

A Phylogenetic Study of *Oxypolis* and *Ptilimnium* (Apiaceae) Based on Nuclear rDNA ITS Sequences

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Abstract—*Oxypolis* and *Ptilimnium* are two endemic genera of North America within tribe Oenantheae (Apiaceae subfamily Apioideae). Both genera are small, with 12 species currently recognized. Some members of each genus share an unusual leaf morphology. Rather than having the pinnately compound leaves that generally characterize apioid umbellifers, they have highly reduced, linear, terete, hollow, septate appendages known as rachis leaves. It has long been questioned whether the species with rachis leaves should be placed in separate genera. In this study, we use data from the internal transcribed spacer region of nuclear ribosomal DNA to explore relationships within and between these genera. A total of 147 internal transcribed spacer sequences were obtained from multiple accessions of all species of *Oxypolis* and *Ptilimnium* and for several other genera from tribe Oenantheae. These included *Lilaeopsis*, which also has rachis leaves, and *Cynosciadium* and *Limnosciadium*, which have rachis-like leaves. These sequence data were analyzed using maximum parsimony, maximum likelihood, and Bayesian methods. The results from each of these analyses were congruent and suggest that neither *Oxypolis* nor *Ptilimnium* as presently defined is monophyletic. *Oxypolis* and *Ptilimnium* are each separated into two clades according to leaf morphology. The rachis-leaved *Oxypolis* species are provisionally recognized as the genus *Tiedemannia* and the rachis-leaved *Ptilimnium* species are provisionally recognized as the genus *Harperella*, pending further investigation. The relationships among these four clades and the genera *Cynosciadium*, *Daucosma*, and *Limnosciadium* are not clear. Nevertheless, it appears that the rachis-leaf habit has evolved multiple times in the tribe. Geographic structure is apparent in the phylogenetic trees and, pending further study, may suggest new taxa; in addition, the presence of *O. occidentalis* on the Queen Charlotte Islands, well-separated from other populations of this species, suggests that it may have survived the last glaciation in a refugium in this area.

Keywords—Oenantheae, Queen Charlotte Islands, rachis leaves, refugium.

Oxypolis Raf. and *Ptilimnium* Raf. are two closely related genera of tribe Oenantheae (Apiaceae subfamily Apioideae) endemic to North America. Both genera are small, with a total of 12 species currently recognized (Table 1). *Oxypolis canbyi* and *Ptilimnium nodosum* are federally endangered. These genera share several traits with other members of tribe Oenantheae, including glabrous leaves and stems, fascicled roots, and a preference for moist or wet habitats. The plants are of particular interest because some members of each genus exhibit a unique morphological trait known as rachis leaves, while others have compound leaves typical of subfamily Apioideae. Rachis leaves are linear, terete, hollow, and septate and are equivalent to the rachis of a pinnately compound leaf in which the pinnae are not fully expressed. Instead, these pinnae are highly reduced and are transformed into nodal appendages that function as hydathodes (Kaplan 1970). Rachis leaves are an adaptation to a semiaquatic habitat, and species of *Oxypolis* and *Ptilimnium* with rachis leaves (i.e. *O. canbyi*, *O. filiformis*, *O. greenmanii*, and *P. nodosum*) spend much of the growing season at least partially submerged. Compound-leaved species of *Oxypolis* have pinnately or ternately compound leaves with pinnae that are generally somewhat broad (except for *O. ternata* in which they are long and narrow). Compound-leaved *Ptilimnium* species have finely dissected, pinnately decomposed leaves with linear or filiform pinnae.

The first *Oxypolis* species with compound leaves to be described was originally placed in the genus *Sium* L. (as *Sium rigidius* L.; Linnaeus 1753). The first described *Oxypolis* species with rachis leaves (now called *Oxypolis filiformis*) was originally placed in the genus *Oenanthe* L. (Walter 1788). Subsequently, the latter was grouped with *Sium rigidius* under the name *Sium teretifolium* Elliott. Although Elliott (1817) stated that “this plant is remarkable for its terete, fistulose leaves,” he grouped it with the compound-leaved species

based on features of the fruit. Similarly, Rafinesque (1825) included taxa with both leaf types in his new genus *Oxypolis*. In contrast, de Candolle (1829) advocated the separation of the rachis-leaved species (*Tiedemannia* DC.) from the compound-leaved taxa (*Archemora* DC.) based on leaf morphology and anther attachment; this classification, however, was never widely accepted. Coulter and Rose (1887) argued that *Tiedemannia* and *Archemora* should not be considered separate genera because they could find no significant differences in fruit characters between them. They believed that the leaves of *Oxypolis ternata* (which are long, narrow, and ternate) were intermediate between compound and rachis leaves. The fruits of all species of *Oxypolis* are remarkably similar in appearance, being dorsally flattened and winged. However, dorsal flattening and wing formation have evolved in several independent lineages of Apiaceae, most likely as a dispersal mechanism (Theobald 1971; Plunkett et al. 1996; Downie et al. 1998; Lee et al. 2001). In addition, dorsal flattening and wing formation can develop in different ways (Theobald 1971), therefore such similarities in fruit morphology cannot be taken as evidence of close relationship without further study.

The genus *Ptilimnium* was first described by Rafinesque (1819, 1825) based on *Ptilimnium capillaceum*, a compound-leaved species. The genus *Harperella* Rose was described by Rose (1906) to include three rachis-leaved taxa (*H. nodosa*, *H. fluviatilis*, and *H. vivipara*). In 1936, however, these species were referred to the genus *Ptilimnium* by Mathias (1936) who argued that *Harperella* differed from *Ptilimnium* “only in its striking vegetative character of leaves reduced to fistulose petioles, its less conspicuous involucre and involucre bracts, and its more attenuated petal tips; characters which cannot be considered generic.” Easterly (1957a) studied *Ptilimnium* by examining morphology, seedling development, and chromosome number. He agreed with Mathias’s assessment and

TABLE 1. Currently recognized species of *Oxypolis* and *Ptilimnium* and their distributions according to Kartesz (1999). *Ptilimnium ahlesii* was described by Weakley and Nesom (2004). The two putative interspecific hybrids are not listed.

<i>Oxypolis canbyi</i> (J. M. Coult. & Rose) Fernald – Delaware, Maryland, Georgia, North Carolina, South Carolina. <i>O. fendleri</i> (A. Gray) A. Heller – Arizona, Colorado, New Mexico, Utah, Wyoming. <i>O. filiformis</i> (Walter) Britton – Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Texas. <i>O. greenmanii</i> Mathias & Constance – Florida. <i>O. occidentalis</i> J. M. Coult. & Rose – California, Oregon, British Columbia (Canada). <i>O. rigidior</i> (L.) Raf. – Alabama, Arkansas, District of Columbia, Delaware, Florida, Georgia, Iowa, Illinois, Indiana, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Missouri, Mississippi, North Carolina, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, Wisconsin, West Virginia, Ontario (Canada). <i>O. ternata</i> (Nutt.) A. Heller – Florida, Georgia, North Carolina, South Carolina, Virginia.
<i>Ptilimnium ahlesii</i> Weakley & G. L. Nesom – Georgia, North Carolina, South Carolina. <i>P. capillaceum</i> (Michx.) Raf. – Alabama, Arkansas, Connecticut, District of Columbia, Delaware, Florida, Georgia, Kansas, Kentucky, Louisiana, Massachusetts, Missouri, Mississippi, North Carolina, New Jersey, New York, Pennsylvania, Puerto Rico, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Virginia. <i>P. costatum</i> (Elliott) Raf. – Alabama, Arkansas, Georgia, Illinois, Kentucky, Louisiana, Missouri, North Carolina, Oklahoma, Tennessee, Texas. <i>P. nodosum</i> (Rose) Mathias – Alabama, Arkansas, Georgia, Maryland, North Carolina, South Carolina, Virginia, West Virginia. <i>P. nuttallii</i> (DC.) Britton – Alabama, Arkansas, Illinois, Kansas, Kentucky, Louisiana, Missouri, Mississippi, Oklahoma, Tennessee, Texas.

recognized five species and one putative hybrid within the genus: *P. nodosum*, *P. fluviatile* (Rose) Mathias, *P. capillaceum*, *P. nuttallii*, *P. costatum*, and *P. × texense* J. M. Coult. & Rose. The latter was considered a hybrid between *P. capillaceum* and *P. nuttallii*. Easterly (1957b) synonymized *P. viviparum* (Rose) Mathias with *P. fluviatile* based on their shared habitat type, phenology, and lack of clear morphological differences. Kral (1981) further grouped the taxa and included *P. fluviatile* and *P. viviparum* within *P. nodosum* sensu lato (s.l.). Later, *P. nodosum* s.l. was recognized as a federally endangered species (U.S. Fish and Wildlife Service 1988). *Ptilimnium ahlesii* was recognized in 2004 (Weakley and Nesom 2004). This entity had generally been assumed under *P. capillaceum*, but Weakley and Nesom believed it was distinctive enough to warrant species status, based primarily on fruit size and flowering phenology.

Several other genera within tribe Oenantheae possess rachis or rachis-like leaves. All species of *Lilaeopsis* Greene have rachis leaves, although in some species the leaves are flattened distally (Affolter 1985). In *Lilaeopsis*, the leaves are linear, septate, terete, and hollow, and similar to those of rachis-leaved *Oxypolis* and *Ptilimnium* species. A study by Kaplan (1970) demonstrated that the leaves of *Lilaeopsis occidentalis* J. M. Coult. & Rose and *Oxypolis greenmanii* follow the same developmental pathways and concluded, “morphologically [*Oxypolis greenmanii*] is essentially a gigantic form of *Lilaeopsis*.” *Cynosciadium* DC. and *Limnoscium* Mathias & Constance have basal leaves that are linear to linear-lanceolate, entire, and septate, however, they are flattened throughout and are not terete or hollow. Moreover, their cauline leaves are generally palmately and pinnately lobed, respectively. We refer to the leaves of *Cynosciadium* and *Limnoscium* as rachis-like. It is not known whether these leaves are truly rachis leaves because anatomical and developmental studies have yet to be carried out for these taxa.

