabamicum	40	9.54	1.80	3.23	6.00	13.70	0.13	-0.44
	ozarkanum	59	10.46	1.86	3.48	7.00	16.00	0.19	0.21
	georgianum	7	7.77	0.41	0.17	7.00	8.20	-1.23	1.16
	pusillum	12	8.52	0.96	0.93	6.70	10.00	-0.35	-0.41
	telmacola	1	9.50			9.50	9.50		
	texanum	11	9.73	1.82	3.32	6.50	13.00	0.31	0.22
	monticola	8	11.23	2.78	7.71	8.80	17.50	1.93	4.45
	palustris	22	9.60	1.81	3.28	5.70	12.50	-0.19	-0.33
	carolinianum	7	7.57	2.58	6.66	5.70	13.20	2.24	5.37
	virginianum	40	8.45	1.17	1.36	6.00	11.00	0.25	0.03
pistil	alabamicum	38	9.58	1.90	3.60	5.90	14.50	0.18	0.07
	ozarkanum	57	10.92	2.54	6.44	4.50	17.70	0.43	0.71
	georgianum	7	7.84	0.45	0.20	7.20	8.50	-0.06	-0.72
	pusillum	12	7.48	1.36	1.85	5.10	10.50	0.76	1.59
	telmacola	1	8.50			8.50	8.50		
	texanum	10	9.67	2.32	5.37	6.20	14.50	0.71	1.14
	monticola	8	10.66	2.25	5.05	8.30	15.00	1.03	0.77
	palustris	21	10.32	2.85	8.11	5.90	19.50	1.59	4.56
	carolinianum	7	7.36	2.04	4.15	5.00	10.00	0.28	-1.73
	virginianum	40	8.53	1.33	1.77	5.30	11.20	0.00	-0.18

Table C.1. continued

	taxon	N	Mean	Std. Dev.	Variance	Min	Max	Skewness	Kurtosis
ratio stem to leaf length	alabamicum	39	3.32	0.76	0.58	1.58	4.91	-0.14	-0.43
	ozarkanum	65	3.61	0.95	0.90	1.81	6.70	0.69	0.58
	georgianum	7	2.67	0.47	0.22	2.02	3.43	0.23	-0.16
	pusillum	12	2.93	0.79	0.63	1.67	3.89	-0.29	-1.65
	telmacola	1	2.31			2.31	2.31		
	texanum	15	3.28	0.43	0.19	2.21	3.94	-0.90	1.40
	monticola	10	3.40	0.69	0.48	2.49	4.57	0.48	-0.43
	palustris	25	3.25	0.59	0.35	2.08	4.39	-0.03	-0.48
	carolinianum	8	3.52	0.95	0.90	2.56	5.19	0.79	-0.62
	virginianum	41	3.71	0.87	0.75	1.88	5.52	0.05	-0.52
ratio leaf length to width	alabamicum	40	3.23	0.55	0.30	2.00	5.10	1.22	3.33
	ozarkanum	65	3.09	0.57	0.32	2.08	4.87	0.81	0.56
	georgianum	7	4.89	0.86	0.74	3.91	6.06	0.30	-1.52
	pusillum	12	3.63	0.62	0.39	2.59	4.82	0.15	-0.15
	telmacola	1	2.95			2.95	2.95		
