AN ABSTRACT OF THE THESIS OF

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A molecular systematic investigation into the *Mimulus moschatus* alliance (Scrophulariaceae) was undertaken to determine phylogenetic patterns and test taxonomic hypotheses among a group of rare endemics centered in western North America. *Mimulus* is a speciose genus of vascular plants composed of many closely allied species complexes divided into ten sections. Section *Paradanthus* contains many such complexes thought to be derived from within other sections of the genus. Therein lies a rare alliance of species complexes centered around the widespread *M. moschatus*. Although various taxonomic treatments and phylogenetic hypotheses have been proposed, most remain untested. In addition, ten of the twelve species comprising this alliance are of conservation concern. We have examined these taxonomic and phylogenetic hypotheses by comparing DNA sequences from the nuclear ITS region and chloroplast *rpl16* intron under both maximum parsimony and maximum likelihood criteria. Outgroups included accessions from sections *Paradanthus, Simiolus and Eunanus*. An exceptionally strong stem-loop structure in ITS1 inhibited PCR and direct sequencing in some accessions. *Rpl16* intron

substitution types included small inversions, mononucleotide repeat length variations, and tandem repeat duplications. The ITS region and *rpl16* intron topologies differ only slightly within the *M. moschatus* alliance. Incongruence between the data sets was isolated to *M. alsinoides*, although a "total evidence" approach provided improved resolution in determining the sister taxon to the *M. moschatus* alliance. River drainage specific phylogeographic patterns among three species complexes within the *M. moschatus* alliance are apparent from the combined analysis. Multiple origins of reduced corolla size, cliff dwelling habit, seed dormancy, and vegetative reproduction are apparent from this molecular phylogeny. The results support taxonomic recognition of many rare species of conservation concern including *M. patulus, M. hymenophyllus, M. ampiatus, M. dudleyi* and *M. evanescens*. Although determination of the conservation implications of a molecular phylogeny relies on a synthesis of available data sources, a molecular phylogeny is an efficient means of testing evolutionary patterns and phylogenetic hypotheses for morphologically difficult species alliances.

A Molecular Phylogeny for the *Mimulus moschatus* Alliance (Scrophulariaceae) and its Conservation Implications.

by

Justen Bryant Whittall

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Justen Bryant Whittall, Author

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CONTRIBUTION OF AUTHORS

This thesis is the result of many peoples' efforts. The molecular systematics research was conducted in the laboratory of Dr. Aaron Liston. In addition, he assisted with methodology, technique, contributed to data interpretation, and made numerous editing suggestions. Dr. Robert J. Meinke developed the original study design and contributed to data interpretation and conservation implications of this research. Matt Carlson helped initiate the study and has been intimately involved through contributing plant material, assisting in the field, helping with the study design, and participating in the data interpretation.

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DEDICATION

The inspiration and dedication of my late grandfather, Ernest Whittall, is a motivating force in my life and work.

PREFACE

"Any discussion of the phylogenetic development of this group, as of any group including a large number of closely related species, must, of necessity, be more or less hypothetical. Nevertheless, ..."

-- Adele Lewis Grant, in A monograph of the genus *Mimulus* (1924)

A MOLECULAR PHYLOGENY FOR THE *MIMULUS MOSCHATUS* ALLIANCE (SCROPHULARIACEAE) AND ITS CONSERVATION IMPLICATIONS

CHAPTER 1

INTRODUCTION

The Endangered Species Act (1973) was designed to protect species from extinction throughout all or a significant portion of their range and includes provisions for designating critical habitat. The primary biological goals of the act are to preserve evolutionary legacy and maintain reservoirs of genetic diversity. Although endangered species includes subspecies and varieties of vertebrates, invertebrates, and plants, the ESA only recognizes distinct populations of vertebrates. Threatened and endangered plants and invertebrates still require taxonomic ranking for ESA protection. Therefore, protection of threatened and endangered plants depends on accurate circumscription of the taxa.

THE STUDY TAXA

A phylogenetic taxonomy attempts to classify species based on phylogenetic hypotheses. Taxonomic rankings represent testable phylogenetic statements. Accordingly, species within a genus ought to be more closely related to one another than to members of another genus. Although often created without explicit phylogenetic data (i.e. gestalt decisions), traditional classification systems provide hypotheses that can be evaluated using explicit systematic methods. Morphometric, cytological, chemotaxonomic, and

molecular techniques provide comparative phylogenetic data to critically examine phylogenetic hypotheses. Improved phylogenetic resolution in rare species complexes that are characterized by taxonomic uncertainty will improve the delineation of taxa, a vital component in determining conservation status.

The speciose genus of vascular plants, *Mimulus* L. with many closely allied species complexes in western North America and elsewhere is an appropriate group in which to consider the union of systematics and conservation biology. Over 100 species have been divided into nine apparently natural sections and one clearly artificial one, section *Paradanthus* Grant. The latter is a grouping of several closely related species alliances likely derived from within other sections (Grant 1924, Meinke 1992). One alliance centered around the widespread *M. moschatus* Lindley remains phylogenetically and taxonomically problematic yet contains many taxa of conservation concern.

Ten of the twelve species ascribed to the *M. moschatus* alliance (Argue 1986, Meinke 1992, Carlson unpublished) have been considered for listing by conservation agencies (California Department of Fish and Game 1999, Oregon Natural Heritage Program 1998, Skinner and Pavlik 1994, Idaho Natural Heritage Database 1999, Washington Department of Natural Resources 1999). This alliance is centered in western North America, the diversity focused between northern Oregon and the southern Sierra Nevada, California. The taxa are united by a combination of bilabiate yellow corollas, viscid pubescence, reduced calyx teeth, and acrescent petioles in fruit. Although each species occupies a unique habitat, all are restricted to vernally moist substrates. Uncertain

phylogenetic affinities within the alliance have led to a range of taxonomic interpretations for many of the species of conservation concern.

TAXONOMIC HISTORY

The most recent monographic work for the genus *Mimulus* is Grant's thorough review (1924) where she provided a phylogenetic chart suggesting the close relationships among nine of the twelve taxa comprising the *M. moschatus* alliance. A tenth member of this alliance, the small flowered *M. patulus* Pennell, first appeared in Pennell's treatment of the genus (1951), which followed Grant's taxonomy except for the exclusion of *M. ampliatus* Grant without note. Hitchcock and Cronquist (1959, 1973) recognized six of the eight taxa within the Pacific Northwest, synonymizing *M. ampliatus* and *M. patulus* with *M. washingtonensis* Gand. Munz (1959) recognized all seven Californian taxa of the *M. moschatus* alliance. *Mimulus norisii* Heckard and Shevock, from the southern Sierra Nevada, was described by Heckard and Shevock (1986) and included in Thompson's treatment of the genus for California (1992). Therein, Thompson recognized only five of the seven taxa in that range, reducing *M. dudleyi* Grant and *M. arenarius* Grant to synonymy within *M. floribundus* Lindley. Meinke (1983, 1992, 1995) described two new taxa *(M. hymenophyllus* Meinke and *M. evanescens* Meinke). Skinner and Pavlik (1996) followed Thompson (1992) and, in addition, did not recognize the newly described Modoc endemic *M. evanescens*.

Currently, appropriate taxonomic ranking for *M. patulus, M. ampliatus, M. evanescens*, *M. dudleyi* and *M. arenarius* remains ambiguous and stems, in part from phylogenetic

uncertainty. Without a foundation from which to build an appropriate conservation status, many of the rare taxa of the *M. moschatus* alliance remain legally unprotected (California Department of Fish and Wildlife 1999, Idaho Natural Heritage Program 1999, Oregon Natural Heritage Program 1998, Washington Department of Natural Resources 1999).

PHYLOGENETIC HYPOTHESES

Phylogenetic hypotheses generated by Grant (1924) have provided a framework for systematic treatments and guidance in affiliating newly described taxa with previously named species. Her proposed relationship of *M. arenarius* with *M. floribundus* is supported by palynological studies (Argue 1985). In addition, the alliance of *M. washingtonensis* with *M. ampliatus* is reflected in Meinke's suggested combination *M. washingtonensis* var. *ampliatus* (Meinke 1992). The phylogenetic affinity of *M. patulus* and *M. washingtonenesis,* as suggested by Hitchcock and Cronquist (1959), has been challenged by Meinke (1992) as he suggests dividing the latter into separate varieties. In the recent description of *M. norisii,* Heckard and Shevock (1986) suggest similarities with *M. floribundus*. Phylogenetic affinities of *M. evanescens* appear intermediate between *M. breviflorus* Piper and *M. latidens* (A. Gray) E. Greene (Meinke 1995). Previously, *M. hymenophyllus* has been compared with *M. jungermannioides* Suksd. based on their cliff dwelling habit (Meinke 1986). From this brief review, it is apparent that phylogenetic hypotheses abound in the *M. moschatus* alliance, but remain speculative (Meinke 1992, 1995). Convergence among morphological traits, especially those influenced by cliff-dwelling habit, breeding system, and seed dispersal may confound phylogenetic reconstructions relying on such characters.

TOTAL EVIDENCE

A total evidence approach to phylogenetic reconstruction is meant to include the many sources of comparative data available in order to capture evolutionary branching patterns. Through a combination of morphological, ecological, biogeographic, and molecular data, we can explicitly infer evolutionary relationships within closely related species alliances. The *M. moschatus* alliance has been well characterized morphologically, ecologically, and biogeographically (Grant 1924, Pennell 1951, Thompson 1992, Meinke 1983, 1992, 1995). Detailed morphological circumscriptions indicate the distinct boundaries between the taxa. Ecological work on selected taxa has further contributed to taxonomic circumscription through an understanding of population dynamics, pollination biology, and breeding systems. Field surveys have revealed the edaphic endemism and habitat threats of many rare taxa in the *M. moschatus* alliance. Even after the extensive systematic and life history studies to date, phylogenetic relationships and taxonomic ranking for many members remain uncertain. Determining phylogenetic relationships in species complexes where characteristic morphological traits can be significantly influenced by fluctuating environmental conditions can be misleading (Meinke 1992). An explicit examination of phylogenetic hypotheses using molecular markers could assist in clarifying evolutionary relationships, and help direct taxonomic ranking by contributing to ongoing efforts in delineating the members of the *M. moschatus* alliance.

NUCLEAR RIBOSOMAL INTERNAL TRANSCRIBED SPACER REGION

Nuclear ribosomal DNA internal transcribed spacers (ITS1 and ITS2) separate cistrons that encode the large and small rRNA ribosomal subunits. Within the last several years, the ITS region has contributed greatly to the understanding of evolutionary patterns among plant species (Baldwin et al. 1995, Soltis and Soltis 1998). The ITS1 and 2 are separated by the 5.8S subunit, transcribed with the entire rRNA repeat, yet spliced out before the rRNA subunits are incorporated into ribosomes. Splicing of the ITS1 and ITS2 involves secondary structures such as stem-loops which can be conserved across families, unlike most of the primary sequences (van der Sand et al. 1992, van Neus et al. 1995, Hershkovitz and Zimmer 1996). Flanked by the conserved ribosomal subunits, the ITS region's manageable size (ca. 650 bp in angiosperms) and length conservation facilitates both PCR and sequence alignment. Intraspecific ITS region variation is uncommon (Soltis and Kuzoff 1993, see Mayer et al. 1996 for exception), but if present can be detected by sampling multiple accessions from the same species representing diverse portions of its range. The rDNA tandem repeats are thought to be homogenized through processes of concerted evolution (Arnheim 1983), although intragenomic ITS heterogeneity is not uncommon (Buckler et al. 1997, Whittall et al. submitted). Such heterogeneity can be identified through RFLP analysis of ITS clones.

RPL16 CHLOROPLAST INTRON

Organellar markers can provide an independent phylogenetic measure to complement nuclear loci, illuminating sources of topological incongruence and verifying well

supported relationships. Its non-recombining nature coupled with low intraspecific diversity (reviewed in Soltis, Soltis and Milligan 1992) make the chloroplast genome an appealing marker for phylogenetic reconstruction. The resolving power of intron and spacer sequences in phylogenetic reconstruction has recently become widely employed in plant systematics. Noncoding regions, including spacers and introns, are the most rapidly evolving contiguous regions of the chloroplast genome (Wolfe and Sharp 1988). The approximately 1000 bp *rpl16* intron separates exons of the gene encoding the large ribosomal protein 16. This intron provides interspecific resolution in *Gossypium* (Small et al. 1998), *Lemna* (Jordan et al. 1996), and the Bambusoideae (Kelchner and Clark 1997). The *trnL* (UAA)-F(GAA) intergenic spacer evolves at rates similar or faster than *rbcL* sequences in Themidaceae (Fay et al. 1996) and Orchidaceae (Whitten et al. 1996), suggesting it too may be informative below the generic level. Among the protein coding regions in the chloroplast genome, *matK* (ca. 1550 bp) is one of the most rapidly evolving (Wolfe 1991). It has provided intergeneric and interspecific resolution in seed plants (Johnson and Soltis 1995, Xiang et al. 1998). A comparison of variation among the *matK* gene, *trnL-F* intergenic spacer and *rpl16* intron sequences for several species of *Thalictrum* indicates the *rpl16* intron offers the most phylogenetically informative characters within the limits of two primer sequencing (Liston, Brunet, and Thorsen unpublished). In addition, Small et al. (1998) reported that *rpl16* had more phylogenetically informative sites than seven other rapidly evolving chloroplast loci.

Herein, we report an investigation into the use of the ITS region and *rpl16* intron sequences for inferring phylogenetic relationships within the *M. moschatus* alliance.

Examination of evolutionary hypotheses and tests of phylogenetic hypotheses have delineated the unique lineages of the *M. moschatus* alliance. Through incorporation of available morphological, ecological, and biogeographic data, appropriate taxonomic rankings have been suggested with implications for conservation status among this group of rare endemics. In addition to developing a molecular phylogeny, we have investigated an exceptional ITS1 secondary structure, identified patterns of sequence evolution in the *rpl16* intron, and localized incongruence between data sets.

CHAPTER 2

A MOLECULAR PHYLOGENY FOR THE *MIMULUS MOSCHATUS* ALLIANCE (SCROPHULARIACEAE) AND ITS CONSERVTION IMPLICATIONS

Justen Bryant Whittall, Aaron Liston, Robert J. Meinke, and Matthew Carlson

ABSTRACT

A molecular systematic investigation into the *Mimulus moschatus* alliance (Scrophulariaceae) was undertaken to determine phylogenetic patterns and test taxonomic hypotheses among a group of rare endemics centered in western North America. Sequences from the nuclear ribosomal DNA ITS region and chloroplast *rpl16* intron were compared under both maximum parsimony and maximum likelihood criteria for the 12 putative species of the *M. moschatus* alliance. Outgroups included accessions from sections *Paradanthus, Simiolus* and *Eunanus*. An exceptionally strong stem-loop structure in ITS1 inhibited PCR and direct sequencing in some accessions. *Rpl16* intron substitution types included small inversions, mononucleotide repeat length variations, and tandem repeat duplications. The ITS region and *rpl16* intron topologies differ only slightly within the *M. moschatus* alliance. Incongruence between the data sets was isolated to *M. alsinoides*, although including this species in a combined ITS and *rpl16* analysis provided improved resolution and increased internal support for the sister taxon to the *M. moschatus* alliance. River drainage specific phylogeographic patterns among three species complexes within the *M. moschatus* alliance are apparent from the combined analysis. Multiple origins of reduced corolla size, cliff dwelling habit, seed dormancy, and vegetative reproduction are apparent from this molecular phylogeny. The results support taxonomic recognition of several rare species of conservation concern including *M. patulus, M. hymenophyllus, M. ampliatus, M. dudleyi,* and *M. evanescens*.