Hardway et al. (2004) recognized a strongly-supported monophyletic group within tribe Oenantheae as the North American Endemics (NAE) clade. This group comprised single exemplars from the genera *Atrema* DC., *Cynosciadium*, *Daucosma* Engelm. & A. Gray ex A. Gray, *Lilaeopsis*, *Limnoscium*, *Neogoezia* Hemsl., *Ptilimnium*, and *Trepocarpus* Nutt. ex DC. The genus *Ptilimnium* was represented by *P. capillaceum*, a compound-leaved species. Hardway et al. (2004) acknowledged, however, that there were taxa outside of this clade that are also endemic to North America, such as *Oxypolis* which was represented by three compound-leaved species (*O. fendleri*, *O. occidentalis*, and *O. rigidior*). The relationship between the NAE clade and *Oxypolis* was unclear because of short branches and weak bootstrap support. Additional sampling and analyses are necessary to evaluate the putative close relationship between *Lilaeopsis occidentalis* and *Oxypolis greenmanii*, as suggested by leaf morphology, as well as the relationships among the compound- and rachis-leaved species of *Oxypolis* and *Ptilimnium*.

The major purpose of this study is to produce a phylogenetic hypothesis for *Oxypolis* and *Ptilimnium* to assess their monophyly and to explore infrageneric relationships, especially with regard to the placement of rachis-leaved taxa relative to their compound-leaved congeners. The relationships of rachis-leaved *Oxypolis* and *Ptilimnium* to other genera of tribe Oenantheae exhibiting rachis or rachis-like leaves (i.e. *Cynosciadium*, *Lilaeopsis*, and *Limnoscium*) will also be assessed. The phylogenetic hypothesis has been reconstructed using sequences of the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region. Although the utilization of ITS data has come under scrutiny of late (Álvarez and Wendel 2003), it is widely employed and the benefits of its use in Apiaceae at low taxonomic levels have recently been elaborated (Spalik and Downie 2006).

MATERIALS AND METHODS

Taxon Sampling and Outgroup Selection—Complete sequences of the nrDNA ITS region were obtained for all seven species of *Oxypolis* (70 accessions), all five species of *Ptilimnium* (64 accessions), and the two purported hybrids, “*Oxypolis × intermedia*” ined. and *P. × texense* (Table 1). “*Oxypolis × intermedia*” ined. is not a validly published name, but represents herbarium specimens collected by Walter Judd (University of Florida, Gainesville) which were recognized as putative hybrids between *O. greenmanii* and *O. filiformis* (Judd 1982) and annotated as “intermediate between *O. greenmanii* and *O. filiformis*.” *Ptilimnium texense* was originally described as a species (Coulter and Rose 1909), but Mathias and Constance (1945) and Easterly (1957b) treated it as a hybrid (*P. × texense*) between *P. capillaceum* and *P. nuttallii*. *Ptilimnium × texense* is used in this study to represent herbarium specimens that were given this designation. All *Oxypolis* and *Ptilimnium* taxa were represented by multiple accessions to assess infraspecific variation. Thirteen accessions from eight other oenantheid genera were also included, for a total of 147 accessions. Taxa sampled, taxonomic authorities, voucher information, and GenBank accession numbers are given in Appendix 1. Nomenclature follows Kartesz (1999), except for *Ptilimnium ahlesii* (Weakley and Nesom 2004), *Atrema americanum* (Hardway et al. 2004), and *Lilaeopsis* (Affolter 1985).

Previous phylogenetic studies of tribe Oenantheae (Plunkett et al. 1996; Downie et al. 1998; Downie et al. 2000a; Downie et al. 2000b) have shown the genus *Perideridia* Rchb. to be a sister group to a clade comprising all other members of the tribe; therefore, all trees were rooted with three species of *Perideridia* [*P. americana* (Nutt. ex DC.) Rchb., *P. gairdneri* (Hook. & Arn.) Mathias subsp. *borealis* T. I. Chuang & Constance, and *P. kelloggii* (A. Gray) Mathias]. In addition, the genera *Atrema*, *Cynosciadium*, *Daucosma*, *Lilaeopsis*, *Limnoscium*, *Neogoezia*, and *Trepocarpus* allied with compound-leaved *Ptilimnium* within the NAE clade (Hardway et al. 2004). As such, single accessions of these genera were included in the phylogenetic analyses to explore their placements relative to *Oxypolis* and *Ptilimnium*. Some of these genera have rachis leaves (at least basally),

permitting an assessment of the relationships among the rachis-leaved species.

DNA Extraction, Purification, and Sequencing—Details of DNA extraction, PCR-amplification, purification, and sequencing of previously published ITS sequences are provided elsewhere (Downie and Katz-Downie 1996; Downie et al. 2000a; Hardway et al. 2004). Methods for sequences generated specifically for this study are as follows. Leaf material was taken from either herbarium specimens or field-collected, silica-dried samples. DNA was isolated using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, California) according to the manufacturer's instructions. The entire ITS region (ITS-1, 5.8S rDNA, and ITS-2) was amplified using primers 18S-ITS1-F (Spalik and Downie 2006) and C26A (Wen and Zimmer 1996) or primers 18S-for (5'-GTC CAC TGA ACC TTA TCA TTT AG-3') and C26A. The PCR amplification methods used are described elsewhere (Downie et al. 2000a). PCR products (templates) were purified using either a QIAquick Gel Extraction Kit or a QIAquick PCR Purification Kit (Qiagen Inc.) following manufacturer's instructions. Sequence reactions were carried out using an ABI Prism Big Dye Terminator vers. 3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, California). Sequence reaction products were visualized using an ABI 3730XL high-throughput DNA capillary sequencer at the Genetic Engineering Facility at the University of Illinois at Urbana-Champaign's Biotechnology Center.

Sequence Alignment and Phylogenetic Analysis—Sequences were aligned using Clustal X (Thompson et al. 1997) and manually adjusted as necessary using the alignment editor BioEdit (Hall 1999). Gaps were scored as additional binary characters according to the "simple indel coding" method of Simmons and Ochoterena (2000). Therefore, two matrices were constructed, one that included the aligned nucleotide data and binary-coded indels (combined dataset) and one that omitted the indel characters (nucleotide only dataset). To facilitate analysis, identical sequences were represented by single terminals, except where identical sequences were from individuals from geographic areas of interest (Table 2). Sequence characteristics were obtained for the entire ITS region, as well as for the separate partitions (ITS-1, 5.8S, and ITS-2). Uncorrected pairwise nucleotide distances were calculated using the distance matrix option of PAUP* version 4.0b10 (Swofford 2003). Only positions that were aligned unambiguously were included in the analyses. The aligned data matrix is available in TreeBASE (study number S1939).

Maximum parsimony (MP) analyses of the two data matrices were implemented in PAUP*. All characters were treated as unordered and all character transformations were weighted equally. Heuristic maximum parsimony searches were replicated 1000 times with random addition of taxa and the following options in effect: MULTREES, TBR branch swapping, gaps treated as missing data. Bootstrap (BS) analyses were done on both data sets to assess clade support. One thousand bootstrap replicates were performed using the heuristic search option, with TBR branch swapping, random stepwise addition of taxa, and MULTREES options in effect. In addition, MP analyses were performed on data sets that both included and excluded the putative hybrids ("Oxypolis × intermedia" ined. and *P. × texense*). This was done to assess whether the inclusion of putative hybrids had any effect on the placement of other taxa in the phylogenetic trees. Alternative alignments were also analyzed to assess their effects on the phylogenetic hypothesis. These included alignments created by employing a range of weighting schemes for gap opening and gap extension in Clustal, as well as alignments created by making different manual adjustments to the Clustal-generated alignments. The constraint option of PAUP* was used to determine how many additional steps were needed to force the monophyly of particular groups.

Modeltest version 3.7 (Posada and Crandall 1998) was used to select an appropriate evolutionary model of nucleotide substitution for these ITS sequences. The model that best fit these data, as selected by the Akaike Information Criteria (AIC) estimator, was used. The parameters of this model were input into PAUP* and a maximum likelihood (ML) analysis of the nucleotide only data matrix was implemented. One hundred heuristic searches were performed using random addition sequence and TBR branch swapping. Bootstrap analysis of 1000 replicates was conducted using the NJ method with ML distance estimates using the ML parameters inferred by Modeltest.

MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) was used to conduct Bayesian inference of the nucleotide only matrix. The same model of evolution used in the ML analysis was also used in this analysis. Two independent runs were conducted for 2,000,000 generations each with a sample frequency of 100. The log-likelihood scores of the sample points were plotted against generation time in Microsoft Excel. Stationarity was determined to be reached when the log-likelihood values of the

TABLE 2. A summary of accessions with identical ITS sequences that were represented by a single terminal accession in the phylogenetic analyses, except when sequences were from individuals from geographic areas of interest. Accession numbers in the second column are those identifying terminals in Figs. 1, 2. Accession numbers in the third column have identical ITS sequences to those presented in the second column. Voucher information for all accessions is provided in Appendix 1.