	texanum	15	3.73	0.92	0.84	1.89	5.67	0.04	0.69
	monticola	10	2.22	0.44	0.20	1.33	2.94	-0.34	1.24
	palustris	25	3.36	0.86	0.73	2.36	5.70	1.40	1.52
	carolinianum	9	2.90	0.45	0.20	2.13	3.53	-0.40	-0.66
	virginianum	40	3.50	0.92	0.85	2.33	6.11	1.19	0.86
ratio stem to pedicel	alabamicum	39	8.48	3.66	13.38	3.78	20.71	1.42	2.66
	ozarkanum	65	8.34	3.12	9.75	4.00	19.64	1.11	1.47
	georgianum	7	5.94	1.84	3.38	3.90	8.56	0.44	-1.98
	pusillum	15	10.43	4.44	19.67	3.61	16.50	-0.04	-1.51
	telmacola	1	5.00			5.00	5.00		
	texanum	15	5.90	1.39	1.92	3.39	7.88	-0.18	-0.81
	monticola	7	210.29	107.99	11662.24	86.00	380.00	0.27	-0.81
	palustris	22	113.24	72.42	5244.18	18.14	250.00	0.61	-0.69
	carolinianum	8	54.54	49.88	2487.70	11.36	138.33	1.01	-0.68
	virginianum	38	108.58	73.75	5438.63	21.86	300.00	1.44	1.23
ratio sepal length to width	alabamicum	40	2.96	0.41	0.17	1.82	4.00	0.21	1.06
	ozarkanum	67	3.07	0.69	0.48	1.29	6.00	0.85	4.43
	georgianum	7	2.81	0.49	0.24	2.43	3.80	1.70	3.05
	pusillum	12	3.11	0.46	0.22	2.50	4.00	0.66	-0.39
	telmacola	1	2.33			2.33	2.33		
	texanum	15	3.66	0.96	0.92	2.08	5.67	0.58	0.06
	monticola	9	3.12	0.46	0.21	2.33	4.00	0.28	1.45
	palustris	24	3.66	0.59	0.35	2.60	5.00	0.21	-0.11
	carolinianum	8	3.28	0.63	0.40	2.25	4.00	-0.50	-0.80
	virginianum	40	3.74	0.88	0.78	2.17	6.00	0.87	0.44
ratio pedicel to sepal	alabamicum	40	0.89	0.26	0.07	0.39	1.45	0.41	-0.42
	ozarkanum	65	0.92	0.24	0.06	0.43	1.37	0.01	-0.85
	georgianum	7	1.21	0.32	0.10	0.84	1.60	-0.09	-2.29
	pusillum	12	0.68	0.27	0.07	0.36	1.35	1.56	2.67
	telmacola	1	1.43			1.43	1.43		
	texanum	15	1.44	0.40	0.16	1.00	2.20	0.53	-0.88
	monticola	7	0.04	0.02	0.00	0.02	0.06	0.52	-1.75
	palustris	21	0.09	0.07	0.01	0.03	0.25	1.31	0.63
	carolinianum	8	0.25	0.17	0.03	0.08	0.61	1.77	3.55
	virginianum	37	0.11	0.07	0.01	0.03	0.41	2.31	8.39

