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PHYLOGENETIC RELATIONSHIPS IN *PRIMULA* L. AND RELATED GENERA (PRIMULACEAE) BASED ON NONCODING CHLOROPLAST DNA

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We sequenced the *trnL* and *rpl16* introns of the chloroplast DNA from 95 of the ca. 425 species (30 of 37 sections, seven of eight subgenera) of *Primula* L. in order to reconstruct the phylogenetic history of the group. Among the 24 additional taxa sampled are representatives of all genera that are likely to be embedded in *Primula*, as well as outgroups from the Maesaceae, Theophrastaceae, and Myrsinaceae. In the strict consensus of the most parsimonious trees, *Primula* and the genera embedded in it (*Dionysia* Fenzl., *Sredinskya* [Stein] Fedorov, *Dodecatheon* L., and *Cortusa* L.) are sister to a clade of several genera previously suspected to be embedded in *Primula* (*Hottonia* L., *Omphalogramma* [Franchet] Franch., and *Soldanella* L.). In recognition of this, two new rankless names are defined for these clades (*/Primula* and */Soldanella*). Close relationships are inferred between *Dionysia* and *Primula* subgenus *Sphondylia* (Duby) Rupr., *Sredinskya* and *Primula* subgenus *Primula*, *Dodecatheon* and *Primula* subgenus *Auriculastrum* Schott, and *Cortusa* and *Primula* subgenus *Auganthus* (Link) Wendelbo. The largest subgenus, *Aleuritia* (Duby) Wendelbo, is dispersed among three clades that are not each other's closest relatives. *Primula* sections *Muscarioides* Balf. f., *Soldanelloides* Pax, *Denticulata* Watt, *Armerina* Lindley, and *Aleuritia* Duby are resolved as para- or polyphyletic with moderate to strong support. Throughout, we consider the striking morphological and cytological variation seen in *Primula* within a phylogenetic context, particularly as it relates to the close relationship implied here between *Dionysia* and *Primula* subgenus *Sphondylia*. The homology of involute leaf vernation in *Primula* is reconsidered in light of its two independent origins, and we come to the conclusion that vernation in subgenus *Sphondylia* is better characterized as conduplicate.

Keywords: molecular systematics, chloroplast DNA introns, *Primula*, Primulaceae, heterostyly, leaf vernation, chromosome base number, pollen morphology, homology.

Introduction

The genus *Primula* L. (the primroses) has provided multiple facets for study and enjoyment to taxonomists, ecologists, geneticists, and gardeners. Known in European gardens since the time of the medieval herbalists, *Primula* received early scientific recognition in Darwin's (1877) pioneering work on heterostyly, the dominant breeding system in the group. Today, heterostyly is recognized as a complex reproductive syndrome with significant ecological and evolutionary implications, and it is known from 28 flowering plant families (Barrett et al. 2000). In the Primulaceae, heterostyly and the alternative system, homostyly, have played important roles in systematic treatments at the generic and infrageneric levels (Richards 1993).

Of the ca. 425 species in *Primula*, 75% of them are concentrated in the Himalayan mountain chain and western China (Richards 1993). These species are still incompletely known in the wild, but our understanding of them has been aided by the abundance of material provided by plant hunters for horticultural and scientific purposes. Botanical gardens and private collections have made it possible to observe species from distant locations and, increasingly, to work with live material for cytological, morphological, and molecular studies. Members of *Primula* distributed outside of the Asian highland center of diversity occupy the mountains or high latitudes of North America, Europe, and Asia; a few species also extend into South America, Ethiopia, Java, and Sumatra (Richards 1993).

Traditional systematic treatments of *Primula* have primarily focused on a handful of characters. In addition to heterostyly and homostyly, these characters include chromosome base number ($x=8$, 9, 10, 11, or 12), the presence or absence of "farina" (a powdery flavonoid exudate; Blasdale 1945), leaf vernation (rolling of the leaves as they emerge from bud; "vernation" preferred over "ptyxis" in the relevant literature), and pollen exine morphology. Three main pollen types have been recognized in *Primula*: (i) colporoidate, where the three or four longitudinal furrows do not meet on the grain surface; (ii) stephanocolpate, where the five to seven furrows do not meet; and (iii) syncolpate, where the three to five furrows meet at the poles. Despite their putative importance in elucidating phylogenetic relationships in *Primula*, these five characters do not align consistently with one another. Hence, molecular sampling might provide an indepen-

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dent phylogenetic hypothesis with which to test their evolutionary lability and systematic value.

Systematic Treatments of Primulaceae

The family Primulaceae is closely related to the Myrsinaceae and Theophrastaceae, and together these three are sometimes referred to as the order Primulales (Cronquist 1988). The three families have ovaries with free-central placentation, a rare condition in the angiosperms that Anderberg and Ståhl (1995) considered to be a synapomorphy for the group and independently derived in the order Caryophyllales. Phylogenetic analysis of chloroplast DNA sequence characters supports this supposition of monophyly (Chase et al. 1993; Morton et al. 1996; Anderberg et al. 1998; Källersjö et al. 2000; Savolainen et al. 2000).

Changes to the circumscription of the Primulaceae, Myrsinaceae, and Theophrastaceae were recently suggested by Källersjö and colleagues (2000; Anderberg et al. 2000) based on DNA sequence data. Their realignment differs from previous treatments in (i) moving nine genera (including *Cyclamen* and *Lysimachia*) from the Primulaceae to the Myrsinaceae, (ii) moving one genus (*Samolus*) from the Primulaceae to the Theophrastaceae, and (iii) creating a new family (Maesaceae) for a genus of the Myrsinaceae. The two tribes remaining in the Primulaceae (the Primuleae and the Androsaceae, together ca. 15 genera) share the following six features (Källersjö et al. 2000, p. 1339): (i) a scapose inflorescence, (ii) distinctly tubular flowers with campanulate or hypocrateriform corolla, (iii) imbricate corolla aestivation, (iv) isodiametric corolla epidermal cells, (v) leaves almost always in a basal rosette, and (vi) ovules rarely immersed in the placenta.

Primula is the largest genus in the Primulaceae s.str., and its heterogeneity in morphological and cytological characters has led previous authors to conclude that many, if not most, of the remaining genera are derived members of it. The most extreme view is that of Post and Kuntze (1904), who merged most genera from tribes Androsaceae and Primuleae (as defined by Takhtajan [1997]) into *Primula*. Later, Wendelbo (1961*c*) proposed that tribes Primuleae and Androsaceae are sister groups and that all genera of the Primuleae except *Soldanella*, *Omphalogramma*, and *Bryocarpum* are derived from *Primula*. Richards's (1993) hypothesis for tribe Primuleae went further by also considering *Omphalogramma*, *Bryocarpum*, and perhaps *Soldanella* to be derivatives of *Primula*.

Previous Molecular Systematic Studies

Two molecular studies have been published to date with multiple representatives of *Primula*. Conti et al. (2000) sampled nuclear DNA regions (the two internal transcribed spacers of the nuclear ribosomal DNA) of 19 species from six of Richards's (1993) 37 sections and two of Wendelbo's (1961*b*) eight subgenera, as well as an additional genus in the Primulaceae s.str. Källersjö et al. (2000) sampled chloroplast DNA regions (the *atpB*, *ndhF*, and *rbcL* genes) of seven species from five of Richards's (1993) 37 sections and five of Wendelbo's (1961*b*) eight subgenera, as well as six additional genera in the Primulaceae s.str. Our chloroplast DNA (cpDNA) sampling greatly expands on that of these two earlier studies, both within *Primula* (to include 95 species representing 30 sections and seven subgenera) and within Primulaceae s.str. (to include 11 additional genera).

Objectives

The objective of this study is to develop a phylogenetic hypothesis for *Primula* and its relatives that permits us to address three questions: (i) Which genera are nested in the lineage that contains all sampled members of *Primula*? (ii) Which of the previously recognized infrageneric taxa (the eight subgenera of Wendelbo [1961*b*] and the 37 sections of Richards [1993]) do not appear monophyletic with the current character and taxon sampling? and (iii) What new insights does the phylogeny provide into the evolution of characters historically important in systematic treatments of the group (specifically, leaf vernation, pollen exine morphology, and chromosome base number)?

Material and Methods

Taxonomic Sampling

We sampled molecular characters from 119 accessions representing 95 of the ca. 425 species of *Primula* as well as 22 additional genera in the Primulaceae, Myrsinaceae, Theophrastaceae, and Maesaceae (appendix). The 95 species of *Primula* are distributed in 30 of the 37 sections recognized by Richards (1993) and seven of the eight subgenera recognized by Wendelbo (1961*b*). Of the additional genera, 11 are in Källersjö et al.'s (2000) Primulaceae s.str., eight in their Myrsinaceae s.lat., two in their Theophrastaceae s.lat., and one in their Maesaceae. Källersjö and colleagues' (2000; Anderberg et al. 2000) recent three-gene study involving these families concluded that the Maesaceae is sister to sampled members of Theophrastaceae, Myrsinaceae, and Primulaceae. Consequently, we used *Maesa japonica* from the Maesaceae as the outgroup in this study.

DNA Extraction, Amplification, and Sequencing

We extracted total genomic DNA from 20 to 30 mg of fresh leaf material (dried mass after lyophilization), silica-dried material, herbarium material, or, in the case of *Omphalogramma*, seed. Fresh material was lyophilized for 24–48 h in a Lyovac GT 2 (Leybold-Heraeus). Leaf or seed tissue was disrupted with glass beads using a Retsch MM 2000 Shaker set at an amplitude of 80 for 2 min (dried material) or 4 min (fresh material). For the extractions that followed, we used the DNeasy Plant Mini Kit (Qiagen).

Each DNA region was amplified using the polymerase chain reaction (PCR; Mullis and Faloona 1987): the *trnL* intron with primers "c" and "f" of Taberlet et al. (1991) and the *rpl16* intron with primers "F71" of Jordan et al. (1996) and "R1516" of Baum et al. (1998*b*). The most effective thermal cycling program proved to be 34 cycles of 0.5 min at 95°C, 1 min at 53°C, and 1.7 min at 72°C, with a terminal extension of 10 min at 72°C. A TGradient thermocycler (Biometra) performed all PCR reactions. To detect successfully amplified DNA and the possible contamination of negative controls, we examined PCR products on agarose gels. We purified successful

Table 1

DNA Character Descriptions

Region	Mean sequence length (range)	Aligned length	Informative NT positions (% of total aligned positions)	Informative INDELs (% of total aligned positions)	Ti : Tv	Divergence within /*Primula* (including others)
trnL intron	515.2 (421–550)	748	149 (19.9%)	3 (0.4%)	0.78 : 1	0–7.1 (12.8)%
rpl16 intron	908.8 (732–1018)	1476	347 (23.5%)	6 (0.4%)	0.94 : 1	0–9.1 (23.6)%

Note. Sequence length statistics for the *rpl16* intron were determined after the outlier *Hottonia palustris* (with an intron 121 NTs long) was removed. The ratio of transition to transversion substitutions (Ti : Tv) was estimated by maximum likelihood using an HKY85 substitution model on one of the most parsimonious trees for each region. Divergence calculations also assumed an HKY85 substitution model.

PCR reactions with the QIAquick PCR Purification Kit (Qiagen).