Taxon	Accession number presented in Figs. 1, 2	Accessions with identical sequences
<i>Oxypolis canbyi</i>	2745	2744, 2743
	2747	2746
<i>Oxypolis fendleri</i>	2759	2758
	2764	1509, 2350, 2351, 2353, 2369, 2762
<i>Oxypolis filiformis</i>	2356	2357, 2371
	2653	2652
	2783	2713
<i>Oxypolis occidentalis</i>	2753	1435
	2755	2929
	2898	2897
	2972	2359
<i>Oxypolis rigidior</i>	1653	1652, 1806, 1962
	2771	2768
<i>Oxypolis ternata</i>	2738	2736, 2739
	2741	2740
	2939	2735, 2737
	2940	2360
<i>Ptilimnium capillaceum</i>	2619	2618, 2627, 2628, 2704, 2794, 2795
<i>Ptilimnium costatum</i>	1651	1805
	2625	1503, 1504, 1514, 1646, 1648, 1649, 1650, 1801, 1802, 1970, 2402
	2904	2641
<i>Ptilimnium nodosum</i>	1981	2905, 2906, 2914
	2635	2784, 2785
	2786	2970
	2792	2787
	2900	2901
	2930	2791
	2934	2933
2936	2790	
<i>Ptilimnium nuttallii</i>	2623	2165, 2404, 2617, 2637, 2644, 2708

sample points achieved a stable equilibrium value. The initial 5000 trees from each run were discarded as burn in. A majority rule consensus tree was constructed from the remaining 30,000 trees using PAUP*.

RESULTS

Characteristics of ITS Sequences—Sequence characteristics of the ITS region are provided in Table 3. The length of the entire ITS region for the 83 terminals (147 accessions) included in the phylogenetic analysis ranged from 580–605 bp. Alignment of these sequences resulted in a matrix of 632 positions. Thirty-five positions were removed because of alignment ambiguities. The number of parsimony informative positions was 268 and the number of autapomorphic positions was 47. Alignment of the remaining 597 unambiguous positions required the inclusion of 34 gaps: 30 1-bp gaps, one 2-bp gap, one 3-bp gap, one 6-bp gap, and one 8-bp gap. Fifteen gaps were parsimony informative. The ITS-2 region is generally longer and has more potentially informative sites than the ITS-1 region, even though it has four times as many sites excluded because of ambiguities.

The maximum amount of sequence divergence across the entire ITS region for all 147 terminals was 22.7% (Table 3), which was between *Oxypolis canbyi* and one outgroup accession, *Perideridia americana*. Considering only *Oxypolis* and *Pti-*

TABLE 3. Sequence characteristics of the entire nuclear rDNA internal transcribed spacer region and partitioned ITS-1, 5.8S rDNA, and ITS-2 regions for 83 terminals (representing 147 accessions) used in the phylogenetic analyses.

Sequence characteristic	ITS-1	5.8S	ITS-2	Entire ITS region
Length variation (bp)	201–213	162–165	206–230	580–605
Aligned length (bp)	228	165	239	632
No. of excluded sites	6	2	27	35
No. of sites not variable	81	144	57	282
No. of sites autapomorphic	24	2	21	47
No. of sites parsimony informative	117	17	134	268
No. of unambiguous alignment gaps (indels)	22	1	11	34
No. of unambiguous alignment gaps parsimony informative	7	0	8	15
Maximum pairwise sequence divergence (%):				
All 83 terminals	30.6	5.5	34.4	22.7
<i>Oxyopolis</i> and <i>Ptilimnium</i>	27.1	5.5	29.1	20.7
<i>Oxyopolis</i> only (all, compound-leaved, rachis-leaved)	27.1, 13.0, 10.6	5.5, 2.5, 1.2	29.1, 9.3, 15.9	20.7, 8.5, 9.9
<i>Ptilimnium</i> only (all, compound-leaved, rachis-leaved)	17.0, 4.6, 1.0	3.1, 1.2, 0.0	23.7, 5.3, 1.0	14.9, 3.5, 0.7

limnium, the maximum amount of sequence divergence was 20.7% between *O. fendleri* (a compound-leaved taxon) and *O. canbyi* (a rachis-leaved taxon). The maximum sequence divergence among compound-leaved *Oxyopolis* species was 8.5%, whereas among rachis-leaved *Oxyopolis* species it was 9.9%. Maximum sequence divergence among *Ptilimnium* species only was 14.9%; however, it was just 3.5% among compound-leaved *Ptilimnium* and 0.7% among the rachis-leaved *Ptilimnium*. All species of *Ptilimnium* and *Oxyopolis* showed infra-specific variation in their ITS sequences, except for *P. ahlesii*, *P. capillaceum*, and *P. nuttallii* which showed no variation. All sequences of *P. ahlesii* were identical to all sequences of *P. capillaceum*. Sequence heterogeneity was detected in all accessions of *O. greenmanii* and one of two accessions of “*Oxyopolis* × *intermedia*” ined. These sequences exhibited a C/T polymorphism at position 156 (ITS-1) and an A/G polymorphism at position 537 (ITS-2) in the aligned matrix. None of the accessions of *O. filiformis*, the other putative parent of “*Oxyopolis* × *intermedia*” ined., were polymorphic at these loci, but all had either a C or T at position 156 and either an A or G at position 537.

Phylogenetic Analysis—MP analysis of ITS sequence data (nucleotide only dataset) recovered 350 trees of 843 steps each (CI = 0.5475, without uninformative characters; RI = 0.9173). The strict consensus of these trees is presented with accompanying bootstrap values (MPBS; Fig. 1). MP analysis of the combined dataset (aligned nucleotide data plus 15 parsimony informative binary-scored indels) recovered 353 trees of 864 steps each (CI = 0.5519, without uninformative characters; RI = 0.9181). The strict consensus tree resulting from analysis of combined data was almost identical to that from the previous analysis, with the only difference being the union of *P. nodosum* 2789 and *P. nodosum* 2786 in the former. Bootstrap support values were either identical or within a few percentage points in both analyses; therefore, the combined dataset will not be considered further. Changes to the alignment resulting from employing a variety of costs for gap opening and gap extension or by making different manual adjustments to the Clustal-generated alignments resulted in strict consensus trees similar to the one presented in Fig. 1, with no differences in the five major clades inferred (discussed below).

The ML analysis was carried out using the SYM + I + G model of nucleotide substitution (Akaike weight = 0.9960). Base frequencies were: A = 0.22790, C = 0.25304, G = 0.28357, T = 0.23549. Substitution rates were: A–C = 0.6534, A–G =

1.9859, A–T = 1.2774, C–G = 0.4769, C–T = 4.5628, G–T = 1.000. The assumed proportion of invariable sites was 0.2743 and the gamma distribution shape parameter was 1.4138. ML analysis resulted in 38 trees, each with a $-\ln$ likelihood score of 4962.61109. The strict consensus of these 38 trees is presented in Fig. 2. The majority-rule consensus tree of the 30,000 trees derived from Bayesian analysis is congruent with the ML tree, therefore both bootstrap (MLBS) and posterior probability (PP) values, the latter also expressed as percentages, are given on the ML tree.

The trees resulting from MP, ML and Bayesian analyses are nearly fully congruent except for the placements of the *Limnosciadium pinnatum*/*Daucosma laciniatum* clade and *Cynosciadium digitatum* (cf. Figs. 1, 2). However, the placements of these taxa are not strongly supported and if these weakly supported branches are collapsed the trees become fully congruent. All trees show four major clades which correspond to compound-leaved *Ptilimnium* species (Clade 1), rachis-leaved *Ptilimnium* species (Clade 2), rachis-leaved *Oxyopolis* species (Clade 3), and compound-leaved *Oxyopolis* species (Clade 4). Each of these clades is strongly supported by MP and ML BS and Bayesian PP values (97–100%). The compound-leaved and rachis-leaved *Oxyopolis* species clearly do not form a monophyletic group. A tree of 36 additional steps (879 steps) is required to force the monophyly of *Oxyopolis*. The compound-leaved and rachis-leaved *Ptilimnium* species may also not form a monophyletic group, and constraint analysis showed that a tree of three additional steps is required to force their monophyly (846 steps). Relationships among the compound-leaved *Ptilimnium*, rachis-leaved *Ptilimnium*, and rachis-leaved *Oxyopolis* clades are not resolved. These clades, together with *Limnosciadium pinnatum*, *Daucosma laciniatum*, and *Cynosciadium digitatum*, constitute a moderately (77% MPBS) to strongly (97% MLBS, 100% PP) supported group (Clade 5). In none of the analyses do the *Ptilimnium* clades and the rachis-leaved *Oxyopolis* clade ever form a monophyletic group without *Limnosciadium* and *Daucosma*.