Table C.1. continued.

	taxon	N	Mean	Std. Dev.	Variance	Min	Max	Skewness	Kurtosis
ratio petal length to width	alabamicum	39	2.73	0.50	0.25	1.92	4.00	0.57	-0.49
	ozarkanum	59	2.59	0.52	0.27	1.73	4.17	0.86	0.98
	georgianum	7	2.25	0.54	0.29	1.67	3.20	0.96	0.23
	pusillum	8	3.41	0.51	0.26	2.75	4.30	0.51	-0.22
	telmacola	1	2.50	.	.	2.50	2.50	.	.
	texanum	8	2.49	0.44	0.19	1.83	3.13	0.11	-0.79
	monticola	8	3.59	0.64	0.41	2.56	4.60	-0.09	-0.04
	palustris	23	3.44	0.72	0.51	1.96	5.14	-0.05	0.96
	carolinianum	6	3.88	1.08	1.16	2.50	5.33	0.06	-1.26
	virginianum	34	3.56	0.72	0.52	2.15	5.71	0.69	1.09
ratio sepal length to petal length	alabamicum	39	1.02	0.15	0.02	0.75	1.35	0.55	-0.20
	ozarkanum	63	1.04	0.17	0.03	0.71	1.50	0.29	-0.05
	georgianum	7	0.96	0.11	0.01	0.83	1.13	0.29	-0.87
	pusillum	9	0.92	0.14	0.02	0.77	1.18	0.54	-0.62
	telmacola	1	1.40	.	.	1.40	1.40	.	.
	texanum	10	1.07	0.10	0.01	0.92	1.23	0.07	-1.46
	monticola	9	0.96	0.12	0.01	0.76	1.13	-0.18	-0.76
	palustris	23	1.04	0.17	0.03	0.72	1.50	0.97	1.79
	carolinianum	6	0.92	0.29	0.09	0.51	1.20	-0.59	-1.80
	virginianum	34	0.92	0.11	0.01	0.71	1.24	0.56	1.04
ratio anther to filament	alabamicum	40	1.56	0.45	0.21	0.88	2.60	0.53	-0.50
	ozarkanum	59	1.55	0.47	0.22	0.56	2.75	0.30	0.35
	georgianum	7	1.31	0.22	0.05	1.00	1.73	0.95	2.82
	pusillum	12	1.31	0.36	0.13	0.80	1.86	0.35	-1.19
	telmacola	1	1.38	.	.	1.38	1.38	.	.
	texanum	11	1.64	0.42	0.18	1.13	2.60	1.16	1.52
	monticola	8	1.03	0.14	0.02	0.76	1.20	-0.80	1.31
	palustris	22	1.19	0.27	0.07	0.74	2.00	1.19	3.00
	carolinianum	7	1.02	0.36	0.13	0.67	1.71	1.34	1.96
	virginianum	40	1.02	0.19	0.04	0.58	1.34	-0.15	-0.72
ratio filament to ovary	alabamicum	38	1.19	0.26	0.07	0.67	2.00	0.85	1.46
	ozarkanum	57	1.24	0.48	0.23	0.62	4.00	3.84	19.85
	georgianum	7	1.05	0.15	0.02	0.86	1.30	0.32	-0.12
	pusillum	12	1.44	0.26	0.07	1.14	2.00	0.80	0.37
	telmacola	1	1.00	.	.	1.00	1.00	.	.
	texanum	10	0.94	0.13	0.02	0.71	1.14	0.10	-0.19
	monticola	8	1.43	0.24	0.06	1.00	1.78	-0.31	0.21
	palustris	21	1.34	0.36	0.13	0.79	2.53	1.69	5.54
	carolinianum	7	1.34	0.37	0.13	0.70	1.68	-1.07	0.14
	virginianum	40	1.54	0.36	0.13	0.92	2.94	1.45	4.91
ratio ovary to stigma	alabamicum	38	1.20	0.33	0.11	0.69	2.25	0.91	1.22
	ozarkanum	57	1.41	0.77	0.60	0.38	6.00	3.89	22.00
	georgianum	7	1.62	0.21	0.04	1.23	1.80	-1.43	1.65
	pusillum	12	1.39	0.57	0.33	0.72	2.33	0.62	-1.40
	telmacola	1	1.33	.	.	1.33	1.33	.	.
	texanum	10	2.14	0.51	0.26	1.17	2.92	-0.35	0.20
	monticola	8	3.23	2.67	7.11	1.00	9.00	1.67	3.05
	palustris	21	1.45	0.82	0.67	0.66	4.50	2.77	9.70
	carolinianum	7	1.83	0.75	0.57	0.90	3.00	0.44	-0.92
	virginianum	40	1.31	0.43	0.19	0.53	2.27	0.47	-0.49

Table C.1. continued.