A Gene Amp PCR System 9700 (Perkin Elmer) performed cycle-sequencing reactions that we prepared with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The primers that we used in the sequencing reactions included primers "c" and "d" of Taberlet et al. (1991) and the same primers used to amplify the *rpl16* intron DNA. We cleaned sequenced products with MicroSpin G-50 columns (Amersham Pharmacia Biotech) to remove excess dye terminators before we ran them out on an ABI Prism 377 DNA sequencer (Perkin Elmer). To detect mistakes and correct uncertainties in the computer-generated sequence, we compared aligned trace files in Sequencher 3.0 (Gene Codes 1998).

Defining Substitution and INDEL Characters

We reviewed each variable position in the alignment twice in Sequencher 3.0 to confirm that base calls were consistent at informative positions and that positions of uncertain alignment did not result in informative characters. The aligned matrices are available at TreeBase (http://www.herbaria.harvard.edu/treebase).

We coded insertion or deletion (INDEL) events as additional, binary characters for maximum parsimony (MP) analyses if they were bordered by stretches of unambiguously aligned nucleotides (NT) and were potentially informative. We did not code single-NT INDELs if they were adjacent to strings of the same NT (e.g., four A's present versus five A's). Other researchers have similarly excluded this type of INDEL because it may arise from experimental error (Downie et al. 1998; McDade and Moody 1999), or they have noted its evolutionary lability (Small et al. 1998). We coded insertions as the same state if they were the same size and identical or with no more than one NT substitution or INDEL (when greater than four NTs long). When taxa had long deletions that stretched over positions containing coded INDELs, those taxa were coded as uncertain for the intervening INDELs.

Phylogenetic Reconstruction

We evaluated congruence between the DNA regions using the incongruence-length-difference (ILD) test (Mickevich and Farris 1981; Farris et al. 1995). In PAUP 4.0b8 (Swofford 2000) (where the test has been renamed the "partition homogeneity test"), 100 random partitions of the original data were used to generate a null distribution of tree scores with which to test the null hypothesis that the two data sets are random samples from a single statistical population. MP searches of the permutated data sets employed a simple addition sequence and nearest-neighbor-interchange (NNI) branch swapping with the maximum number of saved trees set at 5000. We included only potentially informative characters in the comparisons, and we weighted all of the characters and character-state transitions equally.

PAUP 4.0b8 (Swofford 2000) reconstructed the phylogeny using the MP optimality criterion. As above, we included only informative NT and INDEL characters in the analyses, and we weighted all of the characters and character-state transitions equally. The heuristic MP search employed a simple addition sequence and tree-bisection-reconnection branch swapping with the maximum number of saved trees set at 25,000. When PAUP (Swofford 2000) saved the maximum number of trees in a search, we ran a reverse-constraint MP analysis with the strict consensus of the 25,000 trees, as suggested by Catalàn et al. (1997) and Rice et al. (1997). That is, PAUP (Swofford 2000) searched for the most parsimonious trees that were not congruent with the strict consensus of the 25,000 trees saved in the first search. The second, reverse-constraint MP analysis employed the same search strategy as the first, unconstrained MP analysis.

To ascertain the relative degree of support for branches in the MP cladograms, we used PAUP 4.0b8 (Swofford 2000) to calculate bootstrap (Felsenstein 1985) values. The program's bootstrap function resampled the data 100 times; MP analyses of each replicate employed a simple addition sequence and NNI branch swapping with the maximum number of saved trees set at 5000.

Results

Defining Substitution and INDEL Characters

The aligned length of the *trnL* intron (748 positions; table 1) is roughly half of the aligned length of the *rpl16* intron (1476 positions). A slightly greater percentage of the *rpl16* intron data (23.5%) than the *trnL* intron data (19.9%) is informative. In comparison with the *trnL* intron sequence of *Nicotiana tabacum* L. (GenBank accession NC_001879), the *trnL* intron data set begins 20 NTs into the 5′ end of the tobacco intron and includes 23 NTs of the *trnL* 3′ exon. In comparison with the *rpl16* intron sequence of *N. tabacum* (same GenBank accession as above), the data set begins ca. 41 NTs into the 5′ end of the intron and ends ca. 11 NTs from its 3′ end. The *trnL* intron matrix is missing sequences for

Primula capitellata and *Primula concinna*, whereas the *rpl16* intron matrix is missing a sequence for *Cortusa matthioli.* These sequences are missing as a result of difficulties in amplifying the regions. The data sets are otherwise complete.

Nine informative INDEL characters were coded, seven of which are most parsimoniously reconstructed as insertions and two as deletions (table 2). Thirteen species from *Primula* subgenus *Aleuritia*, five from *Primula* subgenus *Craibia*, and *Primula cicutarifolia* from subgenus *Auganthus* share a large deletion in their *rpl16* intron that extends across 443 aligned positions. *Hottonia palustris* also has a large, autapomorphic deletion in the 3′ end of the *rpl16* intron that shortens its total raw length to 121 NTs.

Table 2

INDEL Characters

Region/code	Starting position	Approximate length	Insertion (I) or deletion (D)
trnL intron:			
A	197	9	D
B	259	1	I
C	265	5	I
rpl16 intron:			
D	280	8	I
E	325	6	I
F	363	2	I
G	416	6	I
H	812	6	I
I	920	443	D

Note. The alphabetical character code is used when mapping INDEL character changes onto cladograms.

Phylogenetic Reconstruction

Comparison of the data sets with the ILD test does not reject their homogeneity ($P = 0.55$). Additionally, when the relationships are compared on strict consensus trees derived from independent MP analyses of each region, there is only minor topological conflict within *Primula* sections *Aleuritia* and *Auricula* (clades II and V of figs. 1 and 2).

MP analysis of the 152 informative *trnL* intron characters results in 25,000 shortest trees (the maximum number saved; 379 steps; CI = 0.583 and RI = 0.891, excluding uninformative characters). MP analysis of the 353 informative *rpl16* intron characters also results in 25,000 shortest trees (the maximum number saved; 961 steps; CI = 0.549 and RI = 0.873, excluding uninformative characters). Reverse-constraint analyses yielded no alternative topologies that conflict with the strict consensus trees for the initial pools of putatively optimal trees (figs. 1, 2).

MP analysis of the 505 informative characters from the combined data sets results in 25,000 shortest trees (the maximum number saved; 1348 steps; CI = 0.556 and RI = 0.877, excluding uninformative characters). Reverse-constraint analysis yielded no alternative topologies that conflict with the strict consensus tree for the initial pool of putatively optimal trees (fig. 3). This strict consensus tree (fig. 3) supports a sister relationship (99% bootstrap) between a clade composed of *Omphalogramma*, *Soldanella*, and *Hottonia* (99% bootstrap; /*Soldanella*) and a clade composed of *Primula*, *Dionysia*, *Sredinskya*, *Dodecatheon*, and *Cortusa* (87% bootstrap; /*Primula*). A trichotomy is formed at the base of /*Primula* by *Primula* subgenus *Auganthus* (plus *Cortusa* and *Primula* section *Dryadifolia* of subgenus *Aleuritia*; clade VI), *Primula* subgenus *Auriculastrum* (plus *Dodecatheon*; clade V), and the remaining members of /*Primula* (clades I, II, III, and IV). This last clade shows the following relationships: (i) *Primula* subgenus *Sphondylia* is sister to the two sampled representatives of *Dionysia* (clade I); (ii) clade I is sister to a clade composed of *Primula* subgenus *Muscarioides* and part of *Primula* subgenus *Aleuritia* (clade II); (iii) clades I and II are sister to a clade composed of *Primula* subgenus *Primula* and *Sredinskya* (clade III); and (iv) clades I, II, and III are sister to a clade composed of *Primula* section *Pinnatae* of subgenus *Auganthus*, *Primula* subgenus *Craibia*, and the remaining members of subgenus *Aleuritia* (clade IV). The monophyly of each of the clades recognized here by a roman numeral, as well as the interrelationships among these clades (excluding the basal trichotomy), receive 58%–100% bootstrap support.

Naming Clades

We formally name and define /*Primula* and /*Soldanella* but not the major subclades of /*Primula* (which are referred to with roman numerals). The two clades are named here using the "clademark" convention of Baum et al. (1998*a*). Our decision not to name and define subclades of /*Primula* at this time maintains flexibility for the taxonomic revisions expected after a comparison of the chloroplast (presented here) and nuclear (A. Mast and E. Conti, unpublished data) phylogenies. In circumscribing /*Primula* and /*Soldanella*, we are using a stem-based definition (de Queiroz and Gauthier 1994), in which a clade is the most inclusive monophyletic group containing one specifier taxon but not another. In order to maintain continuity, special regard is given to type taxa for the Linnaean names that already exist. Hence, /*Primula* is the most inclusive clade that contains *Primula veris* L. but not *Soldanella alpina* L., and in a complementary way, /*Soldanella* is defined as the most inclusive clade that contains *S. alpina* L. but not *P. veris* L.

Discussion

*/*Primula*, /*Soldanella*, and Tribe Androsaceae*

Members of the family Primulaceae (*sensu* Källersjö et al. 2000) fall into three strongly supported, monophyletic clades in the cpDNA phylogeny: /*Primula*, /*Soldanella*, and tribe Androsaceae (fig. 3). Our choice to recognize a separate /*Soldanella*, rather than a /*Primula* that is expanded to include it (equivalent to tribe Primuleae; Takhtajan 1997), is meant to highlight the position of its members (*Soldanella*, *Omphalogramma*, and *Hottonia*) outside of *Primula*. This distinction is relevant to much historical speculation regarding the circumscription of a monophyletic *Primula*.