Daucosma and *Limnosciadium* form a strongly supported monophyletic group in each analysis (100% BS and PP values). *Limnosciadium* and *Cynosciadium* have rachis-like basal leaves and compound cauline leaves and fall alongside the rachis-leaved and compound-leaved species of Clade 5. The four examined accessions of *Lilaeopsis* form a strongly supported monophyletic group that is clearly separate from *Oxyopolis greenmanii* and other rachis-leaved species with which it shares the rachis-leaf morphology. *Lilaeopsis* is sister

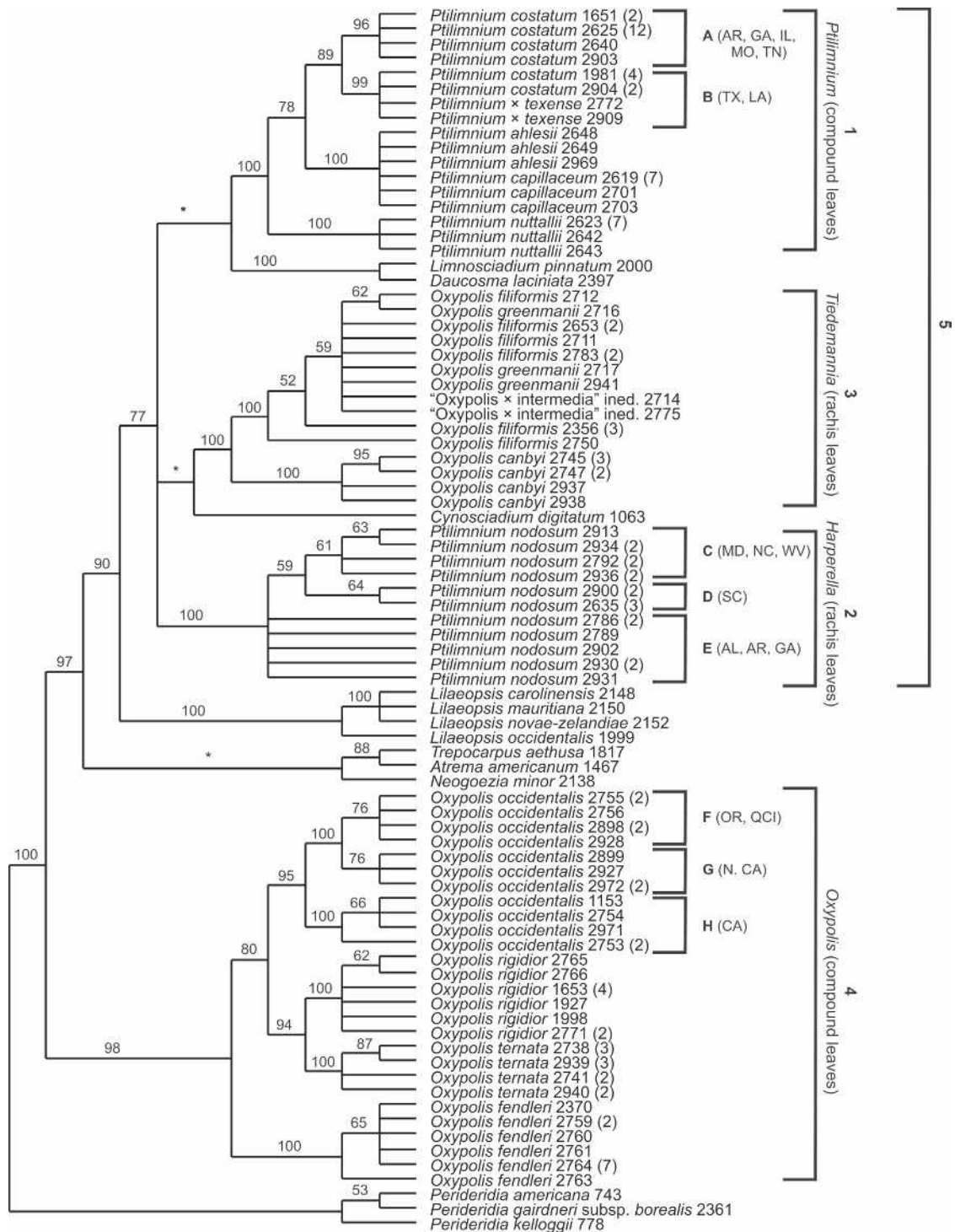


FIG. 1. Strict consensus of 350 minimal length 843-step trees derived from equally weighted maximum parsimony analysis of 147 ITS sequences represented by 83 terminals (CI = 0.5475, without uninformative characters; RI = 0.9173). The numbers in parentheses correspond to the number of identical ITS sequences represented by that terminal (Table 2). Numbers on branches represent bootstrap estimates; a value less than 50% is indicated with an asterisk. Five major clades are identified. These include the compound-leaved *Ptilimnium* species (Clade 1, *Ptilimnium*), the rachis-leaved *Ptilimnium* species (Clade 2, *Harperella*), the rachis-leaved *Oxypolis* species (Clade 3, *Tiedemannia*), and the compound-leaved *Oxypolis* species (Clade 4, *Oxypolis*). Clades 1–3 plus representatives from *Cynosciadium*, *Daucosma*, *Limnoscadium* comprise Clade 5. Subclades or groups A–H represent plants from the indicated geographic regions: AL (Alabama), AR (Arkansas), CA (California), GA (Georgia), IL (Illinois), LA (Louisiana), MD (Maryland), MO (Missouri), NC (North Carolina), N. CA (northern California), OR (Oregon), QCI (Queen Charlotte Islands, British Columbia), SC (South Carolina), TN (Tennessee), TX (Texas), and WV (West Virginia).

group to Clade 5 (90% MPBS, 94% MLBS, 100% PP), while the clade of *Atrema*, *Neogoezia*, and *Trepocarpus* is sister group to the aforementioned assemblage (97% MPBS, 96% MLBS, 100% PP).

Within the compound-leaved *Ptilimnium* clade (Clade 1), all accessions of *P. nuttallii* form a strongly supported monophyletic group (100% BS and PP values) that is sister group to the other compound-leaved *Ptilimnium* taxa. *Ptilimnium* *ahl-*



FIG. 2. Strict consensus of 38 trees resulting from maximum likelihood analysis of 147 ITS sequences represented by 83 terminals (-Ln likelihood score = 4962.61109). This tree was nearly identical to the majority-rule consensus tree of 30,000 trees derived from Bayesian analysis. The numbers in parentheses correspond to the number of identical ITS sequences represented by that terminal (Table 2). Numbers on branches represent bootstrap estimates and Bayesian posterior probability values, respectively; a value less than 50% is indicated with an asterisk. Clade and subclade descriptors and all abbreviations are the same as described in Fig. 1.

esii and *P. capillaceum*, which have identical sequences for all 12 accessions examined, form a monophyletic group. *Ptilimnium costatum* and *P. × texense* also form a monophyletic group (89% MPBS, 71 MLBS, 99% PP) which is divided into two strongly supported subclades: A, comprising all specimens of *P. costatum* from Arkansas, Georgia, Illinois, Missouri, and Tennessee (82% MPBS, 96% MLBS, 100% PP); and B, comprising *P. × texense* and all *P. costatum* specimens from

Texas and Louisiana (99% MPBS, 99% MLBS, 100% PP). The removal of the putative hybrid *P. × texense* from the MP analyses did not affect the relationships among the remaining taxa.

The rachis-leaved *Ptilimnium* clade (Clade 2) comprises all 19 accessions of *P. nodosum*. Some geographic structure is apparent within this clade. Subclade C is made up of specimens from Maryland, North Carolina, and West Virginia,

and subclade D is made up of specimens from South Carolina. Both subclades are moderately to weakly supported in the MP and ML analyses, as is their sister group relationship in all analyses. The remaining *P. nodosum* specimens are from Alabama, Arkansas, and Georgia (group E) and comprise part of a polytomy along with the branch leading to subclades C+D.

Three rachis-leaved species of *Oxypolis* plus the putative hybrid between *O. greenmanii* and *O. filiformis* ("Oxypolis × intermedia" ined.) comprise Clade 3. All seven accessions of *Oxypolis canbyi* form a strongly supported clade (100% BS and PP values) sister group to a clade comprising all accessions of *O. filiformis*, *O. greenmanii*, and "Oxypolis × intermedia" ined. (100% BS and PP values). There is no resolution among *O. filiformis*, *O. greenmanii*, and "Oxypolis × intermedia" ined. When "Oxypolis × intermedia" ined. is excluded from subsequent MP analyses, the relationships among the remaining taxa do not change.

Within the compound-leaved *Oxypolis* clade (Clade 4), *O. fendleri* (13 accessions) is a strongly supported sister group (98% MPBS, 97% MLBS, 100% PP) to the other compound-leaved *Oxypolis* species. In addition, *O. rigidior* (10 accessions) and *O. ternata* (10 accessions) form strongly supported monophyletic sister taxa that collectively form a clade that is sister group to *O. occidentalis* (15 accessions). *Oxypolis occidentalis* is further separated into three subclades showing some geographic structure: subclade F is made up of plants from the Queen Charlotte Islands, British Columbia and the Cascade Mountain region of Oregon; subclade G is made up of plants from the Cascade Range Foothills in northern California; and subclade H is made up of specimens from the Sierra Nevada, San Bernardino, White, and Inyo Mountains of California. The maximum pairwise sequence divergence between subclades F and G is 0.51% and between subclades F + G and subclade H it is 4.86%.

DISCUSSION

Generic Level Phylogenetic Relationships—Classifications separating *Ptilimnium* and *Oxypolis* into two genera each (i.e. *Ptilimnium* and *Harperella*, and *Oxypolis* and *Tiedemannia*) based on the presence of compound or rachis leaves have been proposed (Candolle 1829; Rose 1905, 1906), but this remarkably different leaf morphology has generally not been seen as a worthy character on which to base generic separation. Rather, similarities in fruits and flowers have been emphasized, resulting in the two genera recognized today. The ITS-based phylogenies clearly show that neither *Oxypolis* nor *Ptilimnium* as currently delimited is monophyletic. The four major clades recognized, comprising the compound-leaved *Ptilimnium* species (Clade 1), the rachis-leaved *Ptilimnium* species (Clade 2), the compound-leaved *Oxypolis* species (Clade 4), and the rachis-leaved *Oxypolis* species (Clade 3), coincide precisely with the four aforementioned genera. Each of these groups is strongly supported as monophyletic. The separation of the compound-leaved *Oxypolis* species from its rachis-leaved congeners is most dramatic, with trees of 36 steps longer than those maximally parsimonious required to force monophyly of the genus. Maximum pairwise sequence divergence values between these two *Oxypolis* clades is 20.7%. Differences between the clades are also apparent in chromosome number. The three rachis-leaved *Oxypolis* species are all $n = 14$, whereas the compound-leaved *Oxypolis*

species are $n = 16$ or $n = 18$ (Bell and Constance 1957; Bell and Constance 1960; Crawford and Hartman 1972; Tucker et al. 1983; Pimenov et al. 2003).