	taxon	N	Mean	Std. Dev.	Variance	Min	Max	Skewness	Kurtosis
ratio stigma to anther	alabamicum	38	0.51	0.12	0.02	0.26	0.76	0.06	-0.56
	ozarkanum	57	0.49	0.24	0.06	0.13	1.60	1.92	6.92
	georgianum	7	0.46	0.07	0.00	0.38	0.55	-0.14	-1.27
	pusillum	12	0.45	0.14	0.02	0.23	0.71	0.45	-0.17
	telmacola	1	0.55			0.55	0.55		
	texanum	10	0.35	0.15	0.02	0.18	0.57	0.65	-1.56
	monticola	8	0.35	0.22	0.05	0.08	0.66	0.31	-1.52
	palustris	21	0.54	0.19	0.04	0.15	1.00	0.48	0.85
	carolinianum	7	0.53	0.26	0.07	0.22	0.83	0.15	-2.27
	virginianum	40	0.56	0.19	0.04	0.27	1.12	1.18	1.27
ratio anther to ovary	alabamicum	38	1.77	0.26	0.07	1.17	2.30	-0.08	-0.36
	ozarkanum	57	1.92	1.31	1.70	0.63	11.00	6.19	43.42
	georgianum	7	1.36	0.15	0.02	1.14	1.56	-0.20	-1.46
	pusillum	12	1.86	0.50	0.25	1.14	2.75	0.55	-0.69
	telmacola	1	1.38			1.38	1.38		
	texanum	10	1.53	0.40	0.16	1.00	2.17	-0.05	-0.95
	monticola	8	1.47	0.33	0.11	1.00	2.11	0.80	1.29
	palustris	21	1.57	0.42	0.18	1.00	2.61	1.19	1.26
	carolinianum	7	1.28	0.30	0.09	0.94	1.79	0.67	0.02
	virginianum	40	1.55	0.40	0.16	1.06	3.35	2.48	10.17
ratio stigma to style	alabamicum	38	0.97	0.59	0.35	0.36	3.85	3.32	14.64
	ozarkanum	57	0.77	0.43	0.19	0.10	2.80	2.10	7.55
	georgianum	7	0.85	0.33	0.11	0.41	1.47	0.92	1.96
	pusillum	12	0.85	0.36	0.13	0.38	1.53	0.52	-0.61
	telmacola	1	2.00			2.00	2.00		
	texanum	10	0.60	0.27	0.07	0.40	1.14	1.32	0.55
	monticola	8	0.45	0.31	0.09	0.08	0.88	0.06	-1.93
	palustris	21	0.76	0.38	0.15	0.14	1.67	0.60	0.02
	carolinianum	7	0.77	0.45	0.21	0.33	1.67	1.49	2.28
	virginianum	40	0.74	0.30	0.09	0.29	1.43	0.81	-0.02
ratio stamen to pistil	alabamicum	38	1.01	0.11	0.01	0.82	1.28	0.50	-0.01
	ozarkanum	57	0.99	0.18	0.03	0.40	1.56	-0.24	1.93
	georgianum	7	0.99	0.08	0.01	0.89	1.11	0.13	-0.74
	pusillum	12	1.16	0.16	0.03	0.90	1.43	0.01	-1.06
	telmacola	1	1.12			1.12	1.12		
	texanum	10	1.03	0.15	0.02	0.79	1.30	-0.03	-0.05
	monticola	8	1.06	0.13	0.02	0.88	1.29	0.58	0.10
	palustris	21	0.96	0.17	0.03	0.53	1.27	-0.55	1.29
	carolinianum	7	1.04	0.21	0.04	0.76	1.35	0.09	-1.13
	virginianum	40	1.00	0.12	0.01	0.81	1.30	0.29	-0.18

Appendix D

Discriminant Analyses

Table D.1. Wilks lambda illustrating the significance of the functions calculated for the clusters of the Pusillum Complex.

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	0.088	376.054	50	0
2	0.615	75.386	24	0

Table D.2. Eigenvalues and % variance for the functions for the clusters of the Pusillum Complex showing the amount of variation attributable to each function.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	5.957	90.5	90.5	0.925
2	0.626	9.5	100.0	0.621

Table D.3. Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the functions used in separating the clusters of the Pusillum Complex. The largest values for each function are bold, and the single largest positive and negative coefficients are *bold-italic*.

character	Function	
	1	2
stem	-0.442	-0.474
leaf length	0.536	1.732
leaf width	0.226	-1.618
pedicel	1.200	-0.093
sepal length	-0.858	1.970
sepal width	-0.105	0.530
petal length	-0.559	-1.464
petal width	0.181	-0.183
filament	0.157	2.394
anther	-2.608	3.890
ovary	0.245	-0.593
style	0.142	0.860
stigma	-0.101	0.230
stamen	1.681	-5.218
ratio: stem 2 leaf	0.439	0.770
ratio: leaf length 2 width	0.072	-1.679
ratio: stem 2 pedicel	0.302	0.083
ratio: sepal length 2 width	0.223	0.460
ratio: pedicel 2 sepal	-1.712	-0.008
ratio: petal length 2 width	0.237	-0.321
ratio: sepal length 2 petal length	-0.066	-1.347
ratio: anther 2 filament	1.135	0.276
ratio: ovary 2 stigma	-0.179	-0.223
ratio: stigma 2 anther	-0.105	-0.509
ratio: stigma 2 style	0.075	0.536

Table D.4. Structure matrix indicating the correlations of each character to the functions used in separating the clusters of the *Pusillum* Complex. Variables in *bold italic* were not used in the analysis.