The prior hypothesis of relationships within the Primulaceae s.str. that is most congruent with the cpDNA phylogeny presented here is that by Wendelbo (1961*c*). His proposed sister relationship between tribe Primuleae (/*Primula* and /*Solda-*

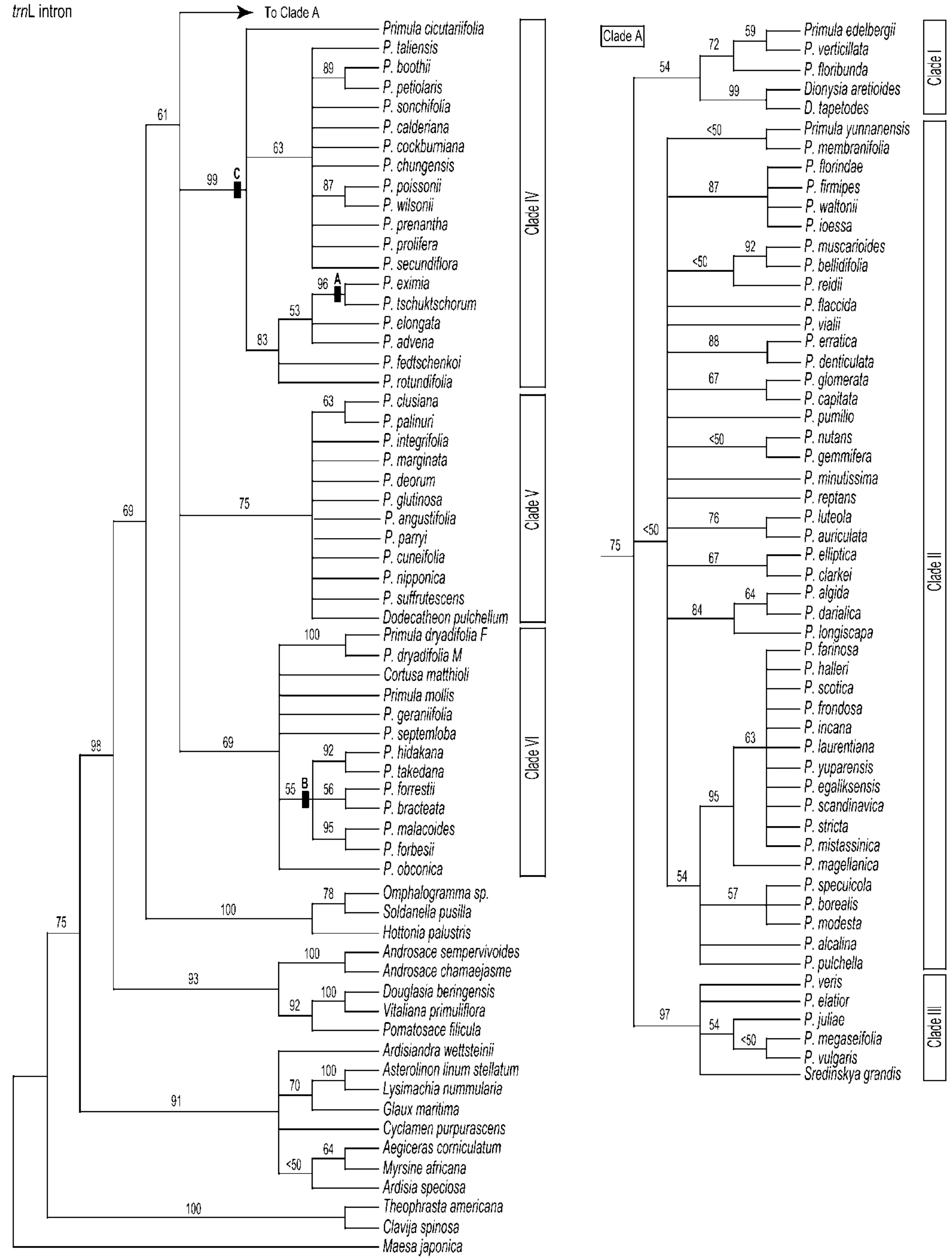

Fig. 1 Strict consensus of 25,000 shortest trees (the maximum number saved; 379 steps; CI = 0.583 and RI = 0.891, excluding uninformative characters) found in maximum parsimony analysis using data from the *trnL* intron (152 informative characters). Bootstrap values are shown above the branches. Unique INDEL changes are shown with solid bars, and changes that occur in parallel are shown with hollow bars. Coding of INDELs is found in table 2. Two specimens of *Primula dryadifolia* were sequenced; the letter following each of these two accessions refers to the source of that material (appendix).

nella) and tribe Androsaceae is supported as is his suggestion that *Soldanella* and *Omphalogramma* are not nested in *Primula* (contra Richards [1993], as well as Halda [1992] in the case of *Omphalogramma*). Further, he not only identified the four genera that are nested in *Primula* in the cpDNA phylogeny, but he also identified subgenera that are closely related to them (*Dionysia* and *Primula* subgenus *Sphondylia*, *Sredinskya* and *Primula* subgenus *Primula*, *Dodecatheon* and *Primula* subgenus *Auriculastrum*, and *Cortusa* and *Primula* subgenus *Auganthus*). His position that *Hottonia* is nested in genus *Primula* is not supported, however.

Our resolution of relationships among genera in the Pri-

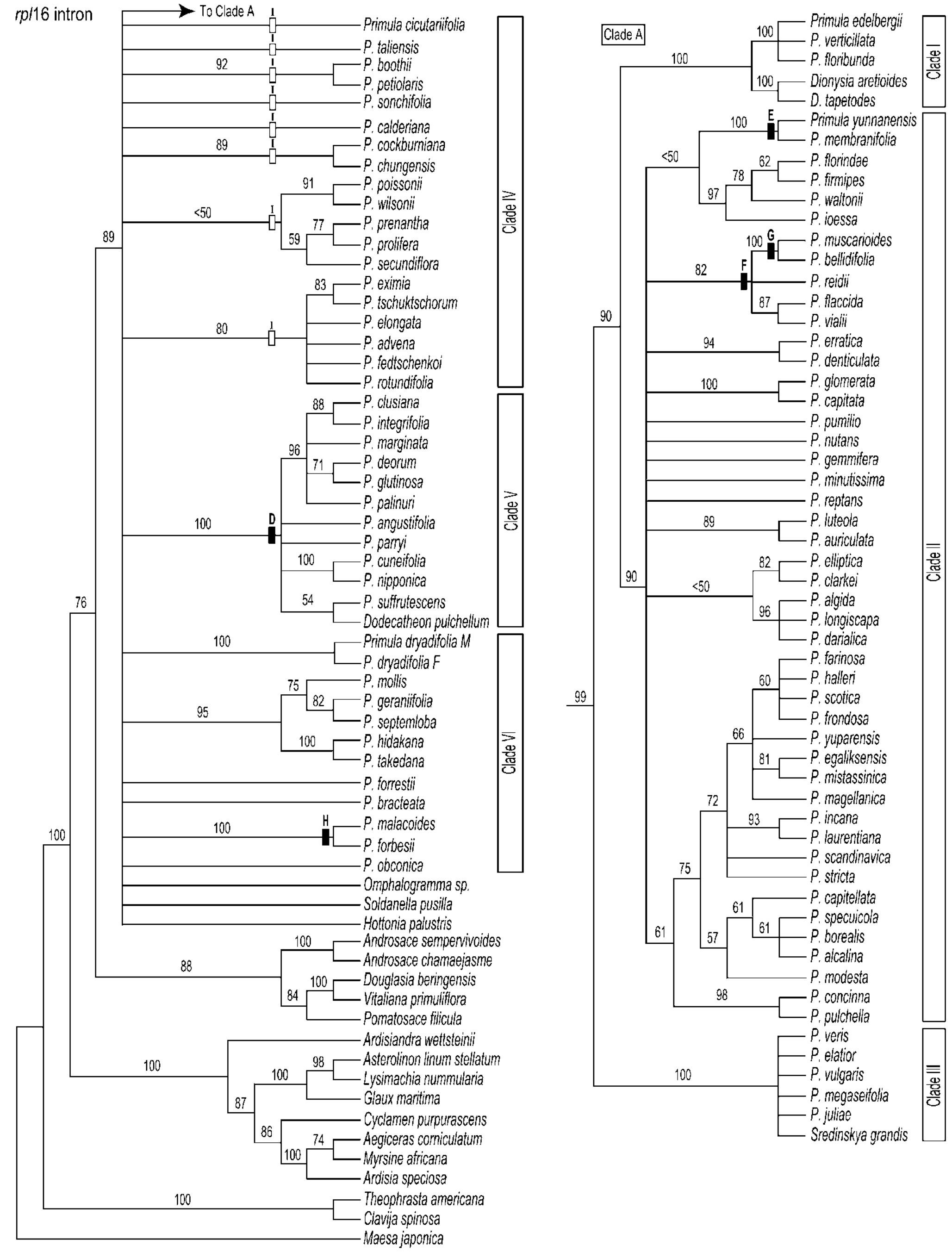

Fig. 2 Strict consensus of 25,000 shortest trees (the maximum number saved; 961 steps; CI = 0.549 and RI = 0.873, excluding uninformative characters) found in maximum parsimony analysis using data from the *rpl16* intron (353 informative characters). Bootstrap values are shown above the branches. Unique INDEL changes are shown with solid bars, and changes that occur in parallel are shown with hollow bars. Coding of INDELs is found in table 2. Clades IV and VI are not resolved as monophyletic in this result.

mulaceae s.str. is congruent with the cpDNA results of Källersjö et al. (2000), although they sampled five fewer genera in the family and many fewer species in *Primula*. In both studies, a clade composed of *Primula*, *Cortusa*, and *Dodecatheon* (*/Primula*) is sister to one of *Soldanella* and *Omphalogramma* (*/Soldanella*), although our study also includes *Dionysia* and *Sredinskya* in the former clade and *Hottonia* in the latter. In both studies, the clade composed of */Primula* and */Soldanella* is sister to a clade of *Androsace* and *Douglasia* (tribe Androsaceae), although our study also adds *Vitaliana* and *Pomatosace* to the latter clade. Their realignment of genera from the Primulaceae to the Myrsinaceae is supported by our results, for in our cpDNA phylogeny, exemplars from five of those genera (*Ardisiandra*, *Lysimachia*, *Asterolinon*, *Glaux*, and *Cy-*

clamen) are paraphyletic with respect to exemplars from the Myrsinaceae (as it is traditionally recognized).

Three genera of the Primulaceae are not represented in this or prior molecular systematic studies: *Kaufmannia* (one species from central Asia), *Bryocarpum* (one species from the central Himalayas), and *Stimpsonia* (one species from eastern Asia). *Kaufmannia* is likely to join *Cortusa* in */Primula* when it is sampled based on Wendelbo's (1961*c*) observation that the two genera differ only in their flower color and minor, quantitative floral traits. In fact, Wendelbo (1961*c*) relegated *Kaufmannia* to a section of *Cortusa*. *Bryocarpum* is likely to join */Soldanella* when it is sampled. *Bryocarpum* and genus *Soldanella* share a circumscissile capsule morphology (Pax 1889; Pax and Knuth 1905) that is uncommon in the family (though also found in *Pomatosace*), and *Bryocarpum* and *Omphalogramma* are very similar in gross morphology (Wendelbo 1961*c*; Richards 1993). *Stimpsonia* shares with other members of tribe Androsaceae tricolporate pollen (Wendelbo 1961*c*; Richards 1993) and white, stellate hairs (Anderberg and Ståhl 1995), and it is likely to join that clade when sampled.

Wendelbo's Subgenera

Only Wendelbo's (1961*b*; 1965) subgenus *Sphondylia* is currently resolved as monophyletic in the cpDNA phylogeny (fig. 3), but members of his subgenera *Muscarioides* and *Craibia* are resolved in polytomies that could each be resolved to produce their monophyly. However, we note that recognition of subgenus *Craibia* might necessitate the undesirable recognition of multiple new subgenera within clade IV in order to maintain monophyly at that rank. The most dramatic taxonomic changes are anticipated to occur in subgenus *Aleuritia*, the bulk of which will likely be split into two different subgenera or rankless taxa (here recognized as clades II and IV).

Clade I: Primula *Subgenus* Sphondylia *and* Dionysia

The sister relationship. The relationship between *Primula* subgenus *Sphondylia* (eight species in one section; unless otherwise noted, the reported number of species and sections in each taxon of *Primula* is taken from Richards [1993]) and *Dionysia* (41 species in three sections; Grey-Wilson 1989) has been the subject of long debate (reviewed by Wendelbo [1961*a*] and Grey-Wilson [1989]). Some features of *Primula* subgenus *Sphondylia* are strikingly similar to those of *Dionysia* section *Anacamptophyllum* Melchior subsection *Scaposae* Wendelbo, suggesting a close relationship. These include their inflorescences of superimposed verticillate umbels of yellow, long-tubular flowers with foliose bracts and their broad, thin, lobed leaves. Additionally, the two groups are biogeographically contiguous in the mountains of Asia Minor (Wendelbo 1961*a*; Grey-Wilson 1989; Al Wadi and Richards 1992). Several of the species now placed in *Dionysia* were originally described as belonging to *Primula* subgenus *Sphondylia* (then called section *Floribundae*; Smith and Fletcher 1948*b*; Wendelbo 1961*a*).