The separation of the rachis-leaved and compound-leaved *Ptilimnium* species is not as dramatic. Trees of three steps longer are required to force monophyly of *Ptilimnium* in the MP analysis, and the rachis-leaved *Oxypolis* clade is sister group to the compound-leaved *Ptilimnium* clade in both ML and Bayesian trees, although this relationship is very weakly supported. Maximum pairwise sequence divergence between *P. nodosum* and the compound-leaved *Ptilimnium* species is 14.9%, whereas it is just 3.5% among all compound-leaved species of *Ptilimnium* and 0.7% among the accessions of *P. nodosum*. The rachis-leaved species *P. fluviatile* and *P. nodosum* each have a chromosome number of $n = 6$, whereas the compound-leaved species have chromosome numbers of $n = 16$ (*P. costatum*), $n = 7$ (*P. capillaceum* and *P. nuttallii*), and $n = 8$ or $n = 14$ (*P. capillaceum*) (Easterly 1957a; Constance et al. 1976; Weakley and Nesom 2004).

On the basis of these molecular and cytological results and to facilitate communication, we provisionally treat members of the rachis-leaved *Oxypolis* clade as the genus *Tiedemannia*, a name proposed by de Candolle (1829). Members of the rachis-leaved *Ptilimnium* clade are provisionally recognized as the genus *Harperella*, as proposed by Rose (1906). Before nomenclatural changes are effected, however, additional evidence will be obtained from the independently evolving chloroplast genome. It is reassuring that the four major clades recognized herein are similar to those inferred based on preliminary analysis of chloroplast DNA *psbI-5' trnK*^(UUU) sequence data (S.R. Downie, unpubl. data).

The relationships among *Ptilimnium*, *Tiedemannia*, and *Harperella* are confounded by the placements of *Cynosciadium*, *Daucosma*, and *Limnoscium*. The positions of the latter three genera vary depending upon optimality criteria selected, and other than the strongly-supported sister group relationship between *Daucosma* and *Limnoscium*, no analysis resolves relationships among these taxa with strong branch support. The monotypic genus *Daucosma* was transferred to *Ptilimnium* as *Ptilimnium laciniatum* Kuntze (Kuntze 1891), but later returned to *Daucosma* based on morphology (Easterly 1957a). The placement of the *Daucosma* + *Limnoscium* clade as sister group to either *Ptilimnium* (Fig. 1) or *Harperella* (Fig. 2) suggests an affinity of these taxa with *Ptilimnium*, as traditionally circumscribed. Their differences in morphology, however, suggest that *Daucosma* and *Limnoscium* be maintained as separate genera, distinct from *Ptilimnium* and *Harperella*.

The polytomy and weak branch support at the base of Clade 5 in all phylogenetic trees preclude an hypothesis on the number of times the rachis-leaved habit evolved in tribe Oenanthae. Rachis leaves (being defined as linear, septate, terete, and hollow) occur in *Lilaeopsis*, *Tiedemannia*, and *Harperella*. They could have evolved just once in Oenanthae in the immediate ancestors of the *Lilaeopsis* clade + Clade 5, but this would require at least one, and possibly as many as three reversals back to the compound-leaved trait depending upon the reconstruction. Alternatively, rachis leaves could have evolved three times independently within the tribe. The latter scenario seems more likely given that rachis-leaves can also be found in the distantly related *Ottoea oenanthoides* Humb., Bonpl. & Kunth. of Apiaceae tribe Selineae (C.A. Danderson

& S. R. Downie, unpubl. data), as well as in other genera of Apiaceae (Affolter 1985).

Specific and Intraspecific Level Phylogenetic Relationships—*Ptilimnium nodosum* is a semiaquatic plant whose disjunct populations are ecologically distinct (Kress et al. 1994). Although presently recognized as one species (Kral 1981), evidence suggests that it may be a species complex (Kress et al. 1994; Bartgis 1997; Kartesz 1999). Prior to 1957, three species were recognized in the group (Easterly 1957a). Populations from Maryland, North Carolina, and West Virginia were given the name *P. viviparum*, while those from Alabama and Arkansas were called *P. fluviatile*. Only populations from South Carolina and Georgia were recognized as *P. nodosum* sensu stricto (s.s.). A study of isozyme variation found that there was substantial genetic differentiation among rather than within these three geographic groups, suggesting they might be separate, genetically-distinct species (Kress et al. 1994). Reveal and Broome (1982), upon examining morphological differences between *P. fluviatile* and *P. viviparum*, advocated that these should be recognized as *P. fluviatile* and *P. fluviatile* var. *viviparum* (Rose) Reveal & Broome. Kral (1981), however, also conducted a morphological study and concluded that *P. fluviatile*, *P. nodosum* s.s., and *P. viviparum* should be treated as one taxon.

In our study, all accessions of *P. nodosum* from Maryland, North Carolina, and West Virginia form a monophyletic group (subclade C). These plants were formally recognized as *P. viviparum*. This relationship is in accordance with the results of Kress et al. (1994) who showed that populations of *P. nodosum* from these three states were genetically more similar to each other than they were to populations from elsewhere. Further resolution within *Harperella* is not obtained, however. Five accessions from South Carolina also comprise a monophyletic group (subclade D), but other specimens previously attributable to *P. nodosum* s.s. occur at the base of *Harperella* alongside accessions referable to *P. fluviatile* from Alabama and Arkansas. *Ptilimnium nodosum* s.l. is listed as endangered by the U.S. Fish and Wildlife Service (1988) and the determination of boundaries among its constituent taxa will have an impact on management strategies implemented for its conservation.

Ptilimnium × *texense* was described as a distinct species (*P. texense* J. M. Coult. & Rose) by Coulter and Rose (1909), but was later considered to be a hybrid between *P. capillaceum* and *P. nuttallii* (Mathias and Constance 1945; Easterly 1957b) and then finally assumed under *P. costatum* (Mathias and Constance 1961). In the present study, the two specimens from Texas identified by Easterly as *P. × texense* and all specimens of *P. costatum* from Texas and Louisiana comprise a well-supported monophyletic group (*Ptilimnium*, subclade B). This clade is a sister group to subclade A, which is made up of *P. costatum* from Arkansas, Georgia, Illinois, Missouri, and Tennessee. *Ptilimnium* × *texense* allies with neither of its putative progenitors. Its close association with *P. costatum*, however, supports the work of Mathias and Constance (1961) where these plants were considered within this species. While our study was not set up to confirm the hybrid status of *P. × texense* and its parentage, it appears that *P. costatum* should be considered as one of its parents in future studies, if indeed it is a hybrid.

Collectively, subclade A (*P. costatum* from Arkansas, Georgia, Illinois, Missouri, and Tennessee) and subclade B (*P. ×*

texense and *P. costatum* from Texas and Louisiana) comprise a moderately to strongly supported group. Notable morphological differences, however, have been observed between specimens of subclades A and B. Style length and orientation are important characters which have been used to distinguish among species of *Ptilimnium* in numerous keys (Easterly 1957b; Mathias and Constance 1945, 1961). The length and orientation of styles of specimens of subclade A (0.5–1.0 mm in length and strongly recurved) are different from those of subclade B (1.0–3.0 mm long and spreading). *Ptilimnium nuttallii* also has styles that are between 0.5 and 1.0 mm in length and strongly recurved. In addition, plants from subclade B exhibit a unique leaf morphology. They have linear leaflets which are subterete and have no distinct midrib. The leaflets of *P. costatum* from subclade A, *P. nuttallii*, and *P. capillaceum* are all also linear, but they are flattened and have a distinct midrib. Future studies are planned to ascertain if these morphological differences warrant taxonomic recognition.

Ptilimnium capillaceum is the most common and wide-ranging species of *Ptilimnium*. All examined specimens have identical ITS sequences suggesting a recent and rapid radiation throughout its range. Moreover, these sequences are identical to those of *P. ahlesii*, further suggesting a very recent divergence of these species. Despite their identical ITS sequences, we maintain these species as distinct because of their morphological and phenological differences. *Ptilimnium ahlesii* has larger ovaries and fruits, while *P. capillaceum* has more umbellets per umbel and more flowers per umbellet (Weakley and Nesom 2004). In addition, *P. ahlesii* flowers earlier than *P. capillaceum*, although their flowering times do overlap (Weakley and Nesom 2004).

Neither *O. greenmanii* nor *O. filiformis* is monophyletic, but together they form a strongly supported monophyletic group with “*Oxypolis* × *intermedia*” ined., a putative hybrid of these two species. Sequence heterogeneity was detected in all accessions of *O. greenmanii* and one of the two accessions of “*Oxypolis* × *intermedia*” ined., but not in *O. filiformis*. Both *O. filiformis* and *O. greenmanii* share a chromosome number of $n = 14$, however, the chromosomes of *O. greenmanii* are much larger than those of *O. filiformis* (Bell and Constance 1957; Bell and Constance 1960). The larger chromosomes and ITS sequence polymorphisms of *O. greenmanii* suggest that this species could have been derived from *O. filiformis*. Chromosome number and size are not presently known for “*Oxypolis* × *intermedia*” ined. This putative hybrid is found in northwestern Florida where the ranges of *O. greenmanii* and *O. filiformis* overlap. “*Oxypolis* × *intermedia*” ined. exhibits intermediate morphological characters of the two species and are highly fertile, leading Judd (1982) to conclude that *O. greenmanii* should be recognized as a subspecies of *O. filiformis*.