character	function	
	1	2
pedice2lsepel	-0.708	-0.194
pedicel	-0.593	0.117
stem2pedicel	0.466	-0.018
petal_l2w	0.291	-0.074
<i>filament2ovary</i>	0.179	0.059
sepal_l2w	0.171	0.068
leaf_length	-0.170	0.130
sepal2petal	-0.110	0.089
ovary	-0.097	0.056
stigma2style	-0.038	0.036
petal_length	-0.192	0.429
stem	-0.123	0.424
sepal_length	-0.220	0.417
leaf_l2w	-0.007	-0.415
<i>pistil</i>	-0.109	0.381
stem2leaf	0.051	0.377
petal_width	-0.330	0.368
style	-0.050	0.365
anther	-0.205	0.335
leaf_width	-0.140	0.332
stamen	-0.135	0.331
stigma	-0.084	0.322
sepal_width	-0.271	0.311
anther2filament	-0.226	0.228
<i>anther2ovary</i>	-0.041	0.195
ovary2stigma	0.029	-0.161
filament	0.075	0.135
stigma2anther	0.059	0.120
<i>stamen2pistil</i>	0.066	-0.110

Table D.5. Wilks lambda illustrating the significance of the functions calculated for the taxa of the Pusillum Cluster of the Pusillum Complex.

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	0.000	115.563	57	0
2 through 3	0.005	55.428	36	0.020
3	0.152	19.814	17	0.284

Table D.6. Eigenvalues and % variance for the functions used in separating the taxa of the Pusillum Cluster of the Pusillum Complex showing the amount of variation attributable to each function. First 3 canonical discriminant functions were used in the analysis even though only the first 2 were significant.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	306.091	89.9	89.9	0.998
2	28.721	8.4	98.4	0.983
3	5.600	1.5	100.0	0.921

Table D.7. Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the functions used in separating the taxa of the Pusillum Cluster of the Pusillum Complex. The largest values for each function are bold, and the single largest positive and negative coefficients are *bold-italic*.

character	Function		
	1	2	3
stem	-0.487	-0.961	3.176
leaf length	-10.798	-4.788	-4.463
leaf width	14.945	6.914	1.343
pedicel	-1.476	-3.687	-3.325
sepal length	21.412	0.440	4.808
sepal width	-17.030	0.657	-3.049
petal length	7.264	-4.459	-0.817
petal width	3.882	3.590	1.872
filament	-25.929	4.285	-3.858
anther	9.371	0.045	0.619
ovary	22.336	-2.379	3.287
style	-5.390	2.265	1.051
stigma	-12.952	-1.046	-0.211
ratio: stem 2 leaf	-5.987	-0.779	-3.578
ratio: leaf length 2 width	7.008	7.388	1.475
ratio: stem 2 pedicel	4.924	-0.600	2.159
ratio: sepal length 2 width	-22.665	3.222	-1.716
ratio: pedicel 2 sepal	7.120	1.789	4.069
ratio: filament 2 ovary	13.394	-2.053	3.649

Table D.8. Structure matrix indicating the correlations of each character to the function used in separating the taxa of the Pusillum Cluster of the Pusillum Complex. Variables in *bold italic* were not used in the analysis.

character	function		
	1	2	3
stem	0.054	-0.010	-0.024
anther	0.044	-0.028	-0.001
<i>pistil</i>	0.036	-0.001	0.023
<i>ovary2stigma</i>	-0.060	0.144	-0.065
leaf_l2w	-0.024	0.128	-0.013
petal_width	0.037	0.112	0.034
sepal_length	0.064	-0.066	0.035
<i>stamen2pistil</i>	0.025	-0.062	-0.038
filament	0.018	-0.056	0.021
<i>stamen</i>	0.041	-0.048	0.009
<i>sepal2petal</i>	0.066	-0.034	-0.264
petal_length	0.022	-0.066	0.239
style	0.030	-0.006	0.239
<i>stigma2style</i>	-0.013	-0.159	-0.203
leaf_length	0.042	-0.034	-0.198
<i>petal_l2w</i>	-0.055	-0.177	0.197
filament2ovary	-0.049	-0.149	0.188
pedice2lsepel	0.032	0.097	-0.174
sepal_l2w	0.001	-0.023	0.152
stem2pedicel	-0.021	-0.083	0.132
leaf_width	0.051	-0.104	-0.125
pedicel	0.053	0.050	-0.125
<i>anther2ovary</i>	-0.077	-0.101	0.112
sepal_width	0.045	-0.028	-0.110
stem2leaf	0.031	0.023	0.091
ovary	0.048	0.041	-0.081
stigma	0.003	-0.043	-0.066
<i>stigma2anther</i>	-0.030	-0.043	-0.044
<i>anther2filament</i>	-0.008	0.019	-0.035