However, Al Wadi and Richards (1992) proposed that observed similarities between *Primula* subgenus *Sphondylia* and *Dionysia* are due not to a recently shared ancestry but to similar selective regimes. This, they argued, is supported by differences in putatively more important taxonomic characters. They report that *Dionysia* section *Anacamptophyllum*, the only section of the genus that is well known, has (i) revolute leaf vernation, rather than involute vernation (as in *Primula* subgenus *Sphondylia*; fig. 4); (ii) stephanocolpate pollen, rather than colporoidate pollen (*Sphondylia*; fig. 4); (iii) a chromosome base number of $x = 10$, rather than $x = 9$ (*Sphondylia*; fig. 4); (iv) a woody stem, rather than an herbaceous one (*Sphondylia*); and (v) woolly farina, rather than powdery farina (*Sphondylia*). They argue against a close relationship between *Primula* subgenus *Sphondylia* and *Dionysia* because "it is unlikely that so many stable characters altered coincidentally at the evolutionary boundary between *Primula* and *Dionysia*" (Al Wadi and Richards 1992, p. 309). The weight of Al Wadi and Richards's (1992) argument deserves further examination, given that the cpDNA phylogeny strongly supports (87% bootstrap; fig. 3) a sister relationship between *Primula* subgenus *Sphondylia* and *Dionysia*.

Let us first consider what constitutes the evolutionary boundary between these two groups. Within the phylogenetic scenario supported by the cpDNA, the five character-state changes could be split between at least two branches: one leading to subgenus *Sphondylia* and one to *Dionysia*. This requires an assessment of the primitive character state for the two groups' most recent common ancestor, which we have attempted with mixed success. In an analysis using Fitch parsimony, we determined that revolute leaf vernation (fig. 4) and herbaceous growth (A. Mast, unpublished data) are the primitive states. Vernation thus changed state along the branch leading to subgenus *Sphondylia*, while habit changed state along that leading to *Dionysia*. Reconstructions of the character-state changes for two of the remaining three characters, pollen type and chromosome base number, are unfortunately equivocal (A. mast, unpublished data) given the current degree of resolution in the phylogeny and, importantly, their evolutionary lability.

The final character, farina type, is unlikely to have changed state on either of these two branches but rather on a third that is within *Dionysia*. The woolly type of farina in *Dionysia* section *Anacamptophyllum* is also found in section *Dionysia* but not in section *Dionysiastrum*, which has the powdery type of farina typically found in *Primula* (Grey-Wilson 1989). If the woolly type is accepted as a synapomorphy for sections *Anacamptophyllum* and *Dionysia*, then the character would have changed state after section *Dionysiastrum* diverged from the common ancestor of these two sections. Our current taxon sampling includes members from sections *Anacamptophyllum* (*Dionysia aretioides*) and *Dionysia* (*Dionysia tapetodes*) and thus does not test this hypothesized relationship.

As alluded to earlier, it is also informative to examine the stability of these characters within the proposed phylogenetic context. While leaf vernation and farina type appear to have few character-state transitions, pollen type and chromosome base number are quite labile (fig. 4). All major subclades of */Primula*, except clade III, include more than one pollen type, and clade II, which is sister to subgenus *Sphondylia* and *Dionysia* and the most appropriate group for comparison, is particularly diverse in chromosome base number, including no fewer than four states for this character. A similar lability is seen in woodiness in */Primula*, with woody or subwoody growth also seen in members of *Primula* sections *Dryadifolia*

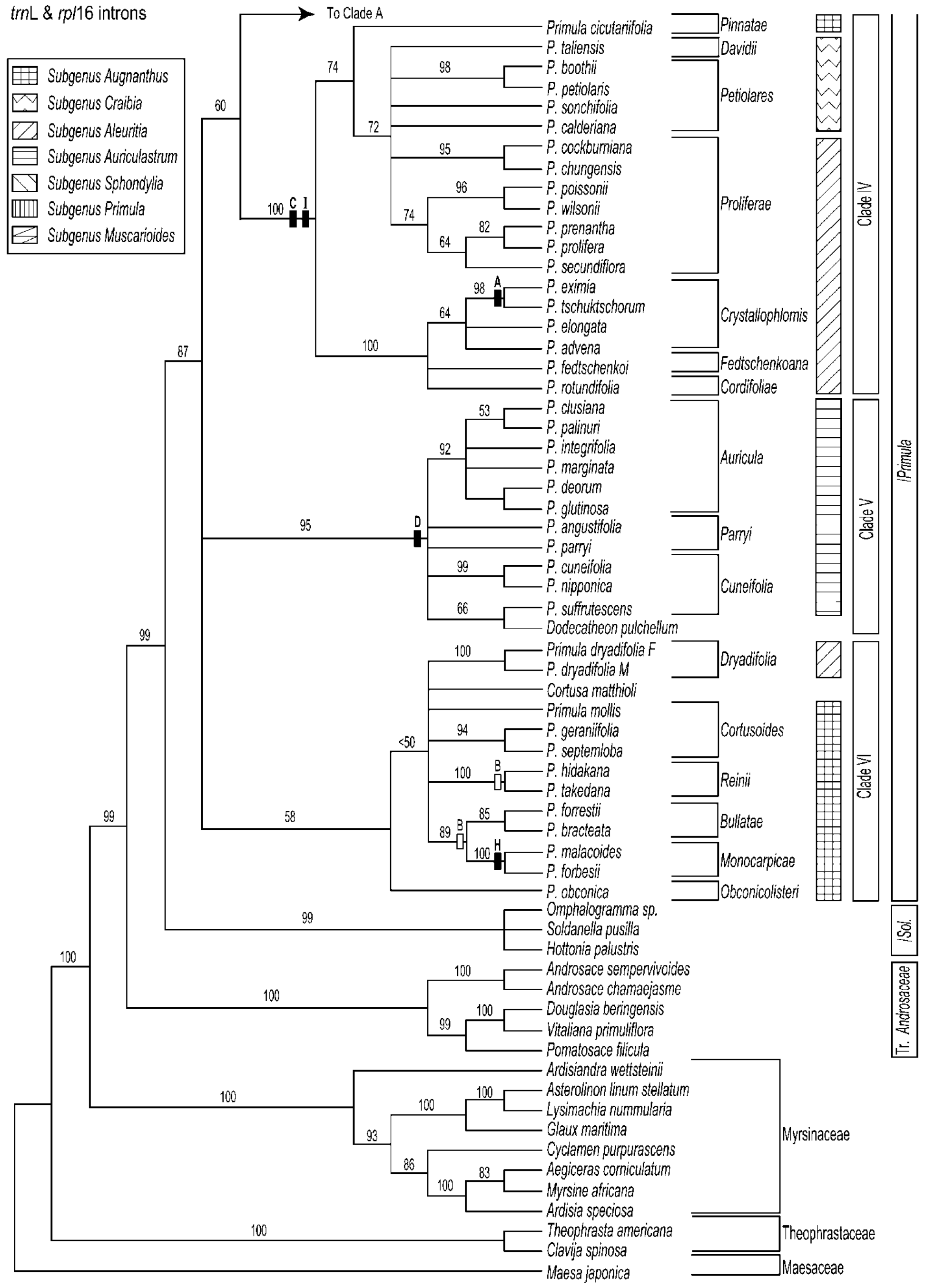

Fig. 3 Strict consensus of 25,000 shortest trees (the maximum number saved; 1348 steps; CI = 0.556 and RI = 0.877, excluding uninformative characters) found in maximum parsimony analysis using data from the *trnL* and *rpl16* introns (505 informative characters). Bootstrap values are shown above the branches. Unique INDEL changes are shown with solid bars, and changes that occur in parallel are shown with hollow bars. Coding of INDELs is found in table 2. Richards's (1993) sectional delimitations are indicated with brackets to the right of the species names. Wendelbo's (1961*b*) subgeneric delimitations are indicated with patterned bars to the right of these. Clade names are provided in the two columns at the far right of the figure. */Sol.* = */Soldanella*. Family delimitations are according to Källersjö et al. (2000).

(two species), *Cuneifolia* (one species), *Auricula* (one species), and *Bullatae* (one species).

Together, these observations seem to suggest a much higher probability of a close relationship between *Primula* subgenus *Sphondylia* and *Dionysia* than is at first apparent given the differences noticed by Al Wadi and Richards (1992). Shifts in five characters at the evolutionary boundary of the groups are likely distributed along three branches, and three of the stable characters are shown to be quite labile when considered in a phylogenetic context.

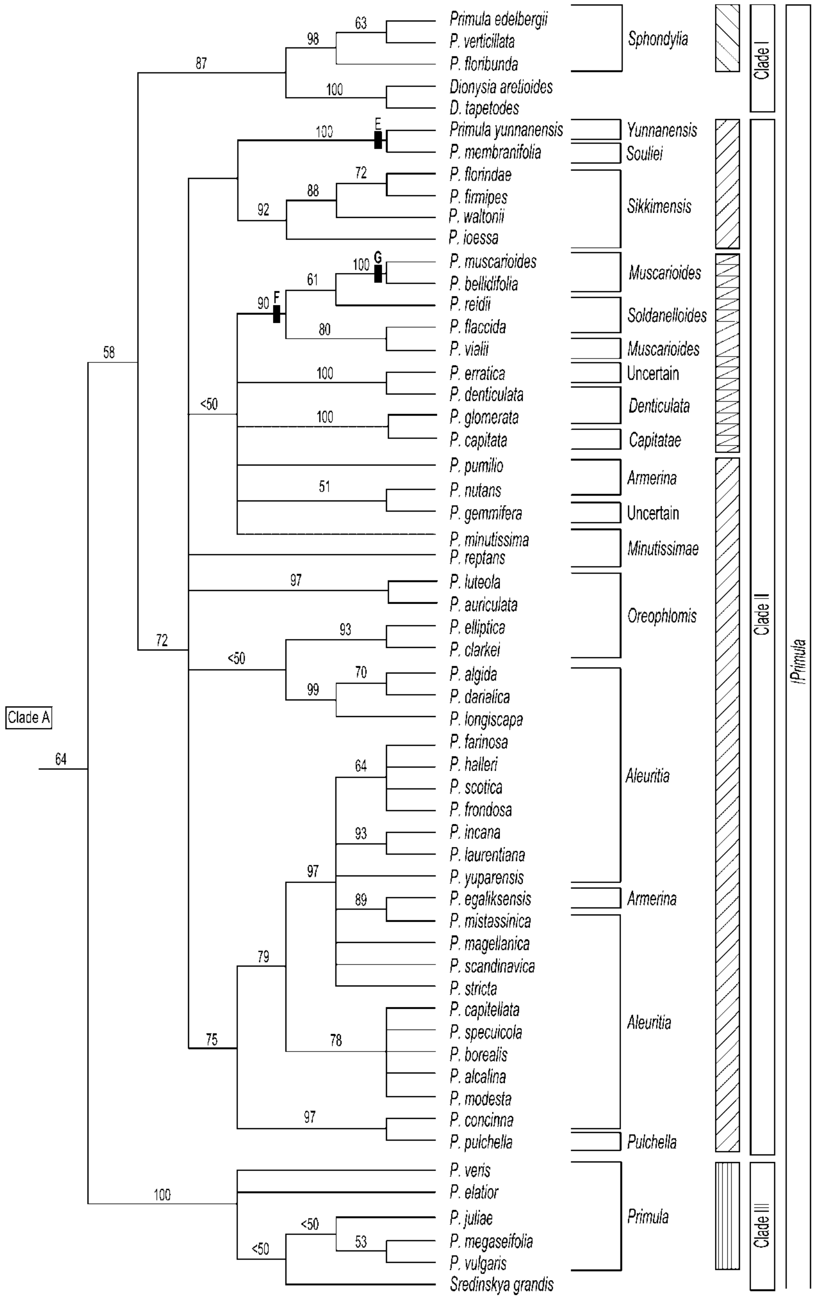

Fig. 3 (*Continued*)