All accessions of *O. occidentalis* form a monophyletic group, which is divided into three subclades that correspond to different geographic locations: F, the Cascade Mountains in Oregon and the Queen Charlotte Islands; G, the Cascade Range Foothills in northern California; and H, the Sierra Nevada, San Bernardino, White, and Inyo mountain ranges. The maximum pairwise sequence divergence between subclades F and G is 0.51%, while between subclades F+G and subclade H it is 4.86%. This latter value is greater than the maximum pairwise sequence divergence between *O. rigidior* and *O. ternata* (4.69%), suggesting that the accessions of subclade H may constitute a new species.

The Queen Charlotte Islands, located approximately 80 km

off the west coast of British Columbia, are an intriguing geographic area. During the last glacial maximum (ca. 15,000 yrs ago), when glaciers covered much of present-day British Columbia and extended into present-day Washington State, it has been proposed that much of the Queen Charlotte Islands remained ice-free (Heusser 1960; Lacourse et al. 2005) and provided a refugium to plants and animals living there. Studies of the threespine stickleback (O'Reilly et al. 1993), song sparrow (Zink and Dittman 1993), and black bear (Byun et al. 1997) have provided evidence for such a refugium. However, Brunfeld et al. (2001) reported that "there are no genetic data for plants that clearly support the Queen Charlotte Islands as a glacial refugium." *Oxypolis occidentalis* could potentially be such an example. The glacial refugium hypothesis may explain why *O. occidentalis* occurs on these islands and its highly disjunct geographic distribution. These Queen Charlotte Island populations are approximately 1,100 km north of the nearest known populations in central Oregon. The distribution of this species might have once extended from Oregon to British Columbia with intervening populations being wiped out by glaciers.

Our results suggest that populations of *O. occidentalis* from the Cascade Mountains of Oregon and the Queen Charlotte Islands (subclades F and G) are quite different from those of the Sierra Nevada and other more southern mountain ranges (subclade H). The Californian populations are within a geographic region known as the California Floristic Province which harbors more endemic plant and animal taxa and more identifiable subspecies than any other area of comparable size in North America (Calsbeek et al. 2003). The genetically divergent populations from this area could represent a new taxon and another example of a California Floristic Province endemic. The morphology and phylogeography of *O. occidentalis* is currently being studied to confirm the taxonomic status of these plants.

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South Carolina: Bamberg Co., Oxypolis Bay Preserve, 28 August 2005, Feist, Molano-Flores, and Glitzenstein 3194 (ILLS), 2938, EF647760. *O. fendleri*, U.S.A. Colorado: Rio Blanco Co., Flat Tops/White River Plateau, Rough Creek, ca. 17 air mi WSW of Oak Creek, 4 August 1991, Vanderhorst 3759 (RM 616920), 1509, AY360253. Colorado: Boulder Co., Forth of July Canyon, 10 July 1962, Jones 34084 (ILL), 2350, EF647767. Colorado: Boulder Co., along Boulder Creek, 24 June 1962, Jones 34450 (ILL), 2351, EF647768. Colorado: Gunnison Co., 0.8 mi W of Kebler Pass near the Crested Butte-Paonia Rd., 27 July 1984, Barrie 849 (MO 3751099), 2353, EF647769. Colorado: Chafee Co., CO 306, 14 mi W of Buena Vista, 2 August 1973, Haber and Given 2049 (CAN 370800), 2369, EF177734. Colorado: Conejos Co., 5 mi N of Sheep Creek, 4 mi SE of McIntyre Peak, 3 mi W of Aspen Glade Campground, 17 June 1986, Douglas 974 (RM 394593), 2762, EF647770. Colorado: Dolores Co., Lone Cone State Wildlife Area, ca. 26 air mi NNE of Dolores, E of FR 526, 9 July 1995, Moore 6628 (RM 706723), 2763, EF647765. Colorado: Summit Co., Boreas Pass Road, 6.5 mi E of CO 9, 21 July 1972, Nelson 771 (RM 345087), 2764, EF647766. New Mexico: Colfax Co., Porcupine Camp, 24 June 1968, Hartman 2087 (RM 296467), 2760, EF647763. New Mexico: Miguel Co., Windsor Cr., Pecos Rd., 9 July 1976, Pase 2090 (RM 406192), 2761, EF647764. Wyoming: Carbon Co., W slope of Sierra Madre, W of Encampment, Battle Creek, 15 July 1966, Porter and Porter 10218 (DAO 456446), 2370, EF177735. Wyoming: Carbon Co., Sierra Madre, Continental Divide Trail, ca. 10.5–11 air mi SW of Encampment, 1 August 1987, Williams 588 (RM 396608), 2758, EF647762. Wyoming: Carbon Co., Sierra Madre, ca. 13 mi SE of encampment, along a trib. of Big Creek at SE base of Bear Mountain, 30 June 1988, Nelson 16083 (RM 570852), 2759, EF647761. *O. filiformis*, U.S.A. Florida: Bay Co., Callaway Bayou, 2 mi E of junction of Rt. 22 and US 98 on FL Rt. 22, 15 September 1979, Judd and Perkins 2428 (FLAS 174230), 2711, EF647775. Florida: Bay Co., N side of narrow river at end of road, SW of US 98, ca. 9.5 mi E of the East Bay Bridge on Tyndall Air Force Base, 12 August 1977, Perkins 469 (FLAS 131850), 2712, EF647776. Florida: Alachua Co., Gainesville, N side of NE 39th Ave. N. just E of Main St., 9 September 1987, Alcorn 155 (FLAS 166610), 2713, EF177737. Florida: Echols Co., along both sides of GA 94, 20.4 mi W of jct. with Clinch Co. Line in roadside ditch, 27 July 1998, Horn 1305 (UGA 229714), 2783, EF647778. Georgia: Lanier Co., alongside US 85 2.5 mi E of Stockton, Coastal Plain Province, 11 August 1965, Faircloth 2730 (NCU 396688), 2652, EF647774. Georgia: Echols Co., 2.7 mi N of Tarver, Coastal Plain Province, 11 August 1965, Faircloth 2728 (NCU 396687), 2653, EF647773. Louisiana: Vernon Parish, along RR and US 171 N of Pinewood, 22 August 1971, Thomas 24700 (ILL), 2356, EF647771. Louisiana: Newton Parish, 0.3 mi W of Bon Wier, Texas, 7 September 1987, Thomas and Allen 101876 (MO 3671227), 2357, EF647772. Louisiana: Vernon Parish, E of Drake's Creek, ca. 2 mi E of Johnsville Church and LA 10, ca. 7 mi E of Pickering, Kisatchie National Forest, 7 September 1987, Thomas 101486 (DAO 574521), 2371, EF177736. South Carolina: Lee Co., Mount Pleasant Church Bay, 2.5 mi SE of Elliott, 21 August 1994, Nifong 513 (NCU 565136), 2750, EF647777. *O. greenmanii*, U.S.A. Florida: Bay Co., along FL Rt. 22, 5.2 mi E of junction with FL Rt. 167, 30 August 1980, Judd and Perkins 2741 (FLAS 172279), 2716, EF647779. Florida: Bay Co., ca. 1 mi E of road to Baker Pt. and 1 mi N of US 98 on Tyndall Air Force Base, 15 September 1979, Judd and Perkins 2439 (FLAS 174274), 2717, EF177738. Florida: Gulf Co., just E of Wetappo Creek and 3.6 mi S of FL 22, 2 September 2005, Feist and Molano-Flores 3244 (ILLS), 2941, EF647780. "*Oxypolis × intermedia*" ined., U.S.A. Florida: Bay Co., along US 231, 1.8 mi N of the junction with FL Rt. 388, N of Youngstown, 29 August 1980, Judd and Perkins 2714 (FLAS 174297), 2714, EF177739. Florida: Bay Co., along FL Rt. 22, 5.2 mi E of junction with FL Rt. 167, 30 August 1980, Judd and Perkins 2741b (FLAS 174318), 2775, EF647781. *O. occidentalis*, U.S.A. California: Fresno Co., Wishon Reservoir Dam, Call 2455 (UC 282880), 1153, EF177740. California: El Dorado Co., Osgood Swamp, Foot of Meyers Grade, 3 August 1982, Follette s.n. (JEPS 82187), 1435, AY360254. Cali-