Table D.9. Eigenvalues and % variance for the function used in separating the taxa of the Ozarkanum Cluster of the Pusillum Complex showing the amount of variation attributed to each function.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	0.766	100.0	100.0	0.659

Table D.10. Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the function used in separating the taxa of the Ozarkanum Cluster of the Pusillum Complex. The largest values for each function are bold, and the single largest positive and negative coefficients are *bold-italic*.

character	Function 1
stem	-2.139
leaf length	1.339
leaf width	-0.410
pedicel	1.882
sepal length	-2.914
sepal width	-1.061
petal length	1.709
petal width	0.359
filament	0.555
anther	1.916
ovary	-0.140
style	-0.306
stigma	0.275
stamen	-2.526
ratio: stem 2 leaf	0.992
ratio: leaf length 2 width	-0.020
ratio: stem 2 pedicel	0.749
ratio: sepal length 2 width	-1.129
ratio: pedicel 2 sepal	-1.020
ratio: petal length 2 width	0.569
ratio: sepal 2 petal	1.757
ratio: anther 2 filament	0.748
ratio: ovary 2 stigma	-0.280
ratio: stigma 2 anther	0.229
ratio: stigma 2 style	-0.166

Table D.11. Structure matrix indicating the correlations of each character to the function used in separating the taxa of the Ozarkanum Cluster of the Pusillum Complex. Variables in *bold italic* were not used in the analysis.

character	function 1
petal_length	-0.438
petal_width	-0.404
stem	-0.400
sepal_length	-0.378
filament	-0.348
leaf_width	-0.335
style	-0.331
leaf_length	-0.283
<i>pistil</i>	-0.281
pedicel	-0.258
sepal_width	-0.237
stamen	-0.206
ovary	-0.188
ovary2stigma	-0.188
leaf_12w	0.171
stigma2style	0.167
<i>stamen2pistil</i>	0.159
stem2leaf	-0.159
sepal_12w	-0.149
petal_12w	0.131
anther	-0.099
anther2filament	0.074
sepal2petal	-0.061
pedice2lsepal	-0.053
stem2pedicel	0.046
<i>anther2ovary</i>	-0.036
stigma	0.014
stigma2anther	0.008
<i>filament2ovary</i>	0.005

Table D.12. Wilks lambda illustrating the significance of the functions calculated for the taxa of the Virginianum Cluster of the Pusillum Complex.

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	0.019	167.740	81	0
2 through 3	0.105	95.806	52	0
3	0.464	32.664	25	0.140

Table D.13. Eigenvalues and % variance for the functions used in separating the taxa of the Virginianum Cluster of the Pusillum Complex showing the amount of variation attributed to each function.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	4.433	49.2	49.2	0.903
2	3.418	37.9	87.2	0.880
3	1.157	12.8	100.0	0.732

Table D.14. Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the functions used in separating the taxa of the Virginianum Cluster of the Pusillum Complex. The largest values for each function are bold, and the single largest positive and negative coefficients are *bold-italic*.

character	Function		
	1	2	3
stem	-0.965	0.465	-0.002
leaf length	-2.736	-2.212	1.048
leaf width	3.329	2.856	-0.436
pedicel	2.844	0.612	2.224
sepal length	-2.388	1.944	-4.701
sepal width	-1.191	-3.691	-1.167
petal length	4.155	-2.801	4.753
petal width	-3.283	4.546	-0.470
filament	-0.524	-1.858	-1.510
anther	-0.170	1.160	0.049
ovary	-1.604	0.833	2.508
style	3.011	2.077	0.672
stigma	1.308	-5.410	-1.828
ratio: stem 2 leaf	0.329	-0.609	-0.730
ratio: leaf length 2 width	2.606	1.662	-0.909
ratio: stem 2 pedicel	0.499	-0.875	-0.193
ratio: sepal length 2 width	-1.141	-2.240	-0.796
ratio: pedicel 2 sepal	-2.440	-1.228	-2.291
ratio: petal length 2 width	-2.161	3.218	-0.354
ratio: sepal 2 petal	1.316	-0.225	3.915
ratio: anther 2 filament	0.786	-0.167	-1.712
ratio: ovary 2 stigma	0.661	-1.505	-0.726
ratio: stigma 2 anther	-0.872	4.394	0.748
ratio: anther 2 ovary	-2.119	-0.290	2.385
ratio: stigma 2 style	1.884	2.398	1.796
ratio: stamen 2 pistil	2.803	1.760	0.478