Apparent reversal to involute leaf vernation in Primula *subgenus* Sphondylia. Early monographers of *Primula* (Schott 1851; Pax 1889; Pax and Knuth 1905; Smith and Forrest 1928) noted a dichotomy between those species with involute and those with revolute leaf vernation and considered it to be of fundamental systematic importance. Modern treatments (Wendelbo 1961*b*; Richards 1993), while considering the character to be important, have recognized that involute vernation is likely primitive in the group and thus does not imply monophyly for those species with the condition. Sections with involute vernation include members of subgenera *Sphondylia* (section *Sphondylia*) and *Auriculastrum* (sections *Auricula*, *Cuneifolia*, and *Parryi*, and possibly section *Amethystina*; see discussion below). While all the members of subgenus *Auriculastrum* show clear morphological and cytological links (each with a chromosome base number of x = 11 and thick, remotely dentate or entire leaves), the subgenus differs in morphology and chromosome base number from subgenus *Sphondylia*, which has a chromosome base number of x = 9 and thin, broad, heavily farinose leaves.

If involute vernation is primitive and revolute vernation is derived, one might recognize the latter condition as a syn-

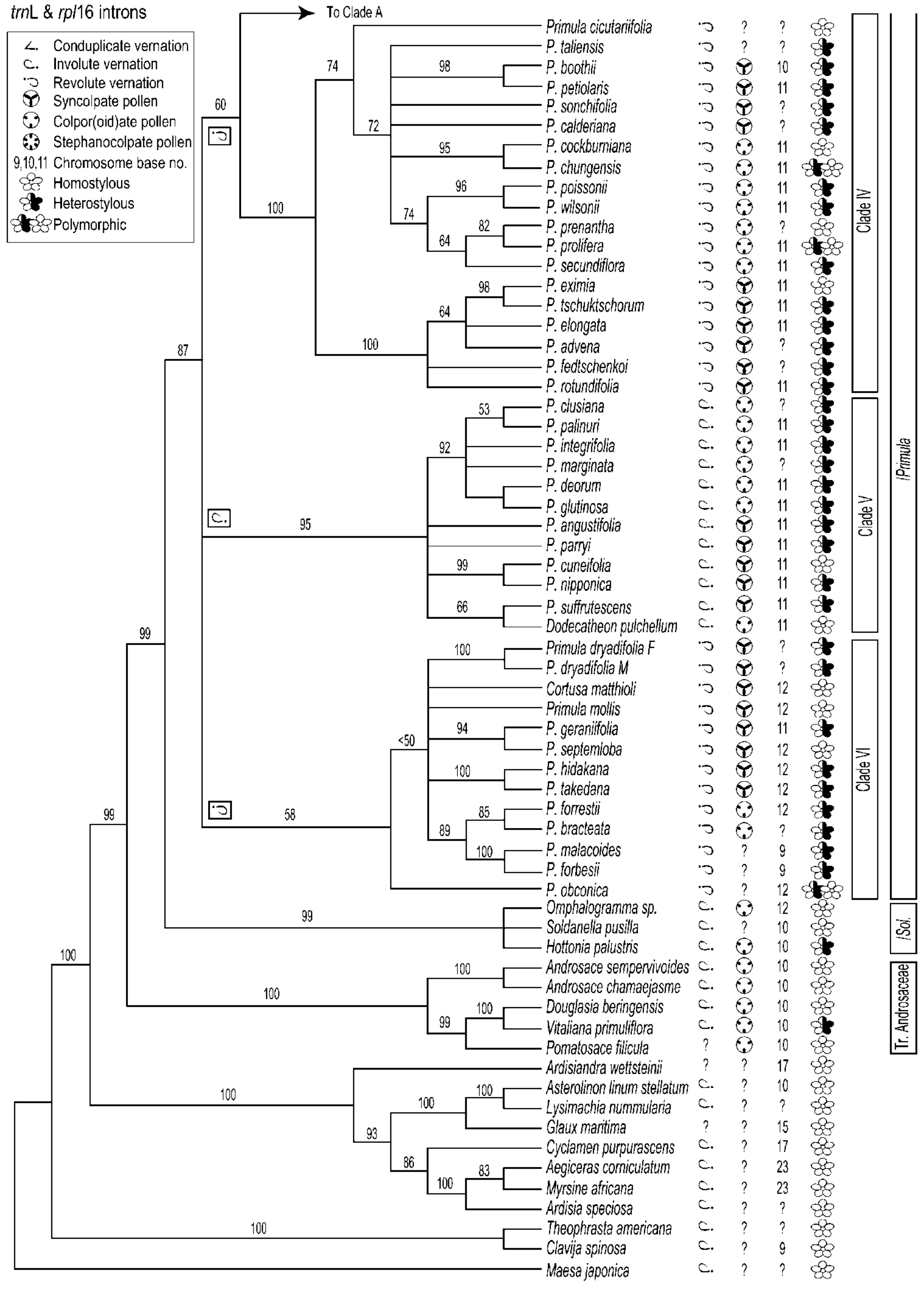

Fig. 4 Morphological and cytological variation in the group, as distributed on the maximum parsimony results of fig. 3. From left to right, these characters are leaf vernation, pollen type, chromosome base number, and heterostylous/homostylous condition. Character states for these four were determined from the taxonomic (Smith and Forrest 1928; Smith and Fletcher 1941, 1942*a*, 1942*b*, 1942*c*, 1943*a*, 1943*b*, 1944*a*, 1944*b*, 1946, 1948*a*, 1948*b*, 1950; Wendelbo 1961*a*, 1961*b*, 1961*c*; Fenderson 1986; Halda 1992; Richards 1993; Anderberg and Ståhl 1995) and chromosome (Bruun 1930, 1932; Ornduff 1967, 1968; Moore 1973, 1974, 1977; Goldblatt 1981, 1984, 1985, 1988; Goldblatt and Johnson 1990, 1991, 1994, 1996, 1998) literature. Our coding of leaf vernation in *Primula* subgenus *Sphondylia* as conduplicate is explained in the text. The evolution of vernation type is plotted onto the topology as reconstructed using Fitch parsimony. Reconstruction of the trait is equivocal at the basal trichotomy of /*Primula*, and thus bars are not placed on those branches. If clade VI is sister to the other two, its involute vernation is reconstructed as the plesiomorphic condition (also found in taxa outside of /*Primula*); if this is not the case, then inheritance of a plesiomorphic involute condition or the independent origin of the involute condition in clade V are equally parsimonious.

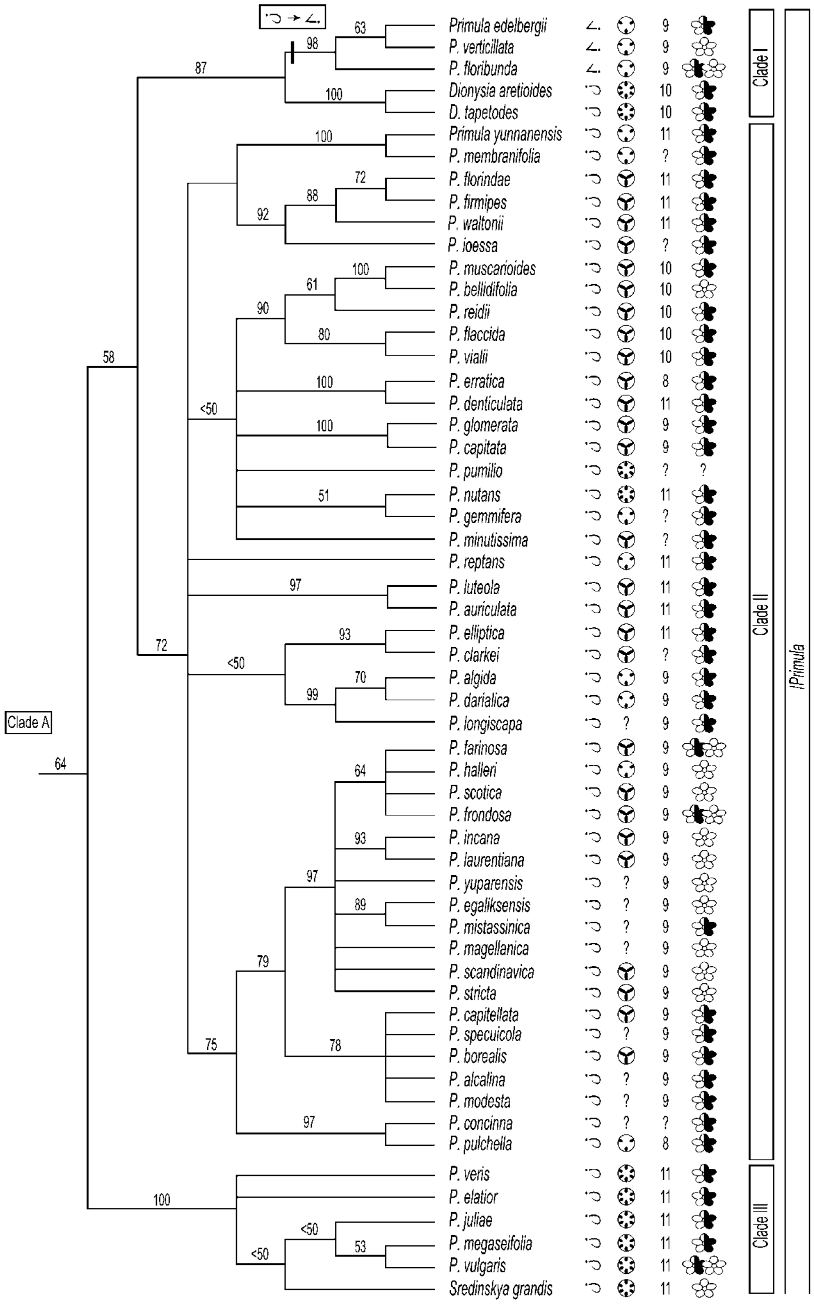

Fig. 4 (*Continued*)

apomorphy for the remaining members of *Primula* and thus expect them to be monophyletic. However, this is not the case in the cpDNA phylogeny (fig. 3) because subgenus *Sphondylia* is nested in one of the two revolute clades forming the trichotomy with the involute clade V (subgenus *Auriculastrum* and *Dodecatheon*) at the base of */Primula*. Given this topology, involute vernation in subgenus *Sphondylia* is likely to be independently derived and thus not homologous to the condition in clade V.