Keywords: *Mimulus moschatus* alliance, section *Paradanthus*, cpDNA, *rpl16* intron, ITS region, stem-loop, phylogeography, molecular phylogeny, conservation.

Mimulus L. (Scrophulariaceae) is a vascular plant genus comprised of over 100 species of predominantly annual herbs characterized by their five-angled calyces, bilabiate corollas, bi-lobed thigmotropic stigmas, four didynamous stamens, and opposite leaves with three palmate main veins. The genus is widely distributed throughout the Old and New Worlds, but most species are restricted to mesic habitats of western North America. Grant's monograph (1924) is the most extensive taxonomic treatment of the genus to date. Therein, she recognized 114 species in two subgenera and 10 sections. Although most of the sections are purported to represent natural groupings, the artificial section *Paradanthus* Grant is likely a polyphyletic grouping of species complexes that may be more appropriately allied with other sections of the genus (Grant, 1924). Interest in section *Paradanthus* has increased following the description of four new rare species from California and Oregon (Meinke, 1983, 1995; Heckard and Shevock, 1985; Heckard and Bacigalupi, 1986), recent nomenclatural changes (Thompson, 1992; von Bohlen, 1995a, 1995b) and the uncertain conservation status of several species (Meinke, 1992; Skinner and Pavlik, 1994; Oregon Natural Heritage Program, 1998).

The *Mimulus moschatus* Lindley alliance of section *Paradanthus* Grant consists of 12 herbaceous species (Grant, 1924; Argue, 1980, 1986; Meinke, 1983, 1992, 1995; Vickery, 1995). These species share 2n=32 (ex. *M. evanescens*, uncounted) and a unique combination of viscid pubescence, reduced calyx teeth, and acrescent fruiting petioles (Grant, 1924; Argue, 1986; Meinke, 1992). Although a few species are widespread, most

of these taxa are rare endemics of mesic basalt and granite substrates of California and the Pacific Northwest (Fig. 1). Various taxonomic rankings have been proposed for rare members of the *M. moschatus* alliance based on morphological (Grant, 1924; Pennell, 1951; Meinke, 1992), ecological and biogeographical (Grant, 1924; Meinke, 1983, 1992, 1995), and palynological characters (Argue, 1980, 1986). Specifically, this study was undertaken to examine the phylogenetic relationships and taxonomic status of six species of conservation concern with uncertain taxonomic ranking: *M. ampliatus* Grant, *M. arenarius* Grant, *M. dudleyi* Grant, *M. evanescens* Meinke, *M. hymenophyllus* Meinke, and *M. patulus* Penn. (Skinner and Pavlik, 1994; California Department of Fish and Wildlife, 1999; Idaho Natural Heritage Program, 1999; Oregon Natural Heritage Program, 1999; Washington Department of Natural Resources, 1999).

Spacer and intron sequences from nuclear rDNA (nrDNA) and chloroplast DNA (cpDNA) have been extensively utilized in interspecific phylogenetic reconstruction (reviewed in Baldwin et al., 1995; Small et al., 1998; Soltis and Soltis, 1998). Many examples of molecular phylogenies describe how methods based on a single locus can generate gene trees that conflict with the species tree (Neigel and Avise, 1986; Rieseberg and Soltis, 1991; Soltis and Kuzoff, 1995; Soltis et al., 1996) due to lineage sorting (reviewed in Wendel and Doyle, 1998) and chloroplast capture (Pamilo and Nei, 1988; see Johnson and Soltis, 1998 for an example). A combination of chloroplast and nuclear loci provides independent phylogenetic estimates that may elucidate areas of topological congruence and conflict. For a cpDNA phylogeny, we chose a rapidly evolving intron in

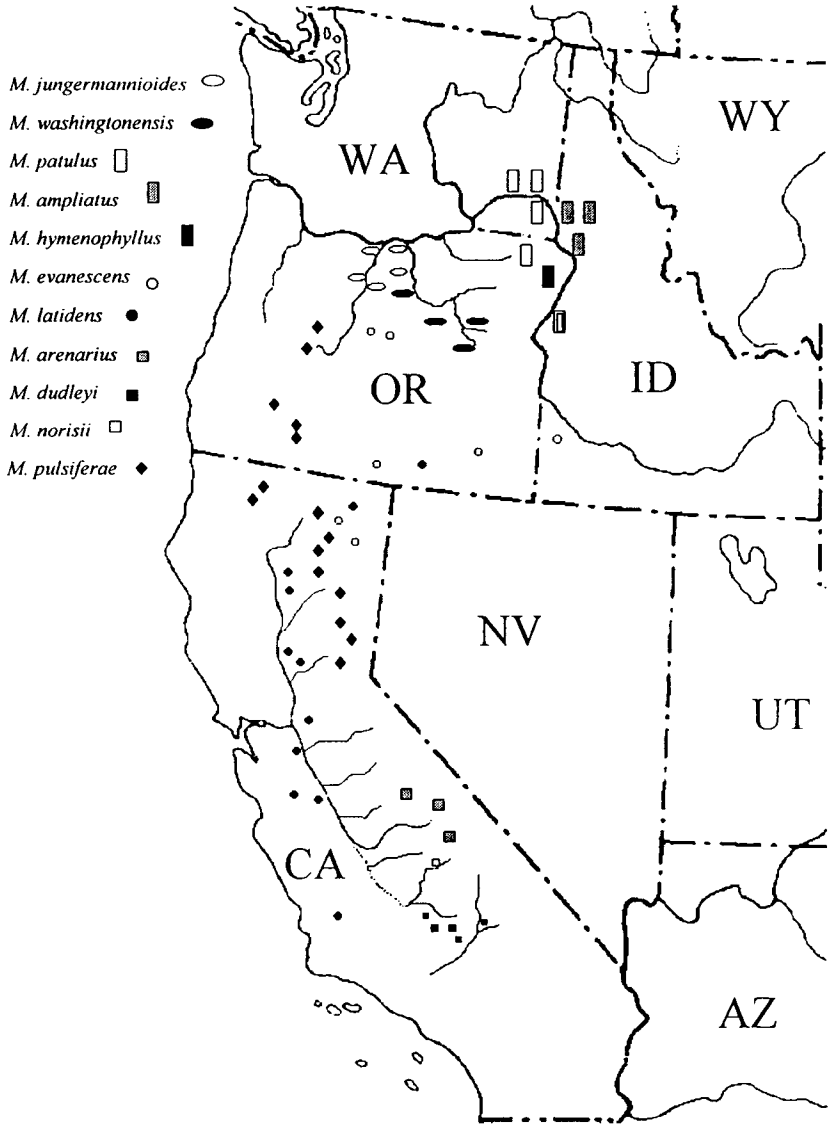

Fig. 1. Distribution of the rare species of the *M. moschatus* alliance and *M. latidens*. Symbols indicate one or more herbarium collections in the vicinity. *Mimulus breviflorus*, *M. moschatus*, and *M. floribundus* are excluded due to their widespread distributions.

the chloroplast gene encoding the large ribosomal protein 16 (*rpl16*) that has provided interspecific phylogenetic resolution in other vascular plants (Downie et al., 1996; Jordan et al., 1996; Small et al., 1998). This chloroplast intron exists as a single copy in plant chloroplast genomes, has a rapid substitution rate, manageable size, and is flanked by conserved exons suggesting it may resolve relationships between closely related species comparable to the nrDNA ITS region (Kelchner and Clark, 1997).

Previous attempts to obtain complete ITS region sequences from *Mimulus* sections *Eunanus* Gray, *Simiolus* Grant, and *Paradanthus* have been hindered by PCR inhibition and incomplete sequencing (A. Liston and W. Messinger, unpublished; P. Beardsley and A. Yen, Univ. of Washington, personal communication; J. Willis and M. Macht, Univ. of Oregon, personal communication). Anomalous ITS region sequences were reported from *Mimulus* section *Simiolus* (Ritland et al., 1993; Ritland and Straus, 1993) that lacked ITS region motifs characteristic of seed plants, and have been subsequently identified as green algal contaminants (Hershkovitz and Lewis, 1996). Partial, genuine ITS region sequences from *Mimulus* sections *Simiolus* and *Paradanthus* (M. Macht and J. Willis, Univ. of Oregon) indicated the presence of a stem-loop structure that appeared to be causing difficulties in amplifying and sequencing some *Mimulus* ITS regions. We have characterized this ITS1 stem-loop in the *M. moschatus* alliance and made limited comparisons to some other Asterid lineages (Liu and Schardl, 1994; Baldwin et al., 1995; Buckler et al., 1997; Mai and Coleman, 1997).

We have examined the utility of the ITS region and *rpl16* intron sequences in reconstructing phylogenetic relationships among rare species of the *Mimulus moschatus* alliance. The phylogenetic patterns are interpreted in light of the available morphological, ecological, and biogeographic data. Taxonomic implications from the phylogenetic results suggest modifications that may influence the conservation status of some species of the *M. moschatus* alliance.

MATERIALS AND METHODS

Sampling strategy and DNA extraction--Taxon sampling followed a broad interpretation of the *M. moschatus* alliance (Grant, 1924; Argue, 1986; Meinke, 1992; M. Carlson, unpublished) (Table 1). A hierarchy of outgroups from within *Paradanthus*, plus section *Simiolus* Grant and the reputedly distant section *Eunanus* Gray (Grant, 1924; Pennell, 1951; Thompson, 1992) were included to confidently examine the monophyly of the *M. moschatus* alliance. Genomic DNA was extracted from 0.2-0.5 g of leaf tissue from field collected material, greenhouse cultures and herbarium specimens (Table 1) following the protocol of the DNeasy Plant Mini Kit (Qiagen, Chatsworth, CA). Genomic DNAs were electrophoresed on 1.2% TBE agarose gels stained with ethidium bromide and quantified using a mass ladder (New England Biolabs, Beverly, MA).

ITS region PCR--Approximately 25 ng of genomic DNA was added to 50 μL aliquots of a PCR master mix consisting of 36 μL ddH_2O, 4 μL 10x *Tfl* polymerase buffer (1.0 M Tris-HCl, 0.4 M ammonium sulfate), 2.5 μL $MgCl_2$ (25 mM), 2.5 μL dNTPs (2.5 mM), 1

Table 1. Collection localities for ITS region and *rpl16* intron accessions from greenhouse cultures (G), field collections (F), herbarium specimens (H), and unpublished sequences (S).

Taxon	ITS	*RPL16*	Source	Locality	Voucher
M. alsinoides Benth.	X	X	F	OR, Douglas Co., Roseburg, N side of West Bank Rd. ca. 6 mi E of I5	OSC Whittall# 40
M. ampliatus A.L.Grant	X	X	F	ID, Lewis Co., 4 mi N of Ferdinand, E side of old Hwy 95	OSC Whittall# 41
M. bicolor Hartw.	X	X	H	CA, Madera Co., E shore Bass Lake between Willow Creek & Beasore Rd.	UCB Dean Taylor #8594
M. breviflorus Piper	X	X	H	OR, Lake Co., Youkum Valley	OSC Rittenhouse #485
M. dentatus Nutt.	X		S	OR, Douglas Co., along small tributary of the Smith Riv., near Suislaw NF	OSC Macht# 7
M. dentatus Nutt.		X	H	OR, Benton Co., Prarie Peak, Alsea	OSC Halse #1977
M. dudleyi A.L.Grant	X	X	H	CA, Tuolumne Co., beside Red Hills Rd., 1.7 mi S of Hwy 120	OSC D.W. McNeal #1132
M. evanescens Meinke	X	X	G	CA, Lassen Co., Moll Reservoir, ca. 20 km E of Adin	Carlson
M. floribundus Lindley	X	X	G	CA, Lassen Co., Moll Reservoir, ca. 20 km E of Adin	OSC Whittall# 42
M. floribundus Lindley	X		F	OR, Wallowa Co., ca. 2 mi S of Troy along Old Troy Rd., banks of Grande Ronde	OSC Whittall# 43
M. guttatus DC.	X		S	OR, Lane Co., Iron Mtn., W Cascades	OSC Macht #9
M. guttatus DC.		X	H	OR, Marion Co., Mt. Jefferson Wilderness, along PCT	OSC Halse #3617
M. hymenophyllus Meinke	X	X	G	OR, Wallowa Co., Horse Creek, Site #3	OSC Whittall#44
M. jungermannioides Suksd.	X	X	G	OR, Sherman Co., Sherman, S side of Hwy 84, 3 mi E of county line	OSC Whittall#45
M. latidens (A.Gray) E.Greene	X	X	H	CA, Yolo Co., beside Road 29, 5 mi W of Hwy 99W, April 22, 1951	OSC Carl Ehlig #37
M. latidens (A.Gray) E.Greene	X		H	OR, Lake Co., E of Warner Valley, ca. 12 mi NW of Adel, SE side of Wool L.	OSC Stephen Shelley #831
M. lewisii Pursh	X	X	S		Beardsley
M. mephiticus E. Greene	X	X	S	CA, Fresno Co., King's Canyon Natl Park, trail to Paradise Meadow	UW Beardsley#98-079
M. moschatus Lindley	X	X	F	OR, Benton Co., Oak Creek Rd., 0.5 mi S of MacDonald Forest	OSC Whittall#46
M. norisii Heckard & J.R.Shevock	X	X	S	CA, Tulare Co., Sequoia Natl Park, near Potwisha campground	UW Beardsley #98-013
M. patulus Pennell	X	X	G	OR, Wallowa Co., Imnaha River Rd., 4 mi S of Imnaha	OSC Whittall#30
M. primuloides Benth.	X	X	F	OR, Klamath Co., Mud Spring, ca. 2 miles S of Hwy 66 on W Branch Rd.	OSC Whittall#47
M. primuloides Benth.	X		G	OR, Baker Co., Anthony Lake	Carlson
M. pulsiferae A.Gray	X	X	F	OR, Jefferson Co., Camp Sherman Rd., 6 mi N of Hwy 20	OSC Whittall#48
M. washingtonensis Gand.	X	X	G	OR, Wheeler Co., Spray, Hwy 19, 3 mi E of Horseshoe Cr.	OSC Whittall#49
M. whitneyi A.Gray	X	X	S	CA, Tulare Co., Western Divide Hwy, 26.7 mi from Kernville	UW Beardsley #98-060

μL BSA (10 mg/mL), 1 μL DMSO, 50 pmol of each primer ITS 5* (5'-GGAAGGAGAAGTCGTAACAAGG-3') (Liston et al., 1996) and ITS 26S-25R (5'-TATGCTTAAACTCAGCGGGT-3') (Nickerent et al., 1994), and 1 unit *Tfl* polymerase (Epicentre Technologies, Madison, WI). Reaction mixtures were covered with two drops mineral oil and cycled on a PTC-100 Programmable Thermal Cycler (MJ Research, Waltertown, MA) beginning at 92°C (2 min), followed by 35 cycles of 94°C (1 min), 55°C (45 s), and 72°C (45 s). After a final extension at 72°C (5 min), reactions were held at 4°C. PCR products were electrophoresed on 1.2% TBE agarose and gel purified following the protocol for QIAquick Gel Purification (Qiagen, Chatsworth, CA). Purificates were quantified and compared with both size and mass ladders on a 1.2% TBE agarose gel prior to sequencing.