Table D.15. Structure matrix indicating the correlations of each character to the functions used in separating the taxa of the Virginianum Cluster of the Pusillum Complex. Variables in *bold italic* were not used in the analysis.

character	function		
	1	2	3
leaf_width	0.349	0.131	0.106
ovary2stigma	0.334	-0.029	-0.248
ovary	0.253	-0.032	0.135
stem2pedicel	0.250	-0.088	0.016
leaf_l2w	-0.219	-0.118	0.171
filament	0.192	-0.146	0.048
style	0.190	-0.159	0.122
stigma2anther	-0.176	0.074	0.081
sepal_width	0.171	-0.020	0.146
pedicel	-0.141	0.095	-0.049
petal_width	0.103	-0.046	0.103
stamen2pistil	0.073	-0.026	-0.059
stem	-0.065	0.018	0.009
sepal_l2w	-0.091	-0.121	0.108
anther	0.268	-0.185	0.438
sepal_length	0.102	-0.097	0.367
sepal2petal	-0.017	-0.038	0.323
stigma	-0.088	-0.043	0.308
anther2filament	0.022	-0.007	0.305
stem2leaf	-0.051	0.017	-0.302
leaf_length	-0.008	-0.012	0.298
<i>stamen</i>	0.281	-0.202	0.286
<i>pistil</i>	0.161	-0.123	0.283
anther2ovary	-0.026	-0.119	0.234
pedice2lsepal	-0.154	0.157	-0.169
petal_length	0.130	-0.097	0.158
stigma2style	-0.122	0.101	0.152
filament2ovary	-0.067	-0.076	-0.088
petal_l2w	-0.014	0.019	-0.059

Table D.16. Wilks lambda illustrating the significance of the functions calculated for the taxa of the Pusillum Complex.

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 9	0.006	786.608	225	0
2 through 9	0.049	456.308	192	0
3 through 9	0.105	340.988	161	0
4 through 9	0.210	236.739	132	0
5 through 9	0.335	165.529	105	0
6 through 9	0.511	101.643	80	0.052
7 through 9	0.688	56.549	57	0.492
8 through 9	0.804	33.050	36	0.610
9	0.924	11.926	17	0.805

Table D.17. Eigenvalues and % variance for the functions used in separating the taxa of the Pusillum Complex showing the amount of variation attributable to each function. First 9 canonical discriminant functions were used in the analysis even though only the first 5 were significant.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	7.848	66.2	66.2	0.942
2	1.141	9.6	75.9	0.730
3	0.990	8.4	84.2	0.705
4	0.600	5.1	89.3	0.612
5	0.525	4.4	93.7	0.587
6	0.347	2.9	96.6	0.507
7	0.168	1.4	98.0	0.379
8	0.150	1.3	99.3	0.361
9	0.082	0.7	100.0	0.275

Table D.18. Standardized canonical discriminant function coefficients indicating the relative importance of the characters to the functions used in separating the taxa of the *Pusillum* Complex. The largest values for each function are bold, and the single largest positive and negative coefficients are *bold-italic*.