fornia: Butte Co., N side of Coon Hollow Rd., 1.2 mi E of junction with Humbug Rd., ca. 12 mi NE of Stirling City, 15 August 1981, *Taylor and Hart* 4193 (MO 3835050), 2359, EF647794. California: El Dorado Co., Sierra Nevada, Osgood Swamp N of highway 50, ca. 1.5 mi SW of Meyers, 7 August 1982, *Tiehm and Follette* 7524 (RM 341173), 2753, EF647782. California: Tulare Co., Sequoia National Forest, on trail between Mineral King P.O. and Cold Spring Public Camp, 22, August 1946, *Ferris and Lorraine* 11205 (RM 210334), 2754, EF647783. California: Tehama Co., Forest Rd. 26N09 at Cascade Creek, NW of Chico Meadows, 1.1 mi SE of Hwy 32, 9 September 1997, *Oswald and Ahart* 8863 (JEPS 94369), 2899, EF647789. California: Sierra Co., ca. 1.25 mi N of Scales, ca. 2 mi (air) SE of Poverty Hill, 28 September 2001, *Ahart* 9295 (JEPS 102455), 2927, EF647790. California: Tulare Co., Southern High Sierra Nevada, middle of headwaters meadow that eventually feeds into Deep Meadow and Boulder Creek, along road 20579 (road to Lewis Camp Trailhead), 30 July 2004, *Janeway* 8214 (CSUC 89572), 2971, EF647792. California: Butte Co., exactly 6.9 mi from the junction of Humboldt Rd. and Hwy 32 (towards Butte Meadows), 11 September 1977, *Hayes and Warner* 74 (CSUC 25438), 2972, EF647793. Oregon: Douglas and Jackson Co., Abbott Creek Research Natural Area, ca. 20 mi W of Crater Lake near Abbott Butte, 29 July 1972, *Mitchell* 348 (USFS 406185), 2755, EF647784. Oregon: Lane Co., Quaking Aspen Swamp, 6 mi W of S end of Cougar Reservoir, 28 July 1979, *Wagner* 2318 (ORE 103195), 2928, EF647791. Oregon: Klamath Co., near W boundary of Crater Lake Park, 24-29 August ?, *Wynd* 1745 (ORE 64782), 2929, EF647785. Canada: British Columbia: Queen Charlotte Islands, Graham Island, 2003, *Cheney s.n.* (ILLS), 2756, EF647786. British Columbia: Queen Charlotte Islands, Graham Island, 08 U 0677503 5915030, June, 2004, *Cheney s.n.* (ILLS), 2897, EF647788. British Columbia: Queen Charlotte Islands, Graham Island, 08 U 0685969 5912636, June, 2004, *Cheney s.n.* (ILLS), 2898, EF647787. *O. rigidior*, U.S.A. Alabama: Covington Co., ca. 4 mi E of Florala, roadside of Co. Hwy 4 at 1 mi E of junction w/ Hwy 54, 4 October 1999, *MacDonald* 13559 (DUKE 382196), 2768, EF647802. Georgia: Rabun Co., Patterson Gap Road, ca 2 mi E of Tumbling Waters Camp, 17 September 1999, *Stiles* 1349 (UGA 226955), 2771, EF647801. Illinois: Cook Co., Paintbrush Prairie, Markham, Millard Ave. off Frontage Rd. of I-294, 12 August 1996, *Masi and Epting* 553 (ILLS 195720), 1652, EF647796. Illinois: Lawrence Co., Chauncey Marsh, ca. 0.5 mi SW of Embarras River and ca. 4.6 mi E of Chauncey, 4 August 1995, *Phillippe, Tate, and Ebinger* 26766 (ILLS 195822), 1653, EF647795. Illinois: Vermilion Co., 3.5 mi SE of Collision, 23 August 1973, *Evers* 111785 (ILLS 152167), 1806, EF647797. Illinois: Vermilion Co., Windfall Hill Prairie Nature Preserve, Windfall Prairie Seep, 17 July 1991, *Phillippe, Morris, and Simon* 19411 (ILLS 177487), 1927, AY360255. Illinois: Livingston Co., Pontiac, 27 July 1978, *Cull s.n.* (ILLS 188204), 1962, EF647798. Louisiana: Winn Parish., along LA 126, 1.2 mi E of Jct. LA 1233, Kisatchie National Forest, 20 September 1981, *Kessler* 1877 (ILL), 1998, EF177743. North Carolina: Polk Co., along C.R.1154 in a small segment of NCWRC Game Lands improvement, 5 October 1973, *DuMond* 1871 (NCSC 57816), 2765, EF647799. North Carolina: Pender Co., just W of the Lanier Marl Quarry, ca. 2.5 mi SE of Maple Hill on CR 1532, 2 October 1990, *Wilbur* 57659 (DUKE 331952), 2766, EF647800. *O. ternata*, U.S.A. Florida: Franklin Co., N side of Ridge Rd., ca. 1.0 air mi NE of Eastpoint, 21 October 1987, *Anderson* 11051 (MO 3681641), 2360, EF647810. Florida: Wakulla Co., Saint Mark's National Wildlife Refuge, Panacea Unit Longterm Burn Plot (P13), ca. 2 km SE of Sopchoppy, 1 September 2005, *Feist, Molano-Flores, and Glitzenstein* 3222 (ILLS), 2940, EF647809. Georgia: Turner Co., N side of CR 249 (S1531) connecting US 41 and GA 33 at head of trib. to West Fork Deep Creek, 10 October 1998, *Horn* 1590 (DUKE 363352), 2740, EF647806. Georgia: Coffee Co., along a small stream at "the Rocks," 7.2 mi E of Pridgen, 17 October 1970, *Faircloth* 6924 (UGA 114062), 2741, EF647805. North Carolina: Harnett Co., Pine View, 27 September 1966, *Diamond* 399 (NCSC 70046) 2736, EF647803. North Carolina: Pender Co., Holly Shelter Game Land, 3 October 1997, *Horn and Dirig* 362 (DUKE 363865), 2738, EF177746. North Carolina: Scotland Co., at the head of a trib. leading to Kinney Cameron Lake, Scotland Lane Annual Burn Site, Sandhills Game Land, 30 September 2000, *Horn* 3416 (DUKE 379607), 2739, EF647804. South Carolina: Horry Co., 3.8 mi S. of Socastee and ca. 1 mi W. on dirt road, 25 October 1970, *Massey and Thomas* 3480 (NCU 422851), 2735, EF177745. South Carolina: Horry Co., Cartwheel Bay Heritage Preserve, between Cartwheel Bay and Mossy Bay, 7 October 1998, *Pittmann and Darr* 10079813 (DUKE 362749), 2737, EF647808. South Carolina: Georgetown Friendfield Plantation, Georgetown Property, 29 August 2005, *Feist, Molano-Flores, and Glitzenstein* 3203 (ILLS), 2939, EF647807.

Ptilimnium ahlesii, U.S.A. North Carolina: Brunswick Co., just E of Brunswick River and just N of the 74-76 causeway, ca. 2 mi W of Wilmington, 10 June 2004, *Weakley and LeBlond* 7317 (sheet 2 of 2) (NCU,