Rpl16 intron PCR--The *rpl16* intron PCR protocol was identical to the protocol for the ITS region, except in the following ways. Each PCR master mix (50 μL) consisted of 25 μL ddH_2O, 22 μL Premix D [4.8 μL ddH_2O, 0.26 μL $MgCl_2$ (25 mM), 1.38 μL dNTPs (2.5 μM), 4.4 μL 10x *Taq* polymerase buffer (1.0 M Tris-HCl, 0.4 M ammonium sulfate), 8.8 μL 10x MasterAmp PCR Enhancer (Epicentre Technologies)], 50 pmol of each primer F71 (5'-GCTATGCTTAGTGTGTGACTCGTTG-3') and R1661 (5'-CGTACCCATATTTTTCCACCACGTC-3') (Jordan et al., 1996; Kelchner and Wendel, 1996), and 1 unit *Taq* polymerase (Epicentre Technologies, Madison, WI). Thermocycling was initiated with 92°C (5 min), followed by 24 cycles of 95°C (1 min), 50°C (1 min), and then ramped at 1 ° per 8 s to 65°C (4 min). After a final extension at 65°C (10 min), reactions were held at 4°C.

Sequencing--Approximately 30 ng purified PCR products were cycle sequenced following the BigDye Terminator protocol (PE, Applied Biosystems, Inc., Foster City, CA) and separated on the ABI Prism 377 (PE, Applied Biosystems) using a 5% polyacrylamide denaturing gel. One or two accessions from each taxon were sequenced in both the forward and reverse directions using the PCR primers except for *rpl16*, an internal reverse primer R1516 (5'-CCCTTCATTCTTCCTCTATGTTG-3') (Kelchner and Clark, 1997) was used in place of R1661. Sequences were compiled using the GAP 4.0 editor (Bonfield et al., 1995) and manually aligned in GCG10 (Wisconsin Package, Version 10.0, 1999). Automated alignments generated with Clustal X and Pile-Up (Wisconsin Package, Version 10.0, 1999) clarified the small proportion of alignment ambiguities from the manual alignment.

ITS region polymorphism--Both intraspecific and intragenomic polymorphism in the ITS region is well documented (Sang, 1995; Mayer et al., 1996; Soltis et al., 1996; Buckler et al., 1997; Campbell et al., 1997; O'Kane et al., 1997; Whittall et al., submitted). Therefore, three taxa were analyzed for intraspecific ITS region variation by sequencing (reverse direction only) one individual from two distinct populations representing a significant portion of the species range (Table 1). Intraspecific sequences were aligned and examined for polymorphisms. In addition, a single accession was analyzed for intra-individual ITS region polymorphism following the TOPO TA Cloning Kit protocol (Invitrogen, Carlsbad CA). *Mimulus pulsiferae* was selected for intragenomic ITS polymorphism due to its unresolved phylogenetic position following initial sequencing

efforts. Adenine overhangs were added to 20 μL gel purified ITS region template. One μL of this mixture was ligated to the TOPO vector and 2 μL were cloned into competent *E. coli* cells. Eight clones were grown overnight in 2 mL media, centrifuged and amplified following the ITS region PCR protocol. The directly sequenced *M. pulsiferae* ITS region was mapped for restriction sites with GCG10 (Wisconsin Package Version 10.0, 1999). A search for available enzymes that cut the ITS region fragment into a few distinctly sized bands identified *Hae*III, *Hin*fI, *Scr*fI, and *Msp*I. A single 16 μL restriction digest consisted of 9 μL ddH20, 1 μL Buffer #2 [10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT (pH 7.9 at 25°C)] (New England Biolabs, Beverly, MA), 1 μL BSA, either 1 unit enzyme (*Hae*III) or 0.48 units enzyme (*Hin*fI, *Scr*fI, *Msp*I) and 5 μL cloned PCR product. Reactions were incubated at 37°C for 1 hour, then separated and visualized on a 3% TBE agarose gel with EtBr stain.

Combinability tests--Templeton's (1983) significantly less parsimonious test for character-state reconstructions on competing topologies (SLP_T in Johnson and Soltis, 1998) is considered a very conservative test of incongruence (Templeton, 1997; Johnson and Soltis, 1998). The most parsimonious tree lengths for one data set (A) is compared with the number of steps it takes to force data set A onto the most parsimonious topologies from another data set (B), thereby testing the null hypothesis of no significant difference between the length of the most parsimonious tree(s) from data set A before and after it is forced onto B's topologies. The Wilcoxan signed-rank test is used to determine statistically significant tree length differences (Johnson and Soltis, 1998). Templeton's

Combinability test was implemented in PAUP* (Swofford, 1998) using the PTEST command.

The incongruence length difference test (ILD, also named the homogeneity partitioning test and HT_F by Johnson and Soltis, 1998) is a less conservative, yet more efficient means of testing for homogeneity between data partitions (Farris et al., 1995; Cunningham, 1997). Specifically, it examines the null hypothesis of homogeneity when the combined data set tree length is compared to a series of random partitions to determine if the two data sets are simply partitions of one homogeneous unit (Farris et al., 1995). Although $p<0.05$ significance is the accepted statistical criteria to reject the null hypothesis in most statistical tests (Johnson and Soltis, 1998; Lecointre et al., 1998), Cunningham's empirical results (1997) have shown that the combined data only suffers when $p<0.01$. Whereas, $p>0.01$ indicates that combining data sets will improve or not reduce phylogenetic accuracy (Cunningham, 1997). In order to detect the cause of incongruence, ILD tests following a series of taxon jackknifing events were performed (Lecointre et al., 1998). All ILD tests considered only variable sites with 1000 replications (Cunningham, 1997; Johnson and Soltis, 1998) implemented in PAUP* (Swofford, 1998) with the HOMPART command.

Phylogenetic analysis--Phylogenetic analyses consisted of branch and bound searches with TBR and ten random sequence additions under maximum parsimony criteria. Maximum likelihood analyses were based on the HKY85 model (Hasegawa et al., 1985) correcting for rate polymorphism. Transition/transversion ratios, base frequencies, and

gamma distributions were estimated from the data set. Starting branch lengths were obtained using the Rogers and Swofford method (1998). Ten random sequence additions in a heuristic search allowed for multiple trees of equally most likely scores (to five decimal places). Bootstrap support for each branch was determined with 500 replicates (Felsenstein, 1985). Decay values for those branches that did not collapse after four additional steps were determined by clade constraint analysis (reviewed in Morgan, 1997). The few parsimony informative indels found among accessions of the ingroup were mapped onto the maximum likelihood topology. All phylogenetic analyses were run with PAUP* version 4.0d64 (Swofford, 1998).

Comparative Asterid ITS1 secondary structure--Gapped BLAST (Altshul et al., 1997) and Entrez (www.ncbi.nlm.nih.gov) were used to search for Asterid nrDNA ITS region sequences (Table2). A selection of complete ITS1 sequences that contained less than 10% ambiguous characters were downloaded into 7(Wisconsin Package Version 9.0, 1992). Sequences were selected in an attempt to represent the major lineages of the Asteridae (Chase et al., 1993; Chase and Albert, 1998) (Table 2). ITS1 sequence boundaries were identified with the conserved 5' sequence of the 5.8S region (Hershkovitz and Lewis, 1996). ITS1 sequences were folded with the mFOLD web-server (http://mfold1.wustl.edu/~mfold/rna/form1.cgi) (Zucker, 1989) after reducing temperature to 25° C. Optimal to five percent suboptimal secondary structures were analyzed for conserved primary sequences, secondary structures, and free energy values.

Table 2. A comparison of ten Asterid ITS1 minimum Gibb's free energy values from the optimal folding algorithms (Zucker 1989) indicates the strength of the *M. hymenophyllus* secondary structure.

Accession	delta G	Family	Order	Genbank #	Reference
Mimulus hymenophyllus	**-83.3**	Scrophulariaceae	Asteridae		
Clerodendrum myricoides ssp. myricoides	-63.4	Lamiaceae	Scrophulariales	U77763	Steane et al. 1999
Oxera macrocalyx	-39.7	Lamiaceae	Scrophulariales	U77775	Steane et al. 1999
Faradaya splendida	-60.8	Lamiaceae	Srophulariales	U77773	Steane et al. 1999
Fraxinus excelsior	-63.7	Oleaceae	Scrophulariales	U82866	Jeandroz et al. 1997
Syringa sp.	-47.8	Oleaceae	Scrophulariales	U82918	Jeandroz et al. 1997
Jasmine sp.	-62.5	Oleaceae	Scrophulariales	U82920	Jeandroz et al. 1997
Solanum	-69.0	Solanaceae	Solanales	X52265	Kiss et al. 1998
Gentiana urnula	-49.6	Gentianaceae	Gentianales	Z48071	Yuan et al. 1996
Sambucus nigra	-60.7	Caprifoliaceae	Dipsacales	U88204	Eriksson and Donoghue 1997
Daucus carrota	-60.7	Apiaceae	Araliales	AF077780	Lee et al. 1997

Table 3. Sequence data and phylogenetic statistics for the ITS region, *rpl16* intron, and combined data sets are indicated for all accessions and only the *M. moschatus* alliance accessions.

All accessions	**ITS**	***RPL16***	**Combined**
Number of Accessions	21	21	21
Alignment Length (bp)	640	956	1596
Average Contig Length (bp)	610 (607-617)	867 (847-881)	1477
GC%	61.9	31.2	43.6
No. of Constant Characters	428	757	1185
No. of Variable Sites	185	199	384
% Variable Sites	28.9	20.8	24.1
No. of Parsimony Informative Sites	112	115	230
% Parsimony Informative	17.5	12.0	14.4
Most Parsimonious Tree Length	256	240	511
Number of Equally Parsimonious Trees	15	96	14
Consistency Index (Ex. Uninf. Chars.)	0.6835	0.8405	0.7273
Maximum Likelihood Values	2404.6231	2685.6473	5090.2704
No. of Maximum Likelihood Trees	6	24	1
No. of Characters Removed Before Analysis	27	0	27
***M. moschatus* alliance accessions**			
Number of accessions	12	12	12
Alignment Length (bp)	590	876	1466
Average Contig Length (bp)	587 (586-588)	863.5 (862-866)	1451.2
GC%	62.592	31.268	43.875
No. of Constant Characters	513	827	1340
No. of Variable Sites	77	49	126
% Variable Sites	13.05	5.59	8.60
No. of Parsimony Informative Sites	33	23	56
% Parsimony Informative	5.59	2.63	3.82
Most Parsimonious Tree Length	80	44	128
Number of Equally Parsimonious Trees	1	48	14
Consistency Index (Ex. Uninf. Chars.)	0.6863	0.8846	0.7160

RESULTS

Sequence data--In all accessions, PCR amplification for both ITS region and *rpl16* intron produced single, sharp bands within the expected size ranges reported for angiosperms (Table 3) (Posno et al., 1986; Baldwin et al., 1995; Hershkovitz and Lewis, 1996; Small et al., 1998). Recent progresses in direct sequencing chemistry (Applied Biological Systems, Inc., Foster City, CA) provided reliable sequencing signal for the majority of the 956 bp *rpl16* intron. Therefore, only a single forward and reverse sequence was required to obtain a contiguous sequence for each accession. The ITS region sequencing was inhibited in the forward direction after bp 63 and terminated in the reverse direction at bp 81 (Fig. 2) even following five-fold increases in DMSO and BSA (Buckler et al., 1997). Sequences in the 5' to 3' direction had reduced fluorescent ddNTP incorporation, but after amplifying the y-axis scale in the Gap Editor (Bonfield et al., 1995), the remaining ca. 587 bps could be salvaged for most accessions. A 25 bp region of unalignable, ambiguous sequence was (Fig. 2) found in the ITS1 of the majority of accessions (Appendix A).

For 21 accessions, the ITS region alignment measured 640 bp and *rpl16* intron measured 956 bp, although the average contig length in the *rpl16* intron data set was significantly smaller due to a few moderate sized indels (Table 3). There were 28% variable positions in the ITS region data set compared to 20% in the *rpl16* intron data set. Except for the sequencing difficulties in ITS1, manual alignment allowed for confident positional homology in both data sets. Many models of sequence evolution have been described for chloroplast introns including slipped strand mispairing in the form of length variations in

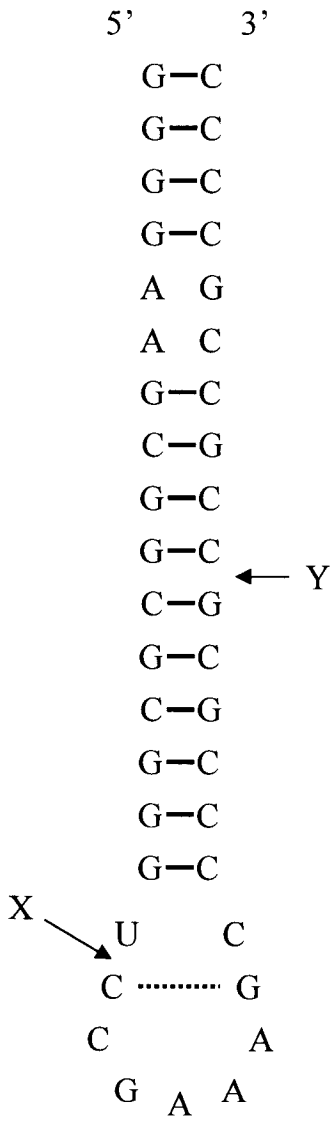

Fig. 2. The proposed stem-loop structure in ITS1 (bp 56-74) identifying the location where the 5'-3' direct sequencing read loses signal (X) and the 3'-5' read terminates (Y).

mononucleotide repeats and extension of short tandem repeats, and small inversions associated with hairpin structures (Kelchner and Wendel, 1996; Kelchner and Clark, 1997). In the 21 accessions alignment, there were and five small inversions (two and four bp), four examples of mononucleotide length variation, two examples of short repeat size variation (one tandem repeat and one dispersed repeat), one long tandem repeat (10 bp), (Fig. 3 and Table 4).

ITS1 secondary structure--The most complete ITS1 sequence spanning the stem-loop within the *M. moschatus* alliance came from the *M. hymenophyllus* accession. Its ITS1 has a significantly higher Gibb's free energy value than that of the other Asterids examined (Table 2). No other ITS1 secondary structures compared produced stem-loop structures in the 5' portion of ITS1 with 10 consecutive complementary base pairs (Fig. 2). There appears to be a bimodal distribution of Gibb's free energy values ranging from -69.0 to -39.7 kcal/mol. All examined structures begin and end with single stranded motifs. Although many suboptimal secondary structures existed for most of the accessions, three stem-loop domains appear conserved across the major Asterid lineages. These stem-loop domains have been previously described by Liu and Schardl (1994) (structures available upon request). Although they ranged in size, base composition and relative strength, they were present in most optimal and suboptimal secondary structures.