character	Function				
	1	2	3	4	5
stem	0.349	0.471	0.212	-1.110	0.378
leaf length	-0.748	0.616	-2.163	1.600	-0.828
leaf width	0.261	-1.006	1.980	-0.697	-0.599
pedicel	-1.211	0.398	-0.632	-0.260	-1.354
sepal length	1.170	0.513	1.645	-4.429	1.169
sepal width	-0.123	0.612	-0.568	1.005	1.019
petal length	0.081	-0.204	-0.927	3.613	-0.002
petal width	0.134	-0.562	0.293	-1.209	-0.501
filament	-0.920	1.488	-1.352	1.157	1.834
anther	1.966	2.937	-2.237	1.011	0.613
ovary	0.051	-0.458	0.282	-0.054	-0.714
style	-0.300	0.707	-0.273	0.158	0.544
stigma	-0.334	0.065	-0.090	-0.053	1.675
stamen	-0.297	-3.288	3.278	-1.448	-2.527
ratio: stem 2 leaf	-0.250	-0.171	-0.194	0.618	-0.451
ratio: leaf length 2 width	0.429	-1.438	1.529	-0.550	0.192
ratio: stem 2 pedicel	-0.327	0.112	0.456	0.247	0.335
ratio: sepal length 2 width	-0.235	0.550	-0.386	0.492	0.400
ratio: pedicel 2 sepal	1.699	-0.214	0.751	0.323	1.150
ratio: petal length 2 width	-0.152	-0.359	0.217	-0.694	-0.587
ratio: sepal 2 petal	0.008	-0.698	-0.616	2.940	-0.505
ratio: anther 2 filament	-1.467	-0.192	-0.217	0.535	0.679
ratio: ovary 2 stigma	0.067	0.146	0.730	-0.128	-0.087
ratio: stigma 2 anther	0.492	-0.354	0.406	-0.070	-1.271
ratio: stigma 2 style	-0.089	0.400	-0.168	0.391	-0.392

Table D.19. Structure matrix indicating the correlations of each character to the functions used in separating the taxa of the Pusillum Complex. Variables in *bold italic* were not used in the analysis.

character	function				
	1	2	3	4	5
pedice2lsepal	0.701	-0.004	0.140	0.120	-0.141
pedicel	0.590	0.313	0.060	0.086	-0.185
stem2pedicel	-0.471	0.047	0.460	0.182	0.090
petal_l2w	-0.282	-0.061	-0.108	-0.047	-0.061
leaf_width	0.130	0.531	0.147	0.179	-0.306
petal_length	0.165	0.522	-0.011	-0.058	0.199
leaf_l2w	0.031	-0.522	-0.087	-0.015	0.313
sepal_length	0.203	0.499	-0.032	0.096	0.054
stamen	0.116	0.451	0.138	0.255	0.135
stem	0.123	0.418	-0.092	-0.088	-0.145
style	0.038	0.418	0.097	-0.023	0.210
<i>pistil</i>	0.098	0.410	0.058	0.117	0.105
petal_width	0.314	0.408	0.075	-0.097	0.135
sepal_width	0.242	0.388	0.045	0.111	-0.036
filament	-0.082	0.291	0.211	0.157	0.110
ovary2stigma	-0.034	0.115	0.581	-0.013	-0.075
stigma	0.070	0.178	-0.224	0.160	0.087
sepal2petal	0.112	0.114	-0.037	0.338	-0.159
<i>anther2ovary</i>	0.028	0.121	-0.109	0.012	0.135
sepal_l2w	-0.151	0.023	-0.123	-0.026	0.140
<i>stamen2pistil</i>	-0.066	-0.070	0.037	0.066	-0.022
stigma2anther	-0.049	-0.043	-0.252	-0.056	-0.047
stem2leaf	-0.043	0.247	-0.067	-0.260	-0.108
leaf_length	0.163	0.210	-0.043	0.211	-0.090
stigma2style	0.037	-0.084	-0.169	0.196	-0.140
anther2filament	0.196	0.175	-0.059	0.133	0.053
anther	0.179	0.386	0.057	0.227	0.104
<i>filament2ovary</i>	-0.184	-0.016	-0.127	-0.054	0.150
ovary	0.101	0.217	0.294	0.175	-0.136

VITA

Susan Baker Farmer was born in Chattanooga, Tennessee on 30 July 1953 and moved to Knoxville approximately two years later where she has lived ever since. She graduated 16th in her class from Halls High School in June, 1971 and entered the University of Tennessee that fall. She graduated in 1978 with a B.A. in Computer Science and worked for a reliability assessment and risk engineering firm for a number of years before. She is married to John Farmer, Jr. of Clinton, Tennessee and the mother of Thomas. After having spent many years with the spring flora of the region, she entered the Master's program at the University of Tennessee where she attended graduate classes, carried out research, and taught Biology for non-majors. She was awarded her Masters of Science in Botany in May, 2000. She continued her studies on *Trillium* and Trilliaceae and was awarded her Doctor of Philosophy in Botany in August of 2007. She has accepted a position with Abraham Baldwin Agricultural College in Tifton, GA.

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