This is not the first suggestion of differences between the vernation of subgenera *Sphondylia* and *Auriculastrum*. Observations of significant differences were made first by Watt (1904), who suggested that representatives of subgenus *Sphondylia* actually showed conduplicate vernation, where the leaf surface is folded inward like a book rather than rolled inward as it is in "true" involute vernation. Smith and Forrest (1928) do not cite Watt's (1904) observation but rather lump the two subgenera together in their "Involutae" group, and Watt's distinction seems to have gone unnoticed by most later workers (except Blasdale [1948], who agreed with it). Recent examination of plants in both subgenera by one of us (S. Kelso) supports Watt's (1904) distinction between the early development of the broad, thin leaves of subgenus *Sphondylia* and that of the narrower, fleshy leaves of subgenus *Auriculastrum*. We believe that his observation should join cytology and morphology in distinguishing the two subgenera.

Clade II: Primula *Subgenus* Muscarioides *and Part of Subgenus* Aleuritia

The largest of Wendelbo's (1961*b*) subgenera is *Aleuritia* (15 sections), which Richards (1993) expanded to include all members of Wendelbo's (1961*b*) subgenus *Muscarioides* and some of his subgenus *Craibia* (section *Petiolares* but not sections *Davidii* or *Chartacea*). Subgenus *Aleuritia* is dispersed among three clades in the cpDNA phylogeny (fig. 3). The largest and most diverse of these three is clade II, which also includes all sampled members of Wendelbo's subgenus *Muscarioides* but no representatives of his subgenus *Craibia*.

The 12 sections in clade II (159 species in total) (estimates of clade size assume that all species of sections that are represented in the clade are part of that clade; species of sections not currently represented in the study are not part of this calculation) are considerably diverse, as noted above in the case of pollen type and chromosome base number (fig. 4). We are unable to suggest a unique morphological synapomorphy for the group with our present understanding of the 12 sections. The small section *Glabra* (two species), one of the two sections of subgenus *Aleuritia* not represented in this study, strongly resembles section *Armerina* in having efarinose, spoon-shaped leaves, although it differs in having a chromosome base number of $x=8$, rather than $x=10$ or 11, and syncolpate, rather than stephanocolpate, pollen (Richards 1993). We expect section *Glabra* to join clade II when it is sampled.

Seven of Richards's (1993) 12 sections in clade II are not monophyletic with the current character and taxon sampling (fig. 3). These are sections *Muscarioides*, *Soldanelloides*, *Denticulata*, *Armerina*, *Aleuritia*, *Minutissimae*, and *Oreophlomis*. We will briefly consider the first five of these sections in turn. Branches disrupting the monophyly of sections *Minutissimae* and *Oreophlomis* are poorly supported (<50% bootstrap), and we await more robust results before commenting on these sections.

Members of section *Muscarioides* are interdigitated with members of section *Soldanelloides*. The two sections uniquely share a chromosome base number of $x=10$ (fig. 4; Bruun 1930) and the presence of articulated hairs (Wendelbo 1961*b*) within clade II. They are also involved in one of two putative intersectional hybrids in the genus, that between *Primula vialii* (section *Muscarioides*) and *Primula flaccida* (section *Soldanelloides*). This evidence led Richards (1993) to consider lumping the sections together—a proposal supported by the cpDNA phylogeny (fig. 3).

The two representatives of section *Denticulata* are each sister to an extrasectional taxon (fig. 3): *Primula denticulata* to the unplaced *Primula erratica* of Richards (1993) and *Primula glomerata* to Richards's monotypic section *Capitatae*. Richards (1993) noticed similarities between section *Denticulata* and *P. erratica* and removed the latter from section *Aleuritia*, where it was traditionally placed. However, rather than placing it in section *Denticulata*, he left it unplaced because of its difference in chromosome base number (fig. 4). The cpDNA phylogeny (fig. 3) offers preliminary support for the placement of *P. erratica* in section *Denticulata* given its sister relationship with *P. denticulata*, the type species for that section.

Chromosome base number similarly played a critical role in Richards's (1993) decision that *P. glomerata* ($2n=44$; Bruun 1930, using the synonym *Primula crispa*) is more closely related to section *Denticulata* ($x=11$) than to section *Capitatae* ($x=9$). However, in this case, incongruence arises between chromosome counts for *P. glomerata* because a later count of $2n=18$ (Kress 1969), which was not considered by Richards (1993), is consistent with the close relationship reported here (fig. 4). This close relationship was suspected by Smith and Fletcher (1944*b*), who placed *P. glomerata* in section *Capitatae* despite Bruun's (1930) count. Treatment of *P. glomerata* as a member of section *Capitatae* is supported by the cpDNA phylogeny (fig. 3).

One of the three sampled members of section *Armerina*, *Primula nutans*, is sister to the unplaced species *Primula gemmifera* of Richards (1993), while a second, *Primula pumilio*, is part of a polytomy with these two and might be resolved as sister to them with further character sampling. Richards (1993) recognized a similarity between the rhizomatous growth forms of *P. gemmifera* and section *Armerina*, removing the former from its traditional position in section *Aleuritia* (Smith and Fletcher 1943*a*) but leaving it unplaced. Inclusion of *P. gemmifera* in section *Armerina* is supported by the cpDNA phylogeny (fig. 3).

The third member of section *Armerina*, *Primula egaliksensis*, is hypothesized to be the product of an intersectional hybridization between *P. nutans* (section *Armerina*) and *Primula mistassinica* (section *Aleuritia*) based on cytology and the morphology of pollen, glands, and vegetative parts (Kelso 1991). If this origin of *P. egaliksensis* is accepted, its position in the cpDNA tree (fig. 3) strongly supports the maternal contribution of *P. mistassinica* to it. Current work by our group on nuclear data sets for the genus will further test the origins of this putative allopolyploid.

Clade III: Primula *Subgenus* Primula *and* Sredinskya

Clade III contains *Primula* subgenus *Primula* (six species in one section) and the monotypic genus *Sredinskya*. These two groups have in common a chromosome base number of $x=11$ and stephanocolpate pollen—a type unusual in the genus (fig. 4). Most recent authors (Wendelbo 1961*b*; Fenderson 1986; Richards 1993) have recognized the similarity of *Sredinskya* to subgenus *Primula* but have maintained it as a separate genus. However, Halda (1992) recognized it as a member of genus *Primula* and placed it in its own subgenus (subgenus *Sredinskya*). *Sredinskya* differs from members of subgenus *Primula* as a homostyle (fig. 4; though some populations of *Primula vulgaris* are also homostylous) with erect petal lobes and a very long style that is exserted beyond the corolla.

Richards's (1993) section *Primula* is composed of species that other recent treatments (Wendelbo 1961*b*; Fenderson 1986; Halda 1992) split into three different sections: *Megaseifolia* Balf. f. (*Primula megaseifolia* and *Primula renifolia* Volg.), *Julia* Fed. & Los. (*Primula juliae*), and *Primula* (the remaining species). In accepting an expanded section *Primula*, Richards treated the group as had Smith and Fletcher (1948*a*) based on calyx and corolla similarities, the shared presence of articulated hairs, and cytology (Bruun 1930, 1932). The cpDNA phylogeny offers weak support for an expanded section *Primula* (fig. 3) because representatives of sections *Megaseifolia* and *Julia* are nested in the clade of section *Primula* s.str., though the bootstrap support of branches placing them there is 53% and lower.

Clade IV: Primula *Subgenus* Craibia *and Part of Subgenus* Aleuritia

Clade IV contains a majority of the remaining members of Wendelbo's (1961*b*) subgenus *Aleuritia*, as well as his subgenus *Craibia* and section *Pinnatae* of subgenus *Auganthus*. Each of the sections sampled in clade IV is either resolved as monophyletic or its representatives are involved in the same polytomy and could be resolved as monophyletic with additional character sampling. Together, the seven represented sections contain 119 species.

Members of this clade, with the exception of *Primula boothii* ($2n=20$; Richards 1993), have in common a chromosome base number of $x=11$ (fig. 4). However, reconstruction of chromosome base number on the cpDNA phylogeny (A. Mast, unpublished data) indicates that this is the primitive condition for the clade and thus not a synapomorphy. As discussed in the section on clade V, membership in this clade of the unrepresented section *Amethystina* ($x=11$) is possible but uncertain at this time.

Members of section *Pinnatae* (four species from eastern China) are poorly known but have been maintained in subgenus *Auganthus* by all recent treatments (Wendelbo 1961*b*; Fenderson 1986; Halda 1992; Richards 1993). Richards (1993) based his placement of the section on its similarity to other members of subgenus *Auganthus* in inflorescence characters and their shared presence of multicellular hairs and absence of farina. Based on pollen observations, Wendelbo (1961*b*) suggested that the section might be better placed in subgenus *Aleuritia*, but he did not have enough confidence in the similarities to make the change. The chromosome base number of the section is unknown (Richards 1993), but a count of $x=11$ would also support its placement in this clade over subgenus *Auganthus*, which typically has a chromosome base number of $x=12$.

Clade V: Primula *Subgenus* Auriculastrum *and* Dodecatheon

Clade V is composed of *Primula* subgenus *Auriculastrum* (30 species) and *Dodecatheon* (ca. 14 species; Thompson 1953). *Dodecatheon* shares with the subgenus involute vernation (fig. 4) and a chromosome base number of $x=11$ (fig. 4), and it is viewed as closely related to the subgenus by recent authors (Thompson 1953; Wendelbo 1961*c*; Richards 1993). However, *Dodecatheon* differs from *Primula* in traits apparently related to its buzz pollination, including its reflexed petals, filaments more or less united into a tube, thick connectives, and poricidal anthers (Wendelbo 1961*c*; Harder and Barclay 1994). Heterostyly appears to have been lost in *Dodecatheon* (fig. 4; based on an unpublished reconstruction by A. Mast using Fitch parsimony)—a change that is consistent with the shift to less precise placement of pollen on the pollinator.

The sister relationship between the North American genus *Dodecatheon* and *Primula suffrutescens* is unexpected, for *Dodecatheon* is more similar to *Primula* section *Parryi*. *Primula suffrutescens*, a member of section *Cuneifolia*, does occur in California, near the Pacific Northwest center of greatest diversity in *Dodecatheon* (Thompson 1953). However, the range of the western North American section *Parryi* (six spp.) is also found nearby, and it shares with *Dodecatheon* broadly cylindrical capsules and somewhat fleshy and lanceolate leaves (Richards 1993). Thompson (1953, p. 75) noted that *Dodecatheon jeffreyi* and *Primula parryi* are "virtually indistinguishable when the corollas and inserted anthers are removed," and one of us (S. Kelso) has observed that misidentifications are common on herbarium sheets with material in the fruiting condition. The habitat of *P. parryi*, along wet, subalpine stream banks, is also similar to that of *Dodecatheon*. One of us (D. M. S. Feller) is currently reexamining morphological similarities of these species to *Dodecatheon*, and finer-scale molecular systematic studies of *Dodecatheon* are currently underway by A. R. Mast, D. M. S. Feller, and E. Conti.