ITS region polymorphism--No ITS region intraspecific variation was detected between the two accessions of *M. floribundus*, *M. latidens*, and *M. primuloides*. In addition, no

```
                    11 333333 3333 33 333333333 55555555 55 66666666666 77777777777
                 99 22 011111 3333 56 666666666 22222222 44 77777777788 11111111112
                 78 67 901234 6789 90 123456789 12345678 45 12345678901 01234567890

M.ampliatus      AT -- ACT--- AAAA GT TTAT----- TTTTTTT- GT TTTTTTT---- AAAAAAAAA--
M.patulus        .. .. ...... .... .. ......... ........ .. N.......... .........AA
M.hymenophyllus  .. .. ...... .... .. ......... ........ .. .......... .........A.
M.breviflorus    .. .. ...... .... A. ......... ........ A. ......-.... R........AA
M.evanescens     .. .. ...... .... A. ......... ........ A. .......... .........AA
M.moschatus      .. .T ...... .... A. ......... ........ A. .......... ........-..
M.floribundus    .. .. ...... .... A. ......... .......T A. .......... ........-..
M.norisii        .. .. ...... .... A. ......... .......T A. .......... ........-..
M.dudleyi        .. .. ...... .... A. ......... .......T A. .......... ..........
M.pulsiferae     .. .. ...... .... A. ....TATTT ........ A. .......... ........--..
M.jungermannioides.. .. ...... .... A. ......... ........ A. .......... .........M.
M.washingtonensis .. .. ...... .... A. ......... ........ A. .......... ........-..
M.latidens       .. .T ...ACT ...- A. ......... ........ A. .......... ....T...-..
M.dentatus       .. .. ...... ...- A. ......... ........ A. .......T... ....T...-..
M.guttatus       .. .T ...... ...- A. ......... ..A..... A. .......TTT. ....T...-..
M.alsinoides     .. TT ...... ...- -- --.....-. ....A... AC .......... ....T...-..
M.primuloides    TA AA .T.... .... -- --.....-. ...C...T AC .....A.TTTT ...CT...-..
M.lewisii        TA TT .T.... ...- -- --.....-. ...A...T AC .......... ...CT...-..
M.bicolor        TA TT .T.... ...- -- --.....-. ...A...T AC .......... ...CT...-..
M.mephiticus     .. .T ..GC.. ..-G AC .......-- ...GA... AC ...----.... ....T...-..
M.whitneyi       .. .T ..ACA. ..-G AC .G.....-- ...GA... AC .....--.... ....T...-..


                 77777777777777777777777777777 88888888888888888888
                 22222233333333334444444445556 44444555555555666666
                 45678901234567890123456789789O 56789012345678901234

M.ampliatus      TTATT-AGAATAATATT-----ATTT---- TTTTGAATCC----------
M.patulus        ..............................  ..................
M.hymenophyllus  ..............................  ..................
M.breviflorus    ..............................  ........T.........
M.evanescens     ..............................  ........T.........
M.moschatus      ..............................  ..................
M.floribundus    ..............................  ..................
M.norisii        ..............................  ..................
M.dudleyi        ..............................  ..................
M.pulsiferae     ..............................  ..................
M.jungermannioides..............................  ........T.........
M.washingtonensis ..............................  ........T.........
M.latidens       ..............................  ..................
M.dentatus       ......................--TATT ..........TTTTTGAATCC
M.guttatus       ..............................  ..................
M.alsinoides     .G.............CAATT....TATT .......G.............
M.primuloides    ..............A.CTATTC.......  ........T.........
M.lewisii        ..............GCCTATTG...TGTT  ..................
M.bicolor        ..............GCCTATTG.......  ..................
M.mephiticus     .....T......G....CTATTT.......  C...AT.AT..........T
M.whitneyi       ............G....CTATT....TATT  C...-T.AT..........T
```

Fig. 3. Three mutation types of indels found in the *rpl16* data set include two and four bp inversions, mononucleotide repeat length variations, and simple sequence repeats. The region spanning bp 724-760 contains a four bp inversion (TATT/AATAA) and up to four TATT repeats across 36 bp.

Table 4. Three mutation types found in the *rpl16* data set include inversions (I), mononucleotide repeat length variations (M), and simple sequence repeats (SSR). The * indicates complex repeat spaced by single bases and the ? indicates uncertain mutation type.

Mutation Type	Position	Sequence A	Sequence B
I	97-98	AT	TA
I	126-127	TT	AA
I	359-360	GT	AC
I	544-545	GT	AC
I	724-728	TTATT	AATAA
M	336-339	$(A)_4$	$(A)_3$
M	521-528	$(T)_6$	$(T)_7$
M	671-681	$(T)_7$	$(T)_{8,10,11}$
M	710-720	$(A)_9$	$(A)_{10,11}$
SSR	309-314	ACT	$(ACT)_2$
SSR*	725-760	TATT	$(TATT)_4$
?	361-368	TTAT	TTATTATT

intra-individual ITS region polymorphism was detected in the RFLP analysis of eight *M. pulsiferae* clones. RFLP patterns matched those expected from restriction site mapping of the direct sequencing results (54 nucleotides analyzed).

ITS region and *rpl16* intron phylogenetic analyses--Among the 21 accessions, ITS region sequences provided 17.5% parsimony informative sites compared to 12% for the *rpl16* intron. The *rpl16* intron data set had three more parsimony informative sites than the ITS region. Maximum parsimony analysis resulted in 15 ITS region trees of 256 steps (CI ex. Uninf. Chars. = 0.6835) and 96 *rpl16* intron trees of 240 steps (CI ex. Unif. Chars. = 0.8405). Under maximum likelihood criteria, a subset (six) of the 15 most parsimonious trees from the ITS region data (gamma shape=0.230889, ts/tv=1.8223) and 24 of the 96 most parsimonious trees from the *rpl16* intron data (gamma shape=0.539225, ts/tv=0.823948) shared equal maximum likelihood values.

In both data sets, the strict consensus maximum likelihood topology indicates a monophyletic *M. moschatus* alliance (Fig. 4), although weakly supported in the ITS region data set (8% bootstrap support). In addition, two clades within the alliance are resolved in both data sets; the "Great Basin Clade" (*M. breviflorus* and *M. evanescens*) and the "Snake River Clade" (*M. ampliatus, M. hymenophyllus,* and *M. patulus*). The placement of *M. latidens*, *M. guttatus,* and *M. dentatus* as the closest sampled outgroups to the *M. moschatus* alliance is consistent in both data sets, but relationships among these accessions are conflicting. It is likely they are not particularly closely related within the genus if more taxa were added.

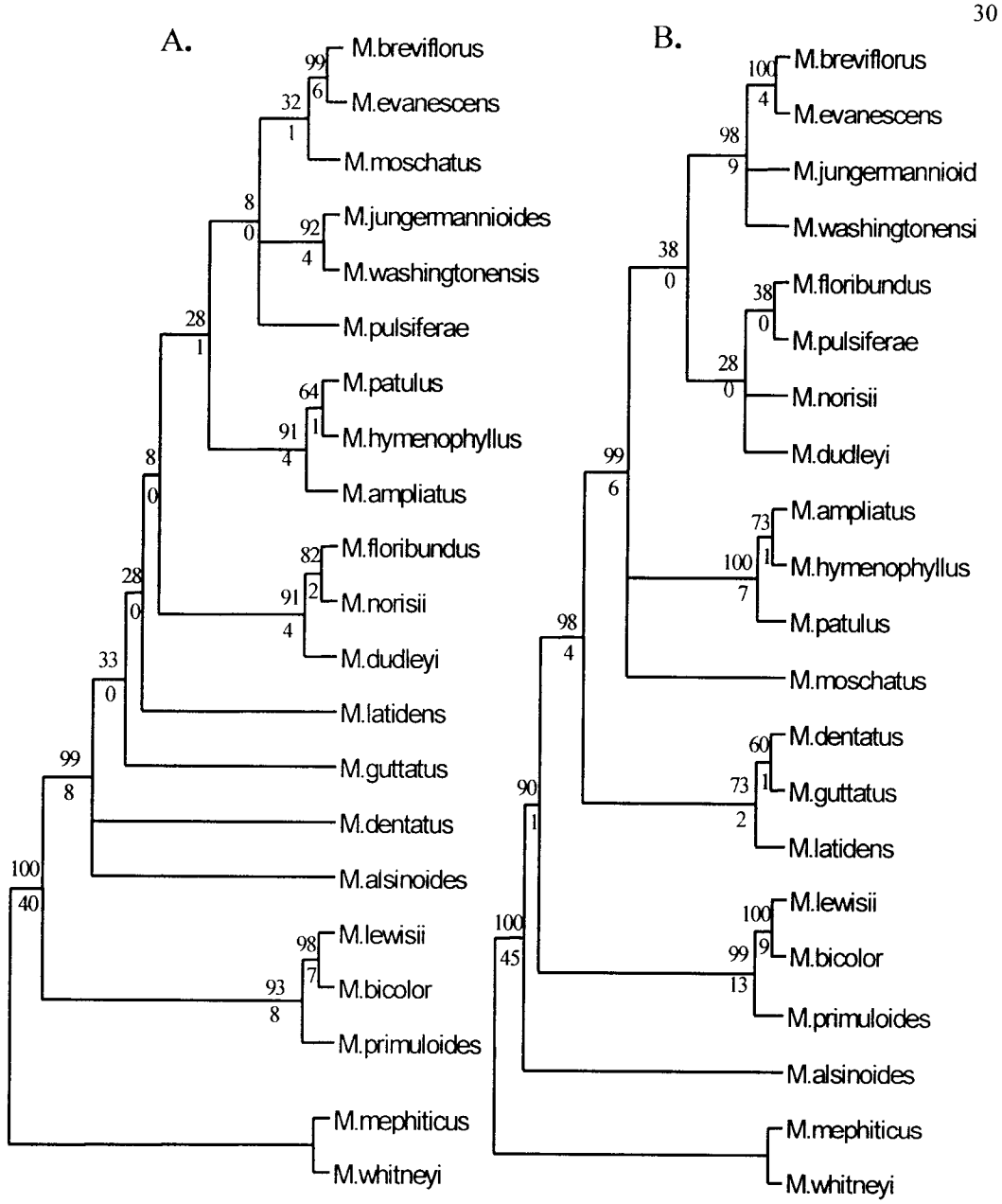

Fig. 4. The strict consensus of the six ITS maximum likelihood trees (A) compared to the strict consensus of the 24 *rpl16* maximum likelihood trees (B). Bootstrap values are indicated above the branches and decay values appear below. Decay value of zero indicates the branch collapses in the maximum parsimony strict consensus tree.

The ITS region data set provides very strong bootstrap support for the two other clades of the *M. moschatus* alliance, the "Sierra Nevada Clade" (*M. floribundus, M. norisii,* and *M. dudleyi*) and the "Columbia River Clade" (*M. jungermannioides* and *M. washingtonensis*) (Fig. 4, A). However, these clades are unresolved in the *rpl16* intron maximum likelihood strict consensus tree (Fig. 4, B). Conversely, *rpl16* intron provides better resolution between clades within the *M. moschatus* alliance (specifically the more distant relationship of the Sierra Nevada clade compared to the other three Pacific Northwestern clades). The placement of *M. pulsiferae* within the "Sierra Nevada Clade" is in conflict with the ITS region topology. Additional topological differences between the strict consensus of the maximum likelihood trees of the ITS region and *rpl16* intron data sets include the conflicting relationships within the Snake River Clade, the unresolved Columbia River Clade and Sierra Nevada Clade (in *rpl16*), and the lability of *M. alsinoides*.

Combinability tests--The Templeton Combinability test comparing ITS region and *rpl16* intron most parsimonious trees rejected the null hypothesis of combined data set homogeneity ($p<0.0001$). When the ITS region data set was forced onto the 96 *rpl16* intron most parsimonious trees, 25-38 additional steps were required. The *rpl16* intron data set required 30-34 additional steps to conform to the 15 most parsimonious ITS region topologies. It appears that the ITS region data cannot give *rpl16* intron topologies without significant tree length increases. Neither can the *rpl16* intron data set be forced to give the ITS region topologies without significant increases in tree length.

The combined ITS region and *rpl16* intron data sets failed the incongruence length difference test (p=0.001), indicating that phylogenetic analysis of the combined data sets would suffer relative to the analysis of individual partitions (Cunningham, 1997). Within the *M. moschatus* alliance there was no evidence to reject the null hypothesis of data set homogeneity (p=0.117) (Table 5). By adding outgroup accessions one at a time, redefining partitions and excluding constant characters, data set incongruence was isolated to *M. alsinoides* (p=0.079 when only *M. alsinoides* was deleted), consistent with its lability in the phylogenetic analysis of the independent data sets (Table 4).

A comparison of combined data set analyses with and without *M. alsinoides* isolates its effect on the ingroup topology (Fig. 5). The only topological difference is the basal relationship of the Sierra Nevada clade compared to the other ingroup accessions. Inclusion of *M. alsinoides* does affect the relationships among the outgroup *sensu lato*. Without *M. alsinoides*, the combined analysis topology reflects the *rpl16* intron pattern. Whereas, when *M. alsinoides* is included, the combined analysis resembles the ITS topology.

Combined phylogenetic analysis--Despite failing both Combinability tests, many authors suggest conditional Combinability (Bull et al., 1993; summarized in Johnson and Soltis, 1998) to elucidate new associations, causes of conflict, and to determine robust clades (Dubuisson et al., 1998). After combining the ITS region and *rpl16* intron data sets

Table 5. Results from a series of nested incongruence length difference tests following selected taxon removal indicate the source of incongruence between data set partitions. $P < 0.01$ is considered evidence to reject congruence between partitions (Cunningham 1997).