2969, EF647814. South Carolina: Berkeley Co., Cooper River at the mouth of Durham Creek, 7 June 1990, *McAninch* 23 (NCU 557199), 2648, EF177747. South Carolina: Colleton Co., 1.4 mi NE of Ashpoo on US 17, 26 June 1956, *Bell* 3703 (NCU 97912), 2649, EF647813. *P. capillaceum*, U.S.A. Arkansas: *Maddox VB-Cap-12*, 2795, EF647821. Georgia: Elbert Co., SE of Elberton on dirt lane to Anthony Shoals on Broad River, 23 June 1977, *Craft Coile* 1000 (GA 140247), 2627, EF647817. Florida: Wakulla Co., Appalachia National Forest, FSR 369, 2 mi from junction with FL 267, SW from Helen Work Center and Springhill, 14 June 1990, *Godfrey* 83887 (GA 185564), 2628, EF647818. Florida: Nassau Co., White Oak Plantation in the wedge formed by the junction of the Little St. Mary's River and St. Mary's River about 8-10 mi NW of Yulee, 19 June 1997, *Wilbur* 67597 (BRIT), 2703, EF647822. Louisiana: LaSalle Parish, N edge of Saline Wildlife Area, off LA 28 ca. 9 mi SW of Archie, 2 June 1979, *Allen and Vincent* 8765 and 2029 (WVA 114846), 2619, EF647815. North Carolina: Jones Co., Croatan National Forest, N floodplain of Hunter Creek, E side of NC Rt. 58, 17 April 1999, *Hill* 31281A (ILLS 200452), 2704, EF647819. North Carolina: Tyrrell Co., SR 1307 at Frying Pan Lake boating access area, 8 August 1980, *McDonald and Nash* 1802 (WVA 114842), 2618, EF647816. North Carolina: *Maddox NC-Cap-15*, 2794, EF647820. Virginia: Lancaster Co., Bellwood Marsh, S of Rt. 3 bridge, W of Lancaster, 22 July 1994, *Weldy* 849 (BRIT), 2701, EF177748. *P. costatum*, U.S.A. Arkansas: Bradley Co., near Lanark Branch along a dirt road, 3.1 mi S of Arkansas 4, SW of Banks, 17 June 1986, *Dale and Leslie* 96999 and 2145 (USF 200503), 2640, EF647837. Georgia: Chattooga Co., Mosely Spring, Lyerly Quadrangle, S of Lyerly, ca. 0.5 mi N of AL state line, 28 June 1970, *Foote* 6043A (UGA 100623), 2903, EF647838. Illinois: Jackson Co., Shawnee National Forest, Oakwood Bottoms, Murphysboro Ranger District, Compartment 15, 14 July 1993, *Phillippe* 22406 (ILLS 181777), 1503, EF647829. Illinois: Jackson Co., Shawnee National Forest, Oakwood Bottoms, Murphysboro Ranger District, Compartment 16, 20 August 1993, *Phillippe* 22870 (ILLS 18338), 1504, EF647830. Illinois: Jackson Co., Shawnee National Forest, 20 September 1989, *Stritch* 2159 (ILLS 172136), 1646, EF177749. Illinois: Jackson Co., 11 September 1989, *Stritch* 2125 (ILLS 172159), 1648, EF647832. Illinois: Johnson Co., in a swamp forest at Grantsburg Swamp (Bell Pond) off of IL Rt. 146 at Grantsburg, 2 July 1992, *Basinger* 4638 (ILLS 179577), 1649, EF647833. Illinois: Jackson Co., 20 September 1989, *Stritch* 2163 (ILLS 172191), 1650, EF647834. Illinois: Johnson Co., N of Karnak, 12 September 1989, *Smith* 1427 (ILLS 172057), 1651, EF647823. Illinois: Jackson Co., 12 September 1989, *Stritch* 2139 (ILLS 172154), 1801, EF647835. Illinois: Alexander Co., Horseshoe Lake Conservation Area, 3 July 1993, *Basinger* 6009 (ILLS 181612), 1802, EF647836. Illinois: Johnson Co., N of Karnak, 12 September 1989, *Smith* 1425 (ILLS 172055), 1805, EF647824. Illinois: Jackson Co., Shawnee National Forest, 11 September 1989, *Stritch* 2124 (ILLS 172160), 1970, EF177750. Illinois: Jackson Co., near Howardton, 16 July 2003, *Feist* 2508, 2625, EF647828. Louisiana: Natchitoches Parish, moist seepage area beside LA 479 at Strange Rd., W of Goldonna in Kisatchie National Forest, 14 August 1989, *Thomas and Bell* 112081 (ILL), 1981, EF177751. Louisiana: Natchitoches Parish, near bridge on Longleaf Vista (FSR 337), ca. 9 mi NW of Gorum, 28 August 1976, *Karl Vincent* 561 (GA 127326), 2641, EF647840. Louisiana: Jefferson Parish., Davis, roadside ditch and RR ROW beside US 165, N of Fenton, 18 October 1975, *Thomas* 47944 (FLAS 168822), 2904, EF647839. Missouri: Wayne Co., Hattie's Ford Fen Area, 12 October 2001, *Brant* 4857 (MO 5573699), 2402, EF177752. Tennessee: Coffee Co., E of I-24 on US 41, ca. 3 mi SE Manchester, 20 August 1971, *Kral* 43657 (MO 3671221), 1514, EF647831. Texas: Anderson Co., Gus Engeling Wildlife Management Area, NW of Palestine, Lake 2 bog area, pasture 2, 16 October 1993, *Dubrulle Reed* 1354 (TAMU 24011), 2905, EF647825. Texas: Anderson Co., Chester's Pitcher Plant Bog, N end of Gus Engeling Wildlife Management Area, on Gibson Branch, S of FM 2961 just down stream from lake, August 1997, *MacRoberts and MacRoberts* 3412 (BRIT), 2906, EF647826. Texas: Hardin, along roadside of Hwy 327 between Silsbee and Kountze, 30 September 1971, *Amerson* 834 (BRIT/SMU), 2914, EF647827. *P. nodosum*, U.S.A. Alabama: Little River, *Maddox LR-35-15*, 2786, EF647845. Alabama: DeKalb Co., Little River near AL Hwy. 35 bridge, DeSoto State Park, 15 July 1987, *Freeman s.n.* (AUA 46749), 2931, EF647853. Alabama: Cherokee Co., on E side of Little River S of AL Hwy 35 SE of Fort Payne, 16 July 1987, *Thomas* 100873 (USCH 42952), 2970, EF647846. Arkansas: *Maddox ARK-7*, 2791, EF647852. Arkansas: Yell Co., Ouachita Mountains, below the Hwy 27 bridge over Irons Fork, 16 October 1990, *Bates* 10558 (UARK), 2930, EF647851. Georgia: *Maddox GA-5*, 2789, EF647847. Georgia: Greene Co., Siloam Outcrop, 12 May 2005, *Feist and Molano-Flores* 2976, EF647849. 2787, Maryland: *Maddox MG-4*, 2902, EF177754. Maryland: Sideling Creek, *Maddox MD-94-6*, 2792, EF647848. North Carolina: *Maddox NC-T-8*, 2790, EF647857. North Carolina: Granville County, Tar River, 26 August 2005, *Feist* 3286 (ILLS), 2936, EF647856.

South Carolina: Aiken Co., Monetta, Windmill High Pond, Carolina Bay Road, 20 July 1992, *Hill* 23921 (USF 206922), 2635, EF647843. South Carolina: Aiken Co., *Maddox* SC-7-4, 2784, EF177753. South Carolina: Saluda Co., *Maddox* SC-7-3, 2785, EF647844. South Carolina: Saluda Co., near Hibernia, Saluda Highpond, 11 May 2005, *Feist and Molano-Flores* 3287 (ILLS), 2900, EF647841. South Carolina: Aiken Co., Janet Harrison Highpond Heritage Preserve, 11 May 2005, *Feist and Molano-Flores* 2967 (ILLS), 2901, EF647842. West Virginia: Morgan Co., along Sleepy Creek, 24 August 2005, *Feist, Harmon, and O'Malley* 3181 (ILLS), 2913, EF647850. West Virginia: Morgan Co., along the Cacapon River, 25 August 2005, *Feist, Harmon, and O'Malley* 3284 (ILLS), 2933, EF647855. West Virginia: Berkeley Co., along Back Creek, 25 August 2005, *Feist, Harmon, and O'Malley* 3285 (ILLS), 2934, EF647854. *P. nuttallii*, U.S.A. Arkansas: Union Co., N of Calion and Chapelle Slough, 8 July 1993, *Thomas and Amason* 134699 (MO 4995947), 2404, EF647858. Arkansas: Ashley Co., SE of Hamburg and NE of Ark. 52, 20 June 1986, *Thomas* 97154 (WVA 114836), 2617, EF177758. Arkansas: Randolph Co., Water Valley, 1 July 1951, *Demaree* 30962 (DUKE 139050), 2644, EF647860. Illinois: Randolph Co., W of Sparta, 16 July 2003, *Feist* 2510 (ILLS), 2623, EF177759. Kansas: Labette Co., Mound Valley, 5 mi W, 2.5 mi S., 19 July 1995, *Freeman* 7322 (WVA 114956), 2637, EF647859. Louisiana: Union Parish, ROW of LA 143 and power line in pine woods, 0.7 mi W of Haile, 10 June 1986, *Thomas* 96932 (USF 200202), 2708, EF647861. Oklahoma: LeFlore Co., 1 mi S of Heavener along Kansas City and South RR, 21 May 1944, *Hopkins, Nelson, and Nelson* 525 (RM 200002), 2643, EF647863. Mississippi: Monroe Co., ca. 3 mi W of Aberdeen, roadside of Hwy 8, 4.7 mi E of junction with Hwy 45 alternate, 6 June 1996, *MacDonald* 9514 (DUKE 375296), 2642, EF647862. Oklahoma: Rogers Co., Claremore, along railroad in moist ground, 12 June 1974, *Jones* 3030 (ILL), 2165, AY360256. *P. × texense*, U.S.A. Texas: Robertson Co., Mill Creek Bog, 4 October 1987, *Starbuck* 1097 (TAMU 15147), 2772, EF647864. Texas:

Smith Co., Swan, 17 September 1902, *Reverchon* s.n. (US 415665), 2909, EF647865.

Outgroup taxa. *Atrema americanum* DC., U.S.A. Texas: Williamson Co., S. of Jarrell, *Nesom and Grimes* 6415 (MO 3691937), 1467, AY360232. *Cynosciadium digitatum* DC., U.S.A. Illinois: Jackson Co., Shawnee National Forest, *Phillippe* 21886 (ILLS 183947), 1063, AY360237. *Daucosma laciniatum* Engelm. & A. Gray, U.S.A. Texas: Kerr Co., 26-30 June 1894, *Heller* 1943 (MO 2535181), 2397, AY360238. *Lilaeopsis carolinensis* J. M. Coult. & Rose, U.S.A. cultivated, origin unknown, 1985, *Bogner* s.n., material sent from *Petersen* GPL4-1 (C), 2148, AF466276. *L. mauritiana* G. Petersen & Affolter, Republic of Mauritius. Le Val Nature Park, 3 May 1992, *Windelov* s.n., material sent from *Petersen* GPL8-1 (C), 2150, AF466277. *L. novae-zelandiae* (Gand.) A. W. Hill, New Zealand. cultivated, origin unknown, material sent from *Petersen* GPL-9 (C), 2152, AF466278. *Lilaeopsis occidentalis* J. M. Coult. & Rose, U.S.A. Oregon: Douglas Co., East Gardiner, *Hill and Dutton* 32982 (ILLS 203634), 1999, AY360242. *Limnoscium pinnatum* (DC.) Mathias & Constance, U.S.A. Illinois: Champaign Co., *Hill* 30580 (ILLS 198706), 2000, AY360243. *Neogoezia minor* Hemsl., Mexico. Oaxaca: Sierra de San Felipe, 1 August 1963, *Molseed* 278 (ISU 1060), 2138, AY360244. *Perideridia americana* (Nutt. ex DC.) Rchb., U.S.A. Illinois: Champaign Co., Urbana, Hart Woods, 14 June 1994, *Downie* 743 (ILL), 743, AY246908. *P. gairdneri* (Hook. & Arn.) Mathias subsp. *borealis* T. I. Chuang & Constance, U.S.A. Wyoming: Yellowstone National Park, 13 July 1961, *Comte* 4300 (MO 3179847), 2361, EF647811. *P. kelloggii* (A. Gray) Mathias, U.S.A. California: Sonoma Co., King Ridge Rd., 5 mi N. of Cazadero, 6 August 1993, *Ornduff et al.*, s.n. (UC), 778, U78373. *Trepocarpus aethusae* Nutt., U.S.A. Illinois: Alexander County, Horseshoe Lake Conservation Area, 8 July 1996, *Basinger* 10891 (ILLS 19458), 1817, AY360264.