A wide geographical disjunction is seen in clade V between the principally western North American *Primula* sections *Parryi* and *Cuneifolia* (also distributed in Japan) and *Dodecatheon* (also in Siberia) and the European *Primula* section *Auricula* that could be bridged by the enigmatic *Primula* section *Amethystina* (Himalayas). Modern treatments (Wendelbo 1961*b*; Fenderson 1986; Halda 1992; Richards 1993) placed section *Amethystina* in subgenus *Aleuritia*, but as pointed out by Smith and Fletcher (1942*b*), its thick, toothed leaves and globose capsules are also strongly suggestive of sections *Cuneifolia* and *Auricula*. As discussed above, the vernation of subgenus *Auriculastrum* is uniquely involute in *Primula*, and leaf vernation could provide support for or against inclusion of section *Amethystina* in it.

Unfortunately, published observations of leaf vernation in section *Amethystina* are conflicting and thus inconclusive. Smith and Fletcher believed section *Amethystina* to have involute vernation based on observations by Cooper (in Smith and Fletcher 1942*b*, p. 209), but they later modified that view based on their own observations of revolute vernation in cultivated specimens of *Primula valentiniana* Hand.-Mazz., *Primula kingii* Watt, and *Primula dickieana* Watt (Smith and Fletcher 1950). Sketches by J. Haldova in Halda (1992) of *Primula amethystina* Franchet, *Primula faberi* Oliver, and *P. kingii* suggest involute vernation for those species. Richards (1993) accepted revolute vernation as the condition of the section, but Wendelbo (1961*b*) suggested that observations of both vernation types might be correct and that the section is thus polymorphic for the character. Clearly, this character state, and the possibly important biogeographic position of section *Amethystina* in clade V, must remain equivocal without further sampling.

Clade VI: Primula *Subgenus* Auganthus *and* Cortusa

Clade VI is composed of five of nine sections of *Primula* subgenus *Auganthus*, *Primula* section *Dryadifolia* (five species) of subgenus *Aleuritia*, and *Cortusa* (one to a few species according to Richards [1993]). Each of the five sections (constituting 65 species) of subgenus *Auganthus* is resolved as monophyletic or its representatives are involved in the same polytomy and could be resolved as monophyletic with additional character sampling. *Cortusa* shares with most sections of subgenus *Auganthus* a chromosome base number of $x=12$ (fig. 4), which is otherwise unknown in *Primula* (Richards 1993), as well as revolute vernation (fig. 4), articulated hairs, and lobed leaves (Wendelbo 1961*b*, 1961*c*). However, it differs from *Primula* in having a fused anther ring (Richards 1993).

The cytology of section *Dryadifolia* is unknown but would strongly support its placement in this clade if it shares the unusual chromosome base number of $x=12$. Richards (1993) suggested that section *Dryadifolia* might be closely related to section *Bullatae* of subgenus *Auganthus* based on their similarly woody, cushion-forming, rhizomatous growth forms. Oddly, these two sections are not sister in the cpDNA phylogeny, but instead, section *Bullatae* is sister to the short-lived and monocarpic section *Monocarpicae* (fig. 3).

Of the three unrepresented sections of subgenus *Auganthus*, sections *Auganthus* (two species from north-central China) and *Pycnoloba* (one species from south-central China) are known to have a chromosome base number of $x=12$, whereas the cytology of section *Malvacea* (five species from south-central China) is unknown. Based on their chromosome number, we expect sections *Auganthus* and *Pycnoloba* to become members of this clade when they are sampled. Richards (1993) contended that section *Malvacea* is typical of subgenus *Auganthus*, but strong support of its membership depends on future cytological or molecular study.

Conclusions

In summary, our cpDNA phylogeny demonstrates that four genera (*Dionysia*, *Sredinskya*, *Dodecatheon*, and *Cortusa*) are descendants of the most recent common ancestor of sampled members of *Primula*, whereas three genera also thought to share this distinction (*Omphalogramma*, *Soldanella*, and *Hottonia*) diverged prior to that most recent common ancestor. In recognition of these results, we define two rankless names for these sister lineages: /*Primula* and /*Soldanella*. The cpDNA results further demonstrate that a number of new subgeneric and sectional delimitations will be necessary, with the most dramatic changes expected in Wendelbo's (1961*b*) subgenus *Aleuritia*. Finally, several characters traditionally viewed as slowly evolving and thus of systematic value (e.g., pollen exine morphology and chromosome base number in the debate over the relationship of *Dionysia* and *Primula* subgenus *Sphondylia*) prove to be quite labile in the context of the cpDNA phylogeny. On the other hand, leaf vernation type (involute, revolute, or conduplicate) proves to be stable in that context. In fact, based on the derived nature of revolute leaf vernation (fig. 4), we predict that further character sampling will resolve the basal trichotomy of /*Primula* with the involute clade V sister to the two revolute clades.

Future sampling of one or more nuclear linkage partitions will be particularly important for a more complete view of the organismal phylogeny (as suggested by Rieseberg and Soltis 1991; Rieseberg and Brunsfeld 1992) prior to taxonomic revisions. Members of six sections are known to hybridize in the wild (compiled from Richards 1993), though only two intersectional hybrids have been suggested (each discussed above). A sampling of single- or low-copy nuclear DNA regions is currently underway by the authors.

Acknowledgments

We thank the many collectors and botanical gardens that generously contributed material for this research and whose names are listed in the appendix. Special thanks is extended to Nikola Repke and Regula Hauser, who collected material for us at private and public gardens in Scotland and northern England in the spring of 2000. We gratefully acknowledge the initial encouragement for this study by Peter Raven. Robert Kuzoff and Sean Graham made helpful comments on the manuscript. This project was funded by the Swiss National Fund grant 3100-061674.00/1 to E. Conti.

Appendix

Table A1

Collection Data

Taxon	Voucher or source	GenBank accession number	
		trnL intron	*rpl16* intron
Subgenus *Sphondylia* (Duby) Rupr.:			
Section *Sphondylia*:			
Primula verticillata Forsskal	Cultivated by A. J. Richards; wild collection in Yemen; photo-vouchered	AF402335	AF402453
P. edelbergii O. Schwarz	Cultivated by A. J. Richards; ex Göteborg Botanic Garden; photo-vouchered	AF402334	AF402452
P. floribunda Wallich	Cultivated by A. J. Richards; ex Royal Botanic Garden Edinburgh; photo-vouchered	AF402336	AF402454

Table A1

(*Continued*)

Taxon	Voucher or source	GenBank accession number	
		trnL intron	*rpl16* intron
Subgenus *Auriculastrum* Schott:			
Section *Auricula* Duby:			
P. palinuri Petagna	A. Anderberg SU-S-00.96.2 (S)	AF402412	AF402532
P. clusiana Tausch.	Cultivated by R. McBeath	AF402408	AF402528
P. marginata Curtis	A. R. Mast 424 (Z)	AF402410	AF402530
P. glutinosa Wulfen in Jacq.	Cultivated by Tromsø Botanic Garden (acc. 95-598.1)	AF402413	AF402533
P. deorum Velen.	Cultivated by P. Eveleigh; wild collection in Bulgaria	AF402411	AF402531
P. integrifolia L.	Cultivated by R. McBeath	AF402409	AF402529
Section *Cuneifolia* Balf.:			
P. cuneifolia Ledeb.	C. Parker 6956 (ALA)	AF402414	AF402534
P. suffrutescens A. Gray	Cultivated by Keith Lever; photo-vouchered	AF402418	AF402538
P. nipponica Yatabe	Cultivated by Ian Scott; ex J. Sutherland	AF402415	AF402535
Section *Parryi* W. W. Smith ex Wendelbo:			
P. parryi A. Gray	Cultivated by R. McBeath; wild seed collection	AF402417	AF402537
P. angustifolia Torrey	T. Kelso s.n. (COCO)	AF402416	AF402536
Subgenus *Primula*:			
Section *Primula*:			
P. veris L. ssp. *veris*	A. R. Mast 423 (Z)	AF402383	AF402503
P. elatior (L.) Hill	A. R. Mast 425 (Z)	AF402384	AF402504
P. vulgaris Hudson	A. R. Mast 421 (Z)	AF402386	AF402506
P. megaseifolia Boiss. & Bal.	Cultivated by J. Mattingley; photo-vouchered	AF402387	AF402507
P. juliae Kusnetsow	A. R. Mast 420 (Z)	AF402388	AF402508
Subgenus *Auganthus* (Link) Wendelbo:			
Section *Auganthus* (Link) Pax ex Balf. f.	Unrepresented		
Section *Monocarpicae* Franchet ex Pax:			
P. malacoides Franchet	Cultivated by Zürich Stadtgärtnerei	AF402421	AF402541
P. forbesii Franchet	Y.-M. Yuan s.n. (Z)	AF402420	AF402540
Section *Obconicolisteri* Balf. f.:			
P. obconica Hance	Cultivated by Zürich Stadtgärtnerei	AF402422	AF402542
Section *Malvacea* Balf. f.	Unrepresented		
Section *Pycnoloba* Balf. f.	Unrepresented		
Section *Pinnatae* Knuth:			
P. cicutariifolia Pax	Luo 609 (MO)	AF402389	AF402509
Section *Reinii* Balf. f.:			
P. takedana Tatew.	Cultivated by J. Mattingley; photo-vouchered	AF402423	AF402543
P. hidakana Miyabe & Kudo	Cultivated by K. Lever; ex J. Sutherland	AF402424	AF402544
Section *Cortusoides* Balf. f.:			
P. mollis Nutt.	Cultivated by A. J. Richards; ex Glasgow Botanic Garden; photo-vouchered	AF402427	AF402547
P. geraniifolia Hook. f.	Cultivated by I. Scott; wild collection; photo-vouchered	AF402426	AF402546
P. septemloba Franchet	Cultivated by K. Lever; ex J. Mattingley	AF402425	AF402545
Section *Bullatae* Pax:			
P. forrestii Balf. f.	Cultivated by A. J. Richards; wild collection in Yunnan; photo-vouchered	AF402430	AF402549
P. bracteata Franchet	Cultivated by A. J. Richards; wild collection in Yunnan; photo-vouchered	AF402429	AF402548
Subgenus *Carolinella* (Helmsley) Wendelbo:			
Section *Carolinella* (Helmsley) Pax	Unrepresented		
Unplaced sections:			
Section *Chartacea* Balf. f.	Unrepresented		
Section *Davidii* Balf. f.:			
P. taliensis Forrest	Cultivated by I. Scott; ex J. Mattingley; photo-vouchered	AF402390	AF402510

Table A1

(*Continued*)