TAXA DELETED	P-value	CONCLUSION
none (0)	0.001	Data set wide incongruence
outgroups *sensu lato* (9)	0.117	Incongruence not in *M. moschatus* alliance
M. alsinoides, M. latidens, M. guttatus, M. dentatus, M. primuloides, M. lewisii	0.167	Incongruence not in *M. mochatus* alliance or outgroup *sensu stricto*
M. alsinoides, M. latidens, M. guttatus, M. dentatus	0.152	Incongruence not in *M. primuloides, M. lewisii,* or *M. bicolor*
M. alsinoides, M. latidens, M. dentatus	0.089	Not *M. guttatus*
M. alsinoides, M. latidens, M. guttatus	0.048	Not *M. dentatus*
M. alsinoides, M. latidens	0.132	Incongruence not caused by *M. guttatus, M. dentatus*
just *M. alsinoides*	0.079	Incongruence not caused by *M. latidens*
just *M. latidens*	0.001	**Incongruence caused by *M. alsinoides***

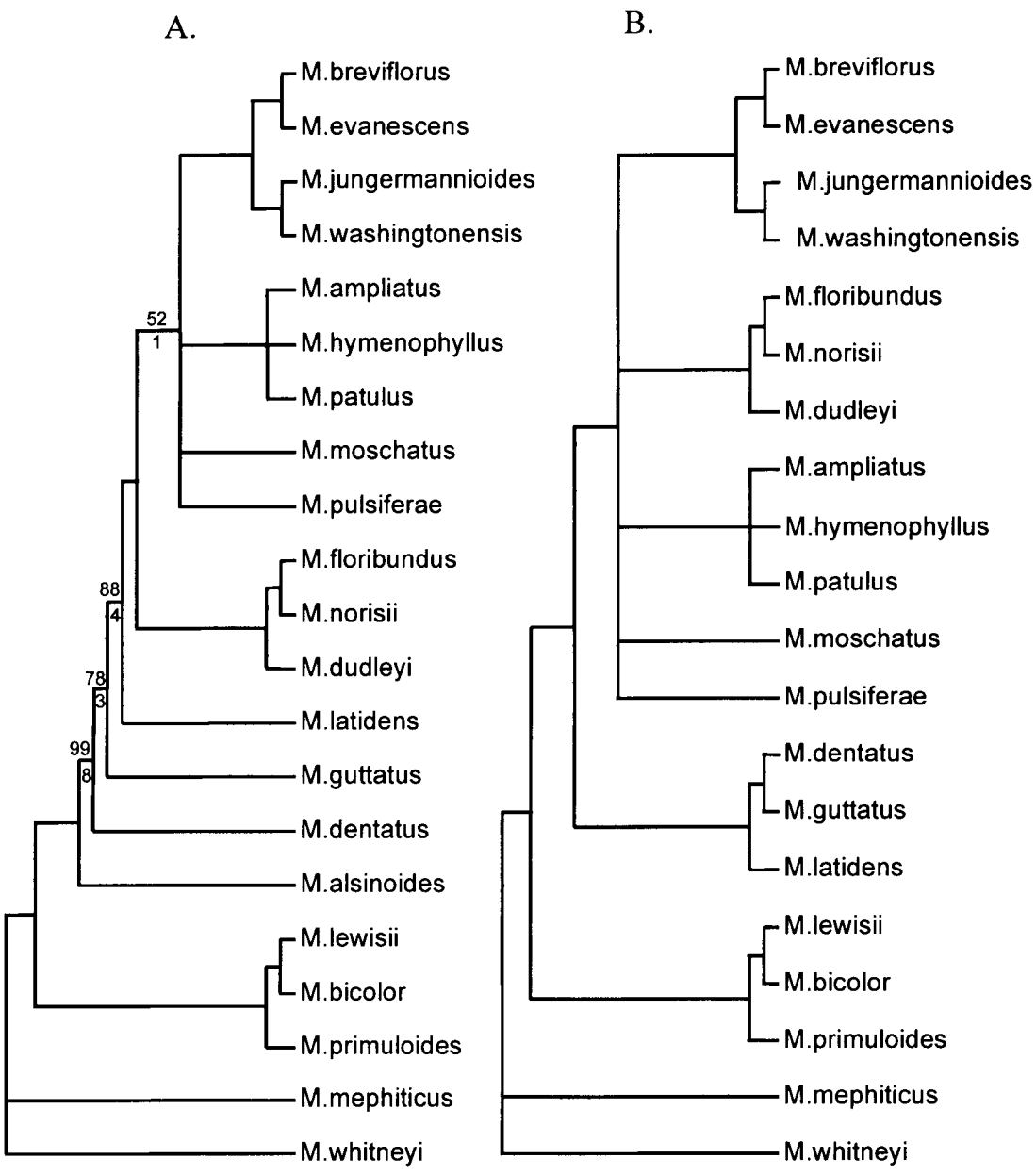

Fig. 5. A comparison of strict consensus trees from maximum parsimony analysis of the combined data sets with (A) and without (B) *M. alsinoides.* Bootstrap and decay values are given above and below the branches influenced by the exclusion of *M. alsinoides.*

(including *M. alsinoides*), maximum parsimony analysis generated 14 trees (CI ex uninf chars = 0.7273). The maximum likelihood analysis identified one of these trees as most likely (Fig. 6) (ts/tv = 1.24097 and gamma shape = 0.23791). The combined analysis supports a monophyletic *M. moschatus* alliance composed of four strongly supported clades of two to three species each plus two unresolved accessions (Fig. 6). There is weak support for the relationships among these clades. *Mimulus latidens* is well supported as the sister species to the *M. moschatus* alliance. Section *Simiolus* represented by *M. guttatus* appears derived from within section *Paradanthus*, although accurate relationships outside the *M. moschatus* alliance await more complete sampling within the genus. A strongly supported outgroup clade including *M. lewisii*, *M. bicolor* and *M. primuloides* serves to provide topological balance between the ingroup and the distant accessions from section *Eunanus* (Smith, 1994).

Indel mapping--Within the ingroup ITS region alignment, there were two insertions in ITS2 totaling three bps, both shared between *M. washingtonensis* and *M. jungermannioides* (Fig. 6, filled rectangles). Whereas, the ingroup of the *rpl16* intron data set had four single bp indels that mapped nonhomoplasiously onto the combined ML tree (Fig. 6, open rectangles). All were mapped to highly supported clades.

DISCUSSION

Phylogenetic utility of the ITS region and *rpl16* intron--The ITS region and *rpl16* intron provide interspecific phylogenetic resolution both within the *M. moschatus* alliance and

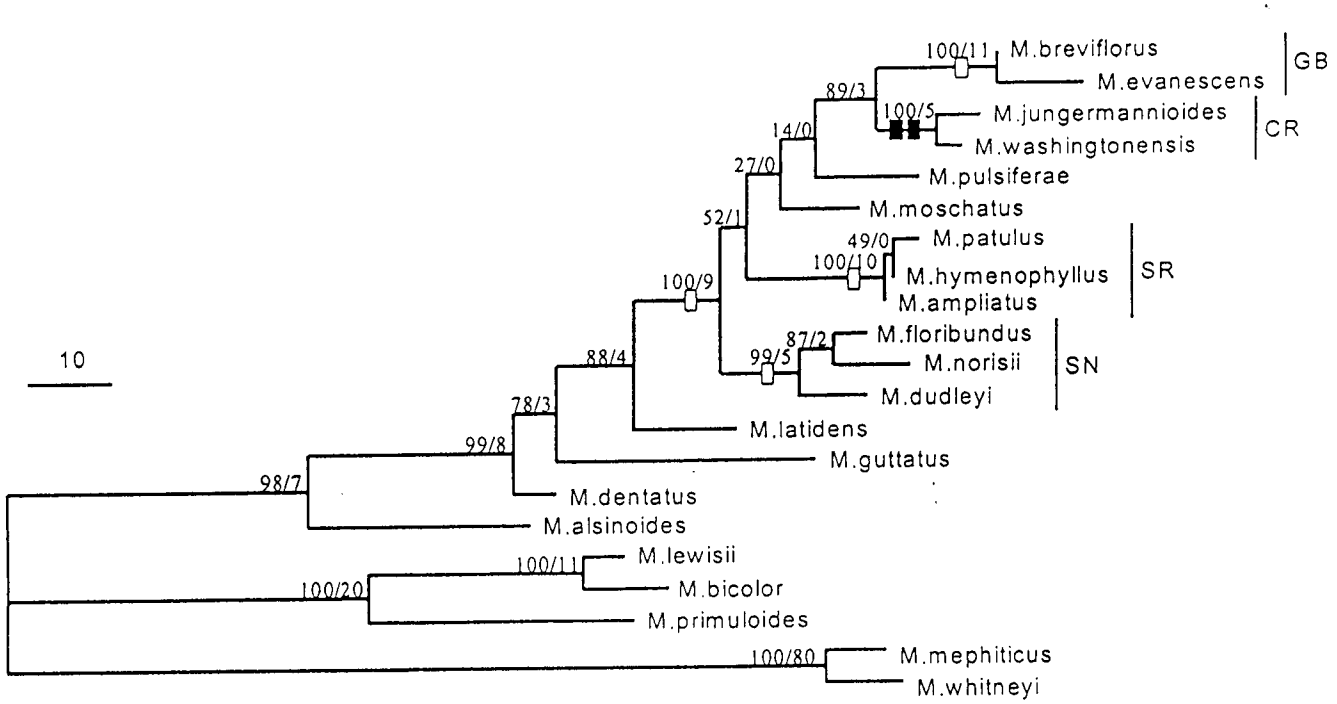

Fig. 6. One of 14 most parsimonious trees with the same topology as the maximum likelihood tree. Branch lengths are proportional to the number of character state changes (see scale bar). Bootstrap percentages are followed by decay values indicated above the branches. Shared insertions and deletions among ingroup taxa are indicated as rectangles (▮=ITS, □=*rpl16*). GB = Great Basin Clade, CR = Columbia River Clade, SR = Snake River Clade, SN = Sierra Nevada Clade.

between it and a hierarchy of outgroups. Sequence variation remains within the limits of confident alignment while still providing variation between sister taxa. A comparison of consistency indices excluding uninformative characters indicates significantly less homoplasy in the *rpl16* intron data set than the ITS region data set (Table 3). In addition, the *rpl16* intron contains more phylogenetically informative indels than the ITS region data set (Fig. 6).

ITS region polymorphism-- Sequence polymorphism among individuals of a species and within individual genomes can confound phylogenetic reconstruction. Detecting such polymorphism can be accomplished through multiple samplings within a species and cloning multiple copies from a single genome. Most chloroplast loci are assumed to be homogeneous within an individual. One example of intraspecific *rpl16* intron polymorphism was found in comparisons of *Lemna* spp. accessions from two populations with extreme ranges within North America (Jordan et al., 1996). However, relatively few studies have employed *rpl16* intron sequences to date (Jordan et al., 1996; Kelchner and Clark,1997; Small et al., 1998). Therefore, the *rpl16* intron was not analyzed for heterogeneity.

No ITS region differences within an individual nor between individuals of the same species were detected, suggesting nrDNA ITS region repeats are homogenized through processes of concerted evolution (Arnheim, 1983). These results are consistent with the relative ITS region homogeneity observed within and among populations of other genera (Soltis and Kuzoff, 1993; see Mayer et al., 1996 for an exception).

ITS1 secondary structure--Buckler et al. (1997) speculated that a high stability stem-loop structure was responsible for previous PCR complications in *Mimulus* section *Simiolus* (Hershkovitz and Lewis, 1996). In *M. hymenophyllus*, this structure lies within the ambiguous region of ITS1, implicating it as the cause of polymerase inhibition in PCR and direct sequencing in *Mimulus* section *Simiolus* (J. Willis and P. Beardsley, personal communications). This structure consists of four complementary bps, followed by two weak bonds, then a series of ten complementary bps ending in a five or nine bp loop (Fig. 2). In mapping substitutions onto this stem-loop for accessions with interpretable sequence through this region, no compensatory substitutions could be identified. This is consistent with other analyses in angiosperms (Baldwin et al., 1995). This observation suggests 1) most substitutions are independent events in the ITS spacer region, 2) cryptic non-independent substitutions were undetected, or 3) secondary structure models based on lowest free energy are not indicative of *in vivo* structures. Furthermore, available Genbank sequences reflect only a subset of taxa successfully sequenced, potentially eliminating some species with high stability secondary structure that may inhibit PCR and direct sequencing.

Although there are examples of primary sequence conservation in plant ITS regions (Liu and Schardl, 1994; Hershkovitz and Zimmer, 1996; Mai and Coleman, 1997), they are short and flanked by hyper-variable stretches. Among Asterids sampled, primary ITS1 sequences were not confidently alignable. However, three stem-loop structures, consistent with those described by Liu and Schardl (1994) appear to be conserved in ITS1 sequences from evolutionarily diverse lineages of Asterids (available upon request).

Combinability tests--Through a series of taxon jackknifing we identified *M. alsinoides* as the source of data set heterogeneity (Table 5) (Lecointre et al., 1998). The long branch leading to *M. alsinoides* in the *rpl16* intron data set (19 autapomorphies compared to 11 autapomorphies in the ITS region data set) could be attracted to the longest branches of the distant *Eunanus* outgroup accessions (*M. mephiticus* and *M. whitneyi*) (Felsenstein, 1978). A comparison of consistency indices along these outgroup branches indicate more homoplasious characters in the *rpl16* data set compared to the ITS region data set (CI ex. Uninf. Chars. = 0.8182, compared to 0.8859 for the ITS region). Although the conditional combinability methodology recommends removing accessions in order to allow for appropriate combinability of data sets (Rodrigo et al., 1993; Johnson and Soltis, 1998), in our case, combining *rpl16* intron and ITS region data sets despite significant heterogeneity due to one accession improves the phylogenetic resolution and internal support. A similar pattern has been in observed in the *Boykinia* Group (Saxifragaceae) (Johnson and Soltis, 1998).

Phylogenetic results--The *M. moschatus* alliance as defined herein, is composed of four clades and two unresolved species. An examination of the clades of the *M. moschatus* alliance suggests that convergence in floral characters and habitat shifts may have led to the phylogenetic uncertainty within this group (Fig. 7). Specifically, multiple origins of small, nearly radiate corollas, parallel adaptations to the cliff habitat, and convergent inflated calyces in fruit appear to have complicated morphologically-based phylogenetic hypotheses. Potential adaptive advantages in delaying seed dispersal through an inflated, yet distally narrowed fruiting calyx (Meinke, unpublished) suggest this trait could be under significant selective pressures. Similarly small corollas found in two of the taxa contribute to a breeding system shift from partial outcrossing to highly autogamous (Stebbins, 1974; Jain, 1976; Barrett and Shore, 1987; Meinke, 1992).

Type IIb pollen grains (those lacking supratectal ornamentation) are found in *M. floribundus* and *M. arenarius* and could be a synapomorphic character for the Sierra Nevada clade, but do arise in the distantly related outgroup taxa *M. alsinoides*, *M. lewisii*, *M. bicolor*, and *M. primuloides* (Argue, 1980, 1986). Pollen type IIc (exhibiting supratectal muriornamentation) is unique to the remaining members of the *M. moschatus* alliance (although *M. evanescens*, *M. dudleyi*, *M. ampliatus*, and *M. norisii* were not sampled) (Argue, 1986).

Few studies to date have integrated seed germination requirements into a phylogenetic framework (Baskin and Baskin, 1998; Smith-Ramirez et al., 1998). Intraspecific

polymophism in germination requirements have been recorded from the widespread *M. guttatus* (Meinke, unpublished), some populations showing varying levels of innate seed dormancy, even though most do not. Such variation may also exist for the widespread *M. moschatus* (Meinke, unpublished) and between individuals of *M. washingtonensis* (M. Carlson, unpublished). *Mimulus pulsiferae, M. washingtonensis, M. ampliatus, M. patulus* (and *M. hymenophyllus, M. breviflorus,* and *M. evanescens* if not germinated within a few weeks of dispersal) all possess seeds that require chilling prior to germination (Meinke, 1992, unpublished). A phylogenetic approach to seed dormancy evolution in the *M. moschatus* alliance suggests required cold stratification may have evolved in an ancestor to *M. pulsiferae*, *M. moschatus,* the Snake River clade, Columbia River clade and Great Basin clade. This pattern requires two later independent losses of seed dormancy in *M. jungermannioides* and *M. moschatus*.

The placement of *M. latidens* as sister taxon to the *M. moschatus* alliance is of potential biogeographic significance. *Mimulus latidens* has been associated with the *M. inconspicuus* complex (Grant, 1924), a group of California vernal pool endemics with glabrous sessile leaves, strongly plicate, inflated mature calyxes, and villous anthers (with the exception of *M. latidens*) (Grant, 1924). The monophyly of such a vernal pool clade would be consistent with recent results in *Navarretia* (Spencer and Porter, 1997). *Mimulus latidens* was chosen as the representative from this complex since it was hypothesized to be a relative of *M. evanescens* and *M. breviflorus* (Meinke, 1995), and has recently been collected in south-central Oregon (Shelley, 1986), suggesting a possible

biogeographic link between a California vernal pool assemblage and the *M. moschatus* alliance (Meinke, 1995).

The Columbia River Clade--*Mimulus jungermannioides* and *M. washingtonensis* are endemic to seasonally moist basalt substrates along tributaries of the Columbia River in north-central Oregon. They are strongly supported sister taxa in both data sets and share the only two parsimony informative ITS region indels in the *M. moschatus* alliance. Although they both have large closed-throated bilabiate corollas (Meinke, 1992), a subtle character that distinguishes them from the Snake River Clade, they are distinct from one another in most other respects. Each has a unique calyx morphology, life history (obligate annual vs. vegetatively reproducing perennial), breeding system (autogamous vs. mixed outcrossing), and habitat type (basalt cliff faces vs. gravel draws) (Grant, 1924; Meinke, 1992; M. Carlson, unpublished). However, seed shape and ornamentation in *M. jungermannioides*, *M. washingtonensis,* and *M. patulus* intergrade (Argue, 1986). Nonetheless, the potential inclusion of *M. patulus* with the Columbia Gorge Clade is not supported by the molecular data presented and its similarity to the other taxa in pollen and seed morphology may result from parallel evolution.

The Great Basin Clade--The sister relationship between *M.breviflorus* and *M. evanescens* has been suggested (Meinke, 1995) based on their small, nearly regular corollas, nonstipitate capsules (sessile ovaries), short puberulent foliage, and papery inflated fruiting calyces. In addition, *M. evanescens* has been allied with *M. latidens* (Meinke, 1995), but the molecular data presented here support a close sister relationship with *M.*

breviflorus. Some ambiguity in the ITS sequences suggest *M. evanescens* may be of hybrid origins, as proposed by Meinke (1995) and P. Beardsley (personal communication). Further investigation of ITS heterogeneity for this accession is underway inspired by the molecular results of Paul Beardsley (University of Washington, unpublished).

The well supported sister relationship between the Columbia River Clade with the Great Basin Clade has been apparently obscured in morphological analyses by the great diversity of life histories between these two groups. The primarily autogamous *M. breviflorus* and *M. evanescens* likely arose from a *M. washingtonenesis*-like ancestor followed by a shift to more dependably mesic sites within xeric landscapes. Specifically, *M. breviflorus* and *M. evanescens* are found in vernal pools, along stream and lake edges as compared to *M. washingtonensis,* which is restricted to gravelly basalts and depends on precipitation runoff and catch basins. The plicate sub-inflated fruiting calyces in *M. jungermannioides* may be a parallel innovation from the inflated fruiting calyces of *M. breviflorus* and *M. evanescens*. *Mimulus washingtonensis* appears to have maintained the presumably ancestral tubular calyx form.

The Snake River Clade--*Mimulus ampliatus*, *M. hymenophyllus*, and *M. patulus* are resticted to drainages of the Snake River. Although *M. ampliatus* has been affiliated with *M. washingtonensis* based on principle components analysis of 26 morphological characters and examination of herbarium specimens (Meinke, 1992), the morphological similarity between these taxa could reflect morphological convergence in similar habitats.

In addition, *M. patulus* has been synonymized with *M. washingtonensis* (Hitchcock and Cronquist, 1969). This synonymy is inconsistent with the molecular phylogeny presented herein. All three species of the Snake River Clade share a relatively open (ampliate) corolla throat, a three-dimensional feature not readily recognized on herbarium specimens. The primarily autogamous breeding system of *M. patulus* (Meinke, 1992) and close relationship with two larger flowered species suggests a progenitor-derivative pair. The ITS region and *rpl16* intron data sets conflict in their placement of *M. patulus*, resulting in a combined analysis with weak support for a *M. patulus-M. hymenophyllus* sister relationship. The longer *rpl16* intron branch leading to *M. patulus* could be an artifact of the small effective population sizes for cpDNA relative to nuclear regions compounded by its highly autogamous breeding system. Although some morphological (corolla shape and pollen size) and habitat similarities (occurring along basaltic seeps and the margins of ephemeral creeks) exist between *M. patulus* and *M. ampliatus*, the former appears more closely related to *M. hymenophyllus*, based on the molecular data and geographic distribution. From the limited number of known populations of *M. patulus* and *M. hymenophyllus*, it appears they are essentially restricted to drainages along the Imnaha and Snake Rivers in northeastern Oregon and adjacent Washington (Meinke, 1983), whereas *M. ampliatus* is only known from the more distant upper tributaries on the opposite side of the Snake River in Idaho. Further phylogenetic resolution is necessary to verify the sister taxon to *M. patulus.*

The Sierra Nevada Clade--All three of these taxa share a distinct multicellular, wiry pubescence, and inflated campanulate calyx in fruit, and occasionally sharp angled

(geniculate) bends in the stems. *Mimulus norisii* and *M. dudleyi* are edaphic endemics to marble outcrops (610 to 1310 m elevation) and granitic boulders (300 to 800m elevation), respectively. Although each possesses a unique calyx morphology, they share relatively large, showy corollas coupled with significant stigma-anther separation (Whittall, unpublished) suggesting a mixed to outcrossing breeding system (Stebbins, 1974; Jain, 1976; Barrett and Shore, 1987). It appears that *M. norisii*, with its diminutive hardened fruiting calyx, is the closest sampled outcrossing sister species to the small flowered *M. floribundus*. *Mimulus floribundus* is the most widespread species in this clade, ranging from British Columbia to central Mexico and disjunctly east to Arkansas. The species is often associated with gravelly, seasonally moist disturbed sites and spreads quickly in such habitats, consistent with Stebbins (1974) conclusion that breeding system shifts from outcrossing to selfing generally occur in species that occupy temporary, pioneer habitats (especially under conditions of drought and pollinator scarcity).

The Sierra Nevada Clade apparently shares morphological, palynological and biogeographic affinities with *M. arenarius*. This species was not sampled in this study. *Mimulus arenarius* shares the distinctive multicelled pubescence, short and wide campanulate calyx in fruit, and pollen type IIb, all traits indicative of the Sierra Nevada Clade (Grant, 1924; Argue, 1980, 1986; Meinke, 1992; Whittall, unpublished). In addition, *M. arenarius* and *M. pulsiferae* share a unique basal rosette, distributional overlap in the northern Sierra Nevada (Grant, 1924; Meinke, 1992), and appear to intergrade morphologically in Northern California (Meinke, unpublished). The

phylogenetic placement of *M. arenarius* within the *M. moschatus* alliance awaits its relocation and sampling.

Mimulus moschatus and *M. pulsiferae*--Of the members in the *M. moschatus* alliance, these are the only two species that are naturally distributed west of the Cascades outside the California Floristic Province. The combination in *M. pulsiferae* of single-celled pubescence, a nearly regular corolla, and slender calyces in fruit are consistent with its unresolved placement within the *M. moschatus* alliance. Grant (1924) suggested that it is most nearly related to *M. washingtonensis*. The placement of this species in the Sierra Nevada clade in the *rpl16* intron data set is consistent with the apparent morphological grade in the northern Sierra Nevada towards *M. arenarius* (Meinke 1992), suggesting a possible biogeographic link between the other three Pacific Northwestern clades of the *M. moschatus* alliance.

Mimulus moschatus, with its multicelled pubescence and elongated calyx teeth, is a widespread species with many local forms, some of which have been taxonomically recognized (Grant, 1924; Pennell, 1946; Hitchcock and Cronquist, 1969). Argue's palynological studies have revealed both pollen type IIc and IIb from *M. moschatus sensu lato* consistent with its unresolved placement in the *M. moschatus* alliance. Although a *M. moschatus* complex may exist, its many ecological forms have not been included in this study because of the inconsistencies in the few characters that distinguish them.

Mimulus alsinoides--Discordant topologies between nuclear and organellar data sets can be caused by chloroplast capture (Soltis et al., 1996), lineage sorting (Neigel and Avise, 1986), or more topological problems such as long branch attraction (Felsenstein, 1978). An autapomorphic load in the *rpl16* intron sequence of *M. alsinoides* gives it an especially long branch, potentially pulling it towards the long branch of the outgroup when compared to its placement in the ITS region analyses. The effects of including *M. alsinoides* on the outgroups *sensu lato* in the combined analysis, indicate that when *M. alsinoides* is included (and the combined data sets are incongruent), the outgroup topology reflects the ITS topology. Whereas, when *M. alsinoides* is deleted, the combined topology is identical to the *rpl16* resutls. This pattern suggests that the ITS could be misleading. In addition, in combination with observed excess deamination substitutions (expected T=110.83, observed T=125), this taxon should be examined for pseudogene behavior (Buckler et al. 1997).

Grant (1924) suggested its closest relative to be *M. pulsiferae*. This is contradictory to both the ITS region and *rpl16* intron results. She also suggests that *M. pachystylus* (only known from Chiapas, Mexico) is similar, although she places this taxon on its own branch in her phylogenetic chart. The unique elongated lower calyx tooth in *M. alsinoides* coupled with broad, winged petioles perplexes phylogenetic hypotheses. Sampling putative relatives (*M. suksdorfii* and *M. pachystylus*) could potentially shorten the long branch and help resolve the relationship of *M. alsinoides* outside the *M.*

moschatus alliance. Examination of intragenomic ITS clones could be used to identify ITS heterogeneity, testing for the presence of pseudogenes.

Within the *M. moschatus* alliance, it appears that there have been multiple origins of seed dormancy, corolla morphology suggesting breeding system shifts to autogamy, and inflated calyces in fuit. One shift to autogamy was followed by widespread dispersal into disturbed habitats (*M. floribundus*), the other, *M. patulus,* remains a narrow endemic. Ecological restrictions, seed dormancy, and generation time in *M. patulus* could contribute to its natural rarity. Two shifts to vegetative reproduction are coupled with unique morphologies; stoloniferous turions in *M. jungermannioides* and bulbils in *M. primuloides*. Synapomorphic morphological characters defining clades of the *M. moschatus* alliance include fruiting calyx morphology, pubescence-type, and subtle corolla throat orifice differences.

Taxonomic and conservation recommendations--The combination of morphological uniqueness and molecular differentiation within the *M. moschatus* alliance can be used to determine appropriate taxonomic ranking. *Mimulus ampliatus* should be resurrected from within *M. washingtonensis* to specific ranking as a member of the Snake River Clade based on its unique corolla morphology. In addition, *M. patulus* should be recognized within this Snake River clade due to its molecular uniqueness coupled with morphologically distinctive diminutive corolla. *Mimulus dudleyi* should be recognized at the specific rank within the Sierra Nevada Clade due to its morphological, habitat and molecular distinctiveness in light of *M. norisii*'s recognition and placement. Extensive

review of herbarium material of *Mimulus arenarius* suggests its ecological, morphological, and biogeographic uniqueness within the Sierra Nevada Clade, but this requires further molecular examination. *Mimulus dudlyei, M. arenarius, M. evanescens* and *M. ampliatus* are currently not considered for conservation in California and Idaho at the specific rank. The combination of molecular and morphological uniqueness for each of these taxa suggests their status be reconsidered.

Recognition of *M. patulus* and *M. ampliatus*, and *M. hymenophyllus* allied in the Snake River Clade, a particularly rare group of these species of conservation concern. *Mimulus jungermannioides* and *M. washingtonensis* together form a closely related pair based on molecular evidence, but represent morphological extremes in the *M. moschatus* alliance. In light of Thompson's (1996) recognition of *M. norisii, M. dudleyi* (a more distant relative of *M. floribundus),* ought to be recognized, as well. Finally, the suggested treatment of *M. evanescens* as a unique species underscores its extreme rarity and further justifies conservation concern for this endangered ephemeral species.

CONCLUSION

A molecular phylogeny for the *M. moschatus* alliance has provided an efficient and independent method for examining phylogenetic hypotheses and testing evolutionary hypotheses based on decades of morphological and life history observations. The current study has clarified phylogenetic relationships and evolutionary patterns in the *M. moschatus* alliance. The ITS region and *rpl16* intron defined four clades, three of which

are phylogeographically well defined. The molecular phylogeny suggests there were multiple origins of highly autogamous taxa and the vegetative mode of reproduction. The conservation status of *M. ampliatus, M. arenarius, M. dudleyi,* and *M. evanescens* should be reconsidered in light of their molecular distinctiveness combined with previously recognized traits. For groups of species previously defined on suites of morphological characters, a molecular phylogeny can provide an explicit test of taxonomic hypotheses, especially important for species of conservation concern.

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CHAPTER 3

CONCLUSION

SUMMARY

Molecular systematics

The nuclear ribosomal ITS region and *rpl16* chloroplast intron provided interspecific phylogenetic resolution in the *M. moschatus* alliance. Although the ITS region contained seven percent more variable sites than the *rpl16* intron, the latter exhibited three more parsimony informative sites. An exceptionally strong stem-loop secondary structure in ITS1 inhibited PCR and interfered with direct sequencing. Comparisons with some available Asterid ITS1 secondary structures suggest the *M. hymenophyllus* stem-loop and ITS1 have distinctively low free energy. The *M. moschatus* ITS1 secondary structure consists of three stem loops consistent with reports from other angiosperms. *Rpl16* intron mutation types include substitutions, insertion/deletion events, slipped strand mispairing causing elongation of mononucleotide repeats and three to four bp extension of tandem repeats, and two and four bp small inversions. Two indels from ITS2 and three from the *rpl16* intron mapped non-homoplasiously onto the ingroup combined topology. Combinability tests indicated incongruence between data sets. Through a series of taxon jackknifing, the source of conflict was isolated to *M. alsinoides*. Removal of this

accession compromised resolution among the ingroup and therefore remained included in the phylogenetic analysis.

Evolutionary patterns

The *M. moschatus* alliance forms a monophyletic group when compared with outgroups from within section *Paradanthus* and three additional sections. Four strongly supported clades consisting of two to three species each have been designated the Snake River Clade, Columbia River Clade, Sierra Nevada Clade, and Great Basin Clade. The phylogeny suggests strong phylogeographic structure of clades restricted to major river drainages of western North America. Synampomorphic characters in the alliance include subtleties of the fruiting calyx shape and texture, corolla orifice shape and size, stem and leaf pubescence, and pollen type. The evidently parallel origins of the cliff habit, reduced corolla size (associated with autogamy), inflated fruiting calyces, and seed dormancy may have been responsible for previous taxonomic and phylogenetic uncertainty. The independent origins of the primarily autogamous *M. patulus* and *M. floribundus* from large flowered endemic relatives offers a comparative system for the study of breeding system evolution. Interestingly, *M. patulus* is narrowly endemic to northeastern Oregon, adjacent Washington and Idaho, whereas *M. floribundus* now occupies mesic habitats from British Columbia to Mexico and east to Arkansas.