Taxon	Voucher or source	GenBank accession number	
		trnL intron	*rpl16* intron
Subgenus *Aleuritia* (Duby) Wendelbo:			
Section *Petiolares* Pax:			
P. petiolaris Wallich in Roxburgh	Cultivated by K. Lever	AF402392	AF402512
P. boothii Craib l.c. ssp. *autumnalis* A. J. Richards	Cultivated by K. Lever, ex Ness	AF402391	AF402511
P. sonchifolia Franchet	Cultivated by J. Mattingley; photo-vouchered	AF402393	AF402513
P. calderiana Balf. f. & Cooper	Cultivated by I. Scott; ex H. & M. Taylor; photo-vouchered	AF402394	AF402514
Section *Crystallophlomis* (Rupr.) Federov:			
P. elongata Watt	B. Dickore 10877 (GOET)	AF402404	AF402524
P. tschuktschorum Kjellman	D. Murray and R. Lipkin 12280 (ALA)	AF402403	AF402523
P. eximia Greene	C. Parker 6961 (ALA)	AF402402	AF402522
P. advena Smith	B. Dickore 9071 (GOET)	AF402405	AF402525
Section *Cordifoliae* Pax:			
P. rotundifolia Wallich	Cultivated by R. McBeath	AF402407	AF402527
Section *Amethystina* Balf. f.	Unrepresented		
Section *Proliferae* Pax:			
P. prolifera Wallich	Cultivated by R. McBeath	AF402401	AF402521
P. chungensis Balf. f. & Ward	Cultivated by J. Mattingley; photo-vouchered	AF402396	AF402516
P. cockburniana Hemsl.	Cultivated by Tromsø Botanic Garden (acc. 91-130)	AF402395	AF402515
P. prenantha Balf. f. & Smith	Cultivated by K. Lever; ex J. Mattingley; photo-vouchered	AF402399	AF402519
P. poissonii Franchet	Cultivated by K. Lever	AF402397	AF402517
P. wilsonii Dunn	Cultivated by R. McBeath	AF402398	AF402518
P. secundiflora Franchet	Cultivated by Tromsø Botanic Garden (acc. 92-1051)	AF402400	AF402520
Section *Sikkimensis* Balf. f.:			
P. florindae Ward	Cultivated by Tromsø Botanic Garden (acc. 92-1063)	AF402379	AF402499
P. waltonii Watt ex. Balf. f.	Cultivated by Tromsø Botanic Garden (acc. 92-1072)	AF402380	AF402500
P. ioessa Smith	Cultivated by Tromsø Botanic Garden (acc. 92-1052)	AF402381	AF402501
P. firmipes Balf. f.	Cultivated by Tromsø Botanic Garden (acc. 92-182)	AF402382	AF402502
Section *Oreophlomis* (Ruprecht) Federov:			
P. auriculata Lam.	Cultivated by R. McBeath; wild collection in Georgia; photo-vouchered	AF402344	AF402462
P. clarkei Watt	Cultivated by I. Scott; photo-vouchered	AF402342	AF402460
P. elliptica Royle	Cultivated by F. Carrie; wild collection in Kashmir; photo-vouchered	AF402341	AF402459
P. luteola Rupr.	M. Davlianidze & G. Azabuli s.n. (Z)	AF402343	AF402461
Section *Fedtschenkoana* Wendelbo in Rechinger:			
P. fedtschenkoi Regel	Cultivated by A. J. Richards; ex Göteborg Botanic Garden; photo-vouchered	AF402406	AF402526
Section *Armerina* Lindley:			
P. nutans Georgi	C. Parker 6966 (ALA)	AF402374	AF402494
P. egaliksensis Wormsk. in Hornem.	T. Kelso 00-150 (COCO)	AF402363	AF402481
P. pumilio Maxim.	B. Dickore 9496 (GOET)	AF402373	AF402493
Section *Glabra* (Smith & Fletcher) A. J. Richards	Unrepresented		
Section *Yunnanensis* Balf. f.:			
P. yunnanensis Franchet	Y.-M. Yuan s.n. (Z)	AF402339	AF402457

Table A1

(Continued)

Taxon	Voucher or source	GenBank accession number	
		trnL intron	*rpl16* intron
Section *Aleuritia* Duby:			
P. farinosa L.	Cultivated by A. J. Richards; wild collection at Gait Barrow, Cumbria, U.K.; photo-vouchered	AF402356	AF402474
P. frondosa Janka	Cultivated by T. Kelso; photo-vouchered	AF402359	AF402477
P. halleri Gmel.	Cultivated by K. Lever; photo-vouchered	AF402357	AF402475
P. algida Adams	M. Davlianidze & M. Bokezia s.n. (Z)	AF402350	AF402468
P. darialica Rupr.	M. Davlianidze & M. Bokezia s.n. (Z)	AF402352	AF402470
P. capitellata Boiss.	Cultivated by K. Lever	…	AF402486
P. longiscapa Ledeb.	Cultivated by A. J. Richards; wild collection at Alma Altai; photo-vouchered	AF402351	AF402469
P. scotica Hooker	Cultivated by K. Lever	AF402358	AF402476
P. scandinavica (Bruun) Bruun	Cultivated by A. J. Richards; wild collection at Bodo, Norway; photo-vouchered	AF402365	AF402483
P. stricta Hornem	Cultivated by J. Mattingley; photo-vouchered	AF402366	AF402484
P. incana M. E. Jones	Cultivated by E. Conti; wild collection at Fairbanks, Alaska	AF402360	AF402478
P. laurentiana Fernald	Cultivated by T. Kelso; ex Berry Botanical Garden; photo-vouchered	AF402361	AF402479
P. borealis Duby	C. Parker 7537 (ALA)	AF402369	AF402488
P. mistassinica Michaux	C. E. Hellquist 939 & E. G. Voss (Z)	AF402367	AF402485
P. specuicola Rydb.	T. Kelso 00-59 (COCO)	AF402368	AF402487
P. alcalina Cholewa & Henderson	R. Lehman s.n. [Z]	AF402370	AF402489
P. magellanica Lehm.	Cultivated by K. Lever; ex J. Dennis	AF402364	AF402482
P. modesta Bisset & Moore	Cultivated by A. J. Richards; photo-vouchered	AF402371	AF402490
P. yuparensis Takeda	Cultivated by K. Lever; ex J. Sutherland	AF402362	AF402480
P. concinna Watt	J. D. A. Stainton 366 (A)	…	AF402491
Uncertain position:			
P. efarinosa Pax	Unrepresented		
Section *Pulchella* (Smith & Fletcher) A. J. Richards:			
P. pulchella Franchet	Cultivated by K. Lever; wild collection in Szechuan, China	AF402372	AF402492
Uncertain position (group A):			
P. gemmifera Batalin	Cultivated by A. J. Richards; wild collection; photo-vouchered	AF402375	AF402495
Uncertain position (group B):			
P. erratica Smith	Cultivated by A. J. Richards; wild collection near Benyilan, China; photo-vouchered	AF402353	AF402471
Section *Souliei* Balf. f.:			
P. membranifolia Franchet	Sino-American Botanical Expedition 1067 (A)	AF402340	AF402458
Section *Minutissimae* Pax:			
P. minutissima Jacq.	Cultivated by Tromsø Botanic Garden (acc. 94-460)	AF402355	AF402473
P. reptans Hook. f.	Cultivated by I. Scott; photo-vouchered	AF402376	AF402496
Section *Dryadifolia* Balf. f.:			
P. dryadifolia Franchet	Cultivated by A. Furness	AF402432	AF402551
P. dryadifolia Franchet	Cultivated by R. McBeath	AF402431	AF402550
Section *Denticulata* Watt:			
P. denticulata J. E. Smith	Cultivated by J. Mattingley; photo-vouchered	AF402354	AF402472
P. glomerata Pax	Cultivated by R. McBeath	AF402377	AF402497
Section *Capitatae* Pax:			
P. capitata Hook. f.	Cultivated by K. Lever	AF402378	AF402498
Section *Muscarioides* Balf. f.:			
P. muscarioides Hemsley	Cultivated by K. Lever	AF402345	AF402463

Table A1

(*Continued*)

Taxon	Voucher or source	GenBank accession number	
		trnL intron	*rpl16* intron
P. bellidifolia King	Cultivated by R. McBeath; photo-vouchered	AF402346	AF402464
P. vialii Franchet	Cultivated by Zürich Botanic Garden	AF402348	AF402466
Section *Soldanelloides* Pax:			
P. reidii Duthie	Cultivated by A. J. Richards; photo-vouchered	AF402349	AF402467
P. flaccida Balakr.	Cultivated by K. Lever	AF402347	AF402465
Other taxa in *Primulaceae* s.str.:			
Androsace sempervivoides Jacq.	Cultivated by Zürich Botanic Garden (acc. 19790157)	AF402436	AF402555
Androsace chamaejasme Wulf.	E. Conti s.n. (ALA)	AF402437	AF402556
Cortusa matthioli L.	Cultivated by Old Göttingen Botanic Garden, photo-vouchered	AF402428	...
Dionysia aretioides (Lehm.) Boissier	Cultivated by University of Copenhagen Botanic Garden (acc. P1975-5514)	AF402337	AF402455
Dionysia tapetodes Bunge	Cultivated by University of Copenhagen Botanic Garden (acc. P1982-5303)	AF402338	AF402456
Dodecatheon pulchellum (Raf.) Merr.	E. Conti s.n. (Z)	AF402419	AF402539
Douglasia beringensis	C. Parker 8098 (ALA)	AF402438	AF402557
Hottonia palustris	Cultivated by Zürich Botanic Garden (acc. 19971659)	AF402435	AF402554
Pomatosace filicula	B. Dickore 9234 (GOET)	AF402440	AF402559
*Omphalogramma*sp.	Alpine Rock Garden Society seed collection acc. 0705	AF402433	AF402552
Soldanella pusilla	Cultivated by Zürich Botanic Garden (acc. 19990226)	AF402434	AF402553
Sredinskya grandis	Cultivated by R. McBeath	AF402385	AF402505
Vitaliana primuliflora	Cultivated by T. Kelso; photo-vouchered	AF402439	AF402558
Myrsinaceae s.l.:			
Aegiceras corniculatum	Cultivated by University of Copenhagen Botanic Garden (acc. S1974-0467)	AF402446	AF402565
Ardisia speciosa	Cultivated by Zürich Botanic Garden (acc. 19963503)	AF402448	AF402567
Ardisiandra wettsteinii R. Wagner	Cultivated by Royal Botanic Garden Edinburgh	AF402441	AF402560
Asterolinon linum-stellatum	Cultivated by Old Göttingen Botanic Garden, photo-vouchered	AF402442	AF402561
Cyclamen purpurascens Miller	Cultivated by Zürich Botanic Garden (acc. 19790026)	AF402445	AF402564
Glaux maritima	Cultivated by Old Göttingen Botanic Garden; photo-vouchered	AF402444	AF402563
Lysimachia nummularia	Cultivated by Zürich Botanic Garden (acc. 19966348)	AF402443	AF402562
Myrsine africana	Cultivated by Zürich Botanic Garden (acc. 19964489)	AF402447	AF402566
*Theophrastaceae*s.l.:			
Clavija spinosa	Cultivated by University of Copenhagen Botanic Garden (acc. P1951-5178)	AF402450	AF402569
Theophrasta americana	Cultivated by University of Copenhagen Botanic Garden (acc. P1997-5273)	AF402449	AF402568
Maesaceae:			
Maesa japonica	Cultivated by University of Copenhagen Botanic Garden (acc. S1982-0919)	AF402451	AF402570

Note. Material from *Primula* organized according to Richards's (1993) treatment.

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