Taxonomic and conservation implications

Specific recognition of all 12 taxa of the *M. moschatus* alliance, based on each one's morphological and molecular uniqueness has implications for the conservation status of

many of the rare taxa. Specifically, taxonomic clarification described herein suggests reexamination of the conservation status of *M. ampliatus*, *M. evanescens*, and *M. dudleyi*. Additionally, the Snake River Clade represents a unique lineage consisting of three species, all under consideration for conservation efforts. Furthermore, the striking life history differences between sister taxa of the Columbia River Clade suggest the great evolutionary potential of each unique lineage within the *M. moschatus* alliance.

FUTURE RESEARCH

Additional phylogenetic signal from other molecular loci would test the weakly supported relationships among the four clades of the *M. moschatus* alliance, resolve the relationships of *M. moschatus* and *M. pulsiferae*, direct the placement of the *M. moschatus* alliance within section *Paradanthus* and contribute to the placement of this section within *Mimulus*.

An independent investigation of inbreeding depression and selfing rates of selected species in the *M. moschatus* alliance will employ this phylogeny to examine patterns of breeding system evolution through character mapping, ancestral state reconstruction, and methods of independent contrasts.

Although the proposed hybrid origins of *M. evanescens* were not apparent from the ITS and *rpl16* intron data sets, explicit morphological comparisons and nuclear ribosomal external transcribed spacer data suggest it. Further investigation of intragenomic ITS heterogeneity for *M. evanescens* could identify potential hybrid origins.

Delineation of evolutionary significant units between populations of *M. jungermannioides,* as suggested by detailed morphological studies, could also benefit from additional microsatellite data.

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APPENDICES

Appendix A. ITS alignment including mask.

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                        1          11         21         31         41
M.ampliatus             TCGAAACCTG CAAAGCAGAC CGCGAATTCG TGACTAAATA CTCTGGGGAA
M.patulus               .......... .......... .......... .......... ..........
M.hymenophyllus         .......... .......... .......... .......... ..........
M.dudleyi               .......... .......... .......... ...T...... GCTC......
M.floribundus           .......... ..G....... .......... ...T....C. GCT.....N.
M.norisii               .NT....... ..G.C..N.. .......... ...T....C. GCTC....G.
M.breviflorus           .......... .......... .......... .......... ...C....G.
M.evanescens            .......... .......... .......... .......... ...C....G.
M.moschatus             .......... .......... .......... ...T...... ...C......
M.pulsiferae            .......... .......... .......... .........G .C.......G
M.jungermannioides      ....G..... ..G....... .......... ...T...N.. ..........
M.washingtonensis       .......... ..G....... .......... ...T...... .........T
M.latidens              .......... ...G...... .......... ...T...... ..T.......
M.dentatus              .......... .......... .......... ..TT...... ..-.......
M.guttatus              .......... .......... .......... ...T...... ...C.....G
M.alsinoides            .......... .......... .......... ..TT...... .........G
M.lewisii               .....G.... .......... .......C.. ..TT....A. ...-.....C
M.arenarius             .....G.... .......... .......C.. ..TT....A. .......-.C
M.primuloides           .....G.... .......... .......... ..TT...GA. ..G.....-C
M.mephiticus            ..A....... .......... ........-. ..TTA..CC. ........-C
M.whitneyi              ..A....... .......... ........-. ..TTA..CC. .......C-C
Mask                    1111111111 1111111111 1111111111 1111111111 1111111111

                        51         61         71         81         91
M.ampliatus             GCG-GCG-CG GGTCC-NAAA G-CCCC--GC GCCGCCGCCC C-TACCC--G
M.patulus               .......... .......... ........N. .NN....NNN N.........
M.hymenophyllus         .......... ......G... .......... .......... ..........
M.dudleyi               ......N... .N..-.G... A.....C.NN N......... N..C......
M.floribundus           .....N.... ........NN ..NNNG...N .NN....... N..C......
M.norisii               .......... ......G..N .G...GCGC. ......-..- -..C......
M.breviflorus           .......... .N..N.C.NG .....N..NN NNN....... N..C......
M.evanescens            .......... ......T..G .......... ..A....N.. ...C......
M.moschatus             .......... ..C...TT.N .......... .......... ...C......
M.pulsiferae            .......... ......TCGG .......... ..A....... ...C......
M.jungermannioides      .......... ....N.GN.. ..NN.N.... .......NNG ...C......
M.washingtonensis       .......... ......G... .......... ......N... ...C......
M.latidens              .......... ......G... .......... .......... ...C......
M.dentatus              .T....N... ......G... .......... ..T..T.... ...C......
M.guttatus              .......... ..G...G... NA........ .......A.. ..A.......
M.alsinoides            .T........ ......G... .G.A...... .......... ...C......
M.lewisii               .GTT...T.. ..G.-.G... .....A.T.. ..T....T.. ...C...C..
M.arenarius             .GCT...T.. ..G.-.G.G. .....A.T.. .......T.. ...C...CC.
M.primuloides           .GTT...C-. ..G..TG... A.....GT.. ..T....T.. .C.C.T.CC.
M.mephiticus            TGCG.TTG-. ..G.TAG--- -.--..TCC. T....T.T-. .C.C-A.CC.
M.whitneyi              TGCG.TCG-. ..G.TAG--- -.--..TCC. T......T-. .C.C-A.CC.
Mask                    1111111111 1111100000 0000000000 0000000000 0011111111
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                    101         111         121         131         141
M.ampliatus         CCGA--TGCG  CACGAAACTG  CGCACCGTGC  GGACTAA-CG  TACCCCGGCG
M.patulus           .......... .......G..  .......... .......... ..........
M.hymenophyllus     .......... .......G..  .......... .......... ..........
M.dudleyi           ...G...... ..........  ....T..... .......... ..........
M.floribundus       .......... ..........  .......... .......... ..........
M.norisii           .......... ..........  .......... .......... ..........
M.breviflorus       ...G...... ..........  ..T....... .......... ..........
M.evanescens        ...G....-. .......N..  N.T.T..... .......... ..........
M.moschatus         ...G...... ..........  .......... .......... ..........
M.pulsiferae        ...G...... ..........  .......... .......... ..........
M.jungermannioides  ...G...... ......G...  .......... .......... ..........
M.washingtonensis   ...G...... ..........  .......... ..G....... ..........
M.latidens          .......... ..........  ..T....... .......... ..........
M.dentatus          .......... ..........  ...CT..... .......... ..........
M.guttatus          .......... ..........  ...CT..... .......... ..........
M.alsinoides        T......... .......G..  ..TCT..... .......... ..........
M.lewisii           ..A....... ..........  ..TCTT.... ..G....... ..........
M.arenarius         ..A....... .......A..  ..TGTT.... ..G....A.. ..........
M.primuloides       ..AG..A... .......N..  ...T.T.... ..G....... C.........
M.mephiticus        A..GCG.... .C..--CG..  ...-.TC.-. ..G....... A.........
M.whitneyi          A..GCG.... .CT.--CGC.  ..-T.TC.-. ..G....... A.........
Mask                1111111111 1111111111  1111111111 1111111111 1111111111


                    151         161         171         181         191
M.ampliatus         CGGAATGCGC  CAAGGAAAAC  TCAACGAAGC  GTCCG-CCCC  CCGTCGCCTC
M.patulus           .......... ..........  .......... .......... ..........
M.hymenophyllus     .......... ..........  .......... .......... ..........
M.dudleyi           .....C.... ..........  .......... .........A ....T.....
M.floribundus       .....C.... ..........  .......... .......... ....T.....
M.norisii           .....C.... ..........  .......... .......... ....T.....
M.breviflorus       .......... ..........  .......... .......... ....T.....
M.evanescens        ...-...... ..........  ....GCG... .......... ....T.....
M.moschatus         .......... ..........  .......... .......... ....T.....
M.pulsiferae        .......... ..........  .......... .......... ....T.....
M.jungermannioides  .......... ..........  .......... .......... ....T.....
M.washingtonensis   .......... ..........  .......... .......... ....T.....
M.latidens          .......... ..........  .......... .......... ....T.....
M.dentatus          .......... ..........  .......... .......... ....T.....
M.guttatus          .......... ..........  ..NN...... .C...C.... ....T..N..
M.alsinoides        .......... ..........  .......... ...A.C..T. ....T.....
M.lewisii           .....C.... ..........  .......... ...T...... .T..T.....
M.arenarius         .....C.... ..........  .......... ...T...... .T..T.....
M.primuloides       .....C.... ..........  ........T. ...N...... .T..T.....
M.mephiticus        .......... ..........  AA........ .......... ...C....C.
M.whitneyi          .......... ..........  GA........ .......... ...C....C.
Mask                1111111111 1111111111  1111111111 1111111111 1111111111
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                    201        211        221        231        241
M.ampliatus         GTTCGCGACG TGCGCGGGGG TGCCGG-GCG T-GTC----- TTGAATGTCA
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.dudleyi           .......... .......... .......A.. .......... .....A....
M.floribundus       .......... .......... .......A.. .......... ......T...
M.norisii           .......... .......... .......A.. .......... ..........
M.breviflorus       .......... .......... ....T..... .......... ..........
M.evanescens        .......... .......... ....T.GA.. .......... .G........
M.moschatus         ..C....... .......... .......... .......... ..........
M.pulsiferae        ..C....... .......... .......... .......... ..........
M.jungermannioides  .......... .......... .......... .......... ..........
M.washingtonensis   .......... .......... .......... .......... ..........
M.latidens          .......... .....-.... .......A.. .......... ..........
M.dentatus          .......... .......... ...GA..A.. .......... ..........
M.guttatus          ..A.N..... .......... ...GA..A.. .......... ..........
M.alsinoides        .......... .......... ...GA..A.. .......... ..........
M.lewisii           ........A. .......... C......A.. .......... .........T
M.arenarius         ........-. .......... C......A.. .......... .........T
M.primuloides       ........A. .......... ...GT..A.. .......... .........T
M.mephiticus        .......--. ......T.C. ..--..GA-. .G...GTCTC .....A....
M.whitneyi          .......--. ......T.C. ..--..GA-. .G...GTCTC .....A....
Mask                1111111111 1111111111 1111111111 1111111111 1111111111
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                    251        261        271        281        291
M.ampliatus         AAACGACTCT CGGCAACGGA TATCTCGGCT CTCGCATCGA TGAAGAACGT
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.dudleyi           .......... .......... .......... .......... ..........
M.floribundus       .......... .......... .......... .......... ..........
M.norisii           .......... .......... .......... .......... ..........
M.breviflorus       .......... .......... .......... .......... ..........
M.evanescens        .......... .......... .......... ..N....... ..........
M.moschatus         .......... .......... .......... .......... ..........
M.pulsiferae        .......... .......... .......... .......... ..........
M.jungermannioides  .......... .......... .......... .......... ..........
M.washingtonensis   .......... .......... .......... .......... ..........
M.latidens          .N........ .......... .......... .......... ..........
M.dentatus          .......... .......... .......... .......... ..........
M.guttatus          .......... .......... .......... .......... .N........
M.alsinoides        .......... .......... .......... .......... ..........
M.lewisii           .......... .......... .......... .......... ..........
M.arenarius         .......... .......... .......... .......... ..........
M.primuloides       .......... .......... .......... .......... ..........
M.mephiticus        T......... .......... .......... .......... ..........
M.whitneyi          T.......N. .......... .......... ..N....N.. ..........
Mask                1111111111 1111111111 1111111111 1111111111 1111111111
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                    301        311        321        331        341
M.ampliatus         AGCGAAATGC GATACTTGGT GTGAATTGCA GAATCCCGTG AACCATCGAG
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.dudleyi           .......... .......... .......... .......... ..........
M.floribundus       .......... .......... .......... .......... ..........
M.norisii           .......... .......... .......... .......... ..........
M.breviflorus       .......... .......... .......... .......... ..........
M.evanescens        ..N....... ......N... .......... .......... .........N
M.moschatus         .......... .......... .......... .......... ..........
M.pulsiferae        .......... .......... .......... .......... ..........
M.jungermannioides  .......... .......... .......... .......... ..........
M.washingtonensis   .......... .......... .......... .......... ..........
M.latidens          .......... .......... .......... .......... ..........
M.dentatus          .......... .......... .......... .......... ..........
M.guttatus          ........N. .......... .......... .......... ......N...
M.alsinoides        .......... .......... .......... .......... ..........
M.lewisii           .......... .......... .......... .......... ..........
M.arenarius         .......... .......... .......... .......... ..........
M.primuloides       .......... .......... .......... .......... ..........
M.mephiticus        .......... .......... .......... .......... ..........
M.whitneyi          .......... ..N....... .......... .......... ..........
Mask                1111111111 1111111111 1111111111 1111111111 1111111111

                    351        361        371        381        391
M.ampliatus         TCTTTGAACG CAAGTTGCGC CCGAAGCCAC TAGGCCGAGG GCACGTCTGC
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.dudleyi           .......... .......... .......... .......... ..........
M.floribundus       .......... .......... .......... .......... ..........
M.norisii           .......... .......... .......... .......... ..........
M.breviflorus       .......... .......... .......... .......... ..........
M.evanescens        ........N. .......... .N........ .......... ..........
M.moschatus         .......... .......... .......... .......... ..........
M.pulsiferae        .......... .......... .......... .......... ..........
M.jungermannioides  .......... .......... .......... .......... .....C....
M.washingtonensis   .......... .......... .......... .......... .....C....
M.latidens          .......... .......... .......... .......... ..........
M.dentatus          .......... .......... .......... .......... ..........
M.guttatus          .......... .......... ..N....... ...N..N..N ......N...
M.alsinoides        .......... .......... .......... .......... ..........
M.lewisii           .......... .......... .........T .......... ..........
M.arenarius         .......... .......... .........T .......... ..........
M.primuloides       .......... .......... .........T .......... ..........
M.mephiticus        .......... .......... .........T .......... ....A.....
M.whitneyi          .......... .......... .........T .......... ....A.....
Mask                1111111111 1111111111 1111111111 1111111111 1111111111
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                    401         411         421         431         441
M.ampliatus         CTGGGCGTCA CGCATTGCGT CG-CCCCC-T T-CCCGCTCC ATCGGG-GCG
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .N........ .......... ..........
M.dudleyi           .......... .......... .......... ...T...... .C........
M.floribundus       .......... .......... .......... ...T...... .C........
M.norisii           .......... .......... .......... ...T...... .C........
M.breviflorus       .......... .......... .........C ...T...C.. ..........
M.evanescens        .......... .......... .........C ...T...C.. ..........
M.moschatus         .......... .......... .......... .......C.. ..........
M.pulsiferae        .......... .......... .......... ...T...... ...C......
M.jungermannioides  .......... .......... .......... ...T...... .-........
M.washingtonensis   .......... .......... .......... ...T...... .-........
M.latidens          .......... .......... .......... ...T...... ..........
M.dentatus          .......... .......... .......... ...T...... ..........
M.guttatus          N......... ....N..N.. ..C.....CC ...TN....N G..N......
M.alsinoides        .......... .......... .......... ...T...... ..T..T....
M.lewisii           .......... .......... .......... ...T.C...G C...A....C
M.arenarius         .......... .......... .......... ...T.C...G C.T......C
M.primuloides       .......... .......... .......... .....C...G C...A....C
M.mephiticus        .......... .....C.... ........G. CA...C...G -..AA.A...
M.whitneyi          .......... .....C.... ........G. CA.......- T..AA.A...
Mask                1111111111 1111111111 1111111111 1111111111 1111111111

                    451         461         471         481         491
M.ampliatus         GGATC---GG GCGCGGACAT TGGTCTCCCG TGCGCTC--G CGCGCGCGGC
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.dudleyi           .......... .......T.. .......... .......... ..........
M.floribundus       .......... .......T.. .......... .......... A.........
M.norisii           .......... .......T.C .......... .......... ..........
M.breviflorus       .......... .......T.A .......... .......... ..........
M.evanescens        ........A. .......T.A .......... .......... ..........
M.moschatus         .......... .......T.. .......... .......... ..........
M.pulsiferae        .......... .......T.. .......... .......... ..........
M.jungermannioides  .......... .......... .......... .......... ..........
M.washingtonensis   .......... .......T.. .......... .......... ..........
M.latidens          .......... ....N..T.. .......... .......... .........N
M.dentatus          .......... .......T.N .......... .......... ..........
M.guttatus          .....N.... .G.....T.. .......... .......... ..........
M.alsinoides        ....T..... .T.....T.. .......... .......... ..........
M.lewisii           ....G..... .T.....T.. .......... .......T.. ....T.....
M.arenarius         ....G..... .T.....T.. .......... .......T.. ..T.T.....
M.primuloides       ....G..... .......T.. .......... .....C.C.. T...T.....
M.mephiticus        T.G.G.GC.. .A.....G.C ...C...... ...A.C.CG. T...--....
M.whitneyi          T.T.G.GC.. .A.....G.C ...C...... ...A.C.CG. T...--....
Mask                1111111111 1111111111 1111111111 1111111111 1111111111
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                    501         511         521         531         541
M.ampliatus         TGGCCCAAAT GAGATCCCTC GGCGATGCAT GTCACGAGCA GTGGTGGTTG
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.dudleyi           .......... .......... .......... .......... .......C..
M.floribundus       .......... .......... .......... .......... ..........
M.norisii           .......... .......... .......... .......... ..........
M.breviflorus       .......... .......... .........C .......... ..........
M.evanescens        .......... .......... .........C .......... ..........
M.moschatus         .......... .......... .........C .......... ..........
M.pulsiferae        .......... .......... .......... .......... ..........
M.jungermannioides  .......... .......... .......... .......... ..........
M.washingtonensis   .......... .......... .......... .......... ..........
M.latidens          .......... .......... .......... ....N..... .N........
M.dentatus          .......... .......... .......... .......... ..........
M.guttatus          .......... .......... .........C .......... ..........
M.alsinoides        .......... .......... .......... .......... ..........
M.lewisii           .......... .......... .....C.... .......... ........G.
M.arenarius         .......... .......... .....C.... .......... ........G.
M.primuloides       .......... .......... .....C.... .......... ..........
M.mephiticus        C......... .C......G. .......... .......... ..........
M.whitneyi          C......... .C......G. .......... .......... ..........
Mask                1111111111 1111111111 1111111111 1111111111 1111111111

                    551         561         571         581         591
M.ampliatus         AATTCTCGAC TCGCTTGCTG CAGTGCTTGA CGGCATCGTC CGCTCGGGCA
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.dudleyi           .......... .....C.... T.A....... T......... ..........
M.floribundus       .......... .......... T.A....... T......... ..........
M.norisii           ..C....... .......... T......... T......... ..........
M.breviflorus       .......... .......... ..A....... .......... ...N......
M.evanescens        .......... .......... ..A......T .......... ...C......
M.moschatus         .......... .......... .......... T......... ..........
M.pulsiferae        .......... .......... .......... ....G..... ........A.
M.jungermannioides  .......... .......... .GA....... .T...C.... ..........
M.washingtonensis   .......... .......... .GA....... .T...C.... ..........
M.latidens          .......... .......... ..A....... T.T....... ..........
M.dentatus          .......... .......... T.A....... T......... ..........
M.guttatus          .......... .......... .G........ .......... ..........
M.alsinoides        .......... .......... T.A....... T......... ..........
M.lewisii           .......... ....C..... TGA....... T...G..... ..........
M.arenarius         .......... ..C......- .GA------- T...G..... ..........
M.primuloides       .......... .......... .GT....... T...G..... ..........
M.mephiticus        ...-...A.. ....G..... T.A....... T......... ........A.
M.whitneyi          ...-...A.. ....G..... T.A....... T......... ........A.
Mask                1111111111 1111111111 1111111111 1111111111 1111111111
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                    601         611         621         631         641
M.ampliatus         TCACCAACGA CCCAACGGCG CCCCTC--GG CGCCTTCGAT T
M.patulus           .......... .......... .......... .......... .
M.hymenophyllus     .......... .......... .......... .......... .
M.dudleyi           .......... .......... .......... .......... .
M.floribundus       .......... .......... ....C..... .......... .
M.norisii           .......... .......... ....C..... .......... .
M.breviflorus       .......... .......... ....C..... ...-...... .
M.evanescens        .........G .......... N...C..... ....A..... N
M.moschatus         .......... .......... ....A..... .......... .
M.pulsiferae        .......... .......... ....AT.... .......... .
M.jungermannioides  .......... .......... ....A.GA.. .......... .
M.washingtonensis   .......... .......... ....A.GA.. .......... .
M.latidens          .......... .......... ...-.TC... .......... .
M.dentatus          .......... .......... ..A..T.... .......... .
M.guttatus          .......... .......... ..A.ATC... .......... .
M.alsinoides        ..G....... .......... ..A..T.... T......... .
M.lewisii           ....G..T.. ........T. .T..AT.... .A........ .
M.arenarius         ....G..T.. ........T. .TA.A..... .A........ .
M.primuloides       ....G..T.. ........T. .TGA...... .A........ .
M.mephiticus        ....AGTT.. ...G...... .NAAA-..-. .........C C
M.whitneyi          ....AGTT.. ...G...... .AAAA-..-. .......N.C C
Mask                1111111111 1111111111 1111111111 1111111111 1
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Appendix B. *Rpl16* alignment.

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                          1          11         21         31         41
M.ampliatus               TAGGGTTAGG ATTCAAAAAA GACCAATCCC ATAGTATCAA AACCAACTCA
M.patulus                 .......... .......... .......... .......... ..........
M.hymenophyllus           .......... .......... .......... .......... ..........
M.breviflorus             .......... .......... ......C... G......... ..........
M.evanescens              .......... .......... ......C... G......... ..........
M.moschatus               .......... .......... ...N.CC..- G..T...... ..........
M.floribundus             .......... .......... ......C... .......... ..........
M.norisii                 .......... ..M..C..Y. ......C... .......... ..........
M.dudleyi                 .......... .......... ......C... .......... ..........
M.pulsiferae              .......... .......... ......C... .......... ..........
M.jungermannioides        .......... .......... ......C... G......A.. ..........
M.washingtonensis         .......... .......... ......C... G......... ..........
M.latidens                .......... .......... ......C... GC........ ..........
M.dentatus                .......... .......... ......C.T. G......... ..........
M.guttatus                .......... .......... ......C... G......... ..........
M.alsinoides              ......N... .......... ......C..G G......... ..........
M.primuloides             ...AN.-... .......... ...T..C..G G......A.. ..........
M.lewisii                 ...A...... .......... --.T..C..G G......A.. ..A.......
M.arenarius               ...A...... .......... A..T..C..G G......A.. ..A.......
M.mephiticus              .......... ...AC..... .....GC..- G--.------ ..........
M.whitneyi                .......... ...AC..... .....GC..- G--.------ ..........

                          51         61         71         81         91
M.ampliatus               TCACTTCGTA TTATCTGAAT CTAAAAAAAC AGTCGAGATA TGCTAAATTA
M.patulus                 .......... .......... .......... .......... ..........
M.hymenophyllus           .......... .......... .......... .......... ..........
M.breviflorus             .......... .......G.. .......... .......... ..........
M.evanescens              .......... .......G.. .......... .......... ..........
M.moschatus               .......... .......G.. .......... ....A..... ..........
M.floribundus             .......... .......G.. .......... .......... ..........
M.norisii                 .......... .......G.. .......... .......... ..........
M.dudleyi                 .......... .......G.. .G........ .......... ..........
M.pulsiferae              .......... .......G.. .......... .......... ..........
M.jungermannioides        .......... .......G.. .......... .......... ..........
M.washingtonensis         .......... .......G.. .......... .......... ..........
M.latidens                .......... .......G.. .....G..C. .......... .........G
M.dentatus                .......... .......G.. .....G..C. .......... .........G
M.guttatus                .......... .......G.. .....G..C. .......... ........GG
M.alsinoides              .......... .......G.. .....G..C. .......... ..........
M.primuloides             .......T.. .......G.. A...G...C. .......... ......TA..
M.lewisii                 .......T.. .......G.. A...GG..C. .......... G.....TA..
M.arenarius               .......T.. .......G.. A...GG..C. .......... G.....TA..
M.mephiticus              .......... .......G.. .....G..C. ....A..... .........G
M.whitneyi                .......... .......G.. .....G..C. ....A..... ........CG
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                    101        111        121        131        141
M.ampliatus         G-TCATATCT TTGTAGCAAC TGAAA--TTT TTTTCACTAA ACTTTAATTC
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.breviflorus       .......... ...G...... ....C..... .......... ..........
M.evanescens        .......... ...G...... ....C..... .......... ..........
M.moschatus         ...T...... .......... ......T... .......... ..........
M.floribundus       .......... .......... .......... ....A..... ..........
M.norisii           .......... .......... .......... ....A..... ..........
M.dudleyi           .......... .......... .......... ....A..... ..........
M.pulsiferae        .......... .......... .......... .......... .....T...T
M.jungermannioides  .......... .......... ....C..... .......... ..........
M.washingtonensis   .......... .......... ....C..... .......... ..........
M.latidens          .......... .......... ......T... .......... ..........
M.dentatus          .......... .......... .......... .......... ..........
M.guttatus          .......... .......... ......TG.. .......... ..........
M.alsinoides        .......... .......... .....TT... ...-..A... ....G.....
M.primuloides       .G........ .......... ...C.AA... ......A... N.........
M.lewisii           .G........ .......... .....TT... ......A... ..........
M.arenarius         .G........ .......... .....TT... ......A... ..........
M.mephiticus        .......... .......... ......T... ....G.A... .....C....
M.whitneyi          .......... .......... ......T... ....G.A... .....C....

                    151        161        171        181        191
M.ampliatus         TAGGTTTAAA GCAAAA---- -----TATAG AAGAAAAGTG TGGATAAATG
M.patulus           .......... .......... .........A .........C ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.breviflorus       .......... .......... .......C.. .......... ..........
M.evanescens        .......... .......... .......C.. .......... ..........
M.moschatus         .......... .......... .......C.. .......... ..........
M.floribundus       .......... .......... .......C.. .......... ..........
M.norisii           .......... .......... .......C.. .......... ..........
M.dudleyi           .......... .......... .......C.. .......... ..........
M.pulsiferae        .......... .......... .......C.. .......... ..........
M.jungermannioides  .......... .......... .......C.. .......... ..........
M.washingtonensis   .......... .......... .......C.. .......... ..........
M.latidens          .......... .......... .......C.. .......... ..........
M.dentatus          .......... .......... .......C.. .......... ..........
M.guttatus          .......... .......... .......C.. ..T....... ..........
M.alsinoides        ..A....... .TT...AAAA AAATA.NC.. .......... ..........
M.primuloides       ..A....... .-........ .......C.. .......... ..........
M.lewisii           ..A....... .A........ .......C.. .......... ..........
M.arenarius         ..A....... .A........ .....-MC.. .......... ..........
M.mephiticus        G.A....... .......... .......C.C .G...GG... C.........
M.whitneyi          G.A....... .......... .......C.C .G...GG... C.........
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                        201        211        221        231        241
M.ampliatus             GAAAGATGAG AGAAAGAGAG AAAA----AT AATATCA--A TGATATAAAA
M.patulus               .......... .......... .......... .......... ..........
M.hymenophyllus         .......... .......... .......... .......... ..........
M.breviflorus           .......... .......... .......... .......... ..........
M.evanescens            .......... .......... .......... .......... ..........
M.moschatus             .......... .......... .......... .......... ..........
M.floribundus           .......... .......... .......... .......... ..........
M.norisii               .......... .......... .......... .......... ..........
M.dudleyi               .......... .......... .......... .......... ..........
M.pulsiferae            ......G... .......... .......... .......... ..........
M.jungermannioides      .......... .......... .......... .......... ..........
M.washingtonensis       .......... .......... .......... .......... ..........
M.latidens              ...G...... .......... .......... .......... ..........
M.dentatus              ...G...... .......... .......... ......T... ..........
M.guttatus              ...G...... .......... .......... .......... ..........
M.alsinoides            ...G...... .......... .........G .......... ..........
M.primuloides           ...G...... .......... .........G .......... ..........
M.lewisii               ...G...... .......... .........G .......... ..........
M.arenarius             ...G...... .......... .........G .......... ..........
M.mephiticus            ...G.....A .......A.. .......T.. C-...G.TT. .......G..
M.whitneyi              ...G.....A .......A.. ....AAAT.. C-...G.TT. .......G..

                        251        261        271        281        291
M.ampliatus             TTCCAATATG TAAGGTCTAC GAATCATCTC ATAAAAGACA ATGTAATAAA
M.patulus               .......... .......... .......... .......... ..........
M.hymenophyllus         .......... .......... .......... .......... ..........
M.breviflorus           .......... .......... .......... .......... ..........
M.evanescens            .......... .......... .......... .......... ..........
M.moschatus             .......... .......... .......... .......... ..........
M.floribundus           .......... .......... .......... .......... ..........
M.norisii               .......... .......... .......... .......... ..........
M.dudleyi               .......... .......... .......... .......... ..........
M.pulsiferae            .......... .......... .......... .......... ..........
M.jungermannioides      .......... .......... .......... .......... ..........
M.washingtonensis       .......... .......... .......... .......... ..........
M.latidens              .......... .......... ..G.T..... .......... ..........
M.dentatus              .......... .......... ..G..... .......... G.........
M.guttatus              .......... .......... ..G..... .......... ..........
M.alsinoides            .......... .........T ..G..... .......... G.........
M.primuloides           .......... .........G ..G..... ......T... G.........
M.lewisii               .......... .........G ..G..... .......... G.........
M.arenarius             .......... .........G ..G..... .......... G.........
M.mephiticus            .......... .......... ..G..... .......... G.........
M.whitneyi              .......... .......... ..G..... .......... G.........
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                    301        311        321        331        341
M.ampliatus         GCATCAATAC T---AAGTCG ATTCATCCAT AATTGAAAAT ATTCAATGAA
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.breviflorus       .......... .......... .......... .......... ...G......
M.evanescens        .......... .......... .......... .......... ...G......
M.moschatus         .......... .......... .......... .......... ..........
M.floribundus       .......... .......... .......... .......... ..........
M.norisii           .......... .......... .......... .......... ..........
M.dudleyi           .......... .......... .......... .......... ..........
M.pulsiferae        .......... .......... .......... .......... ..........
M.jungermannioides  .......... .......... .......... .......... ..........
M.washingtonensis   .......... ....G..... .......... .......... ..........
M.latidens          .......... .ACT....T. .......... ........-. ..........
M.dentatus          ....T..... ........T. .......... ........-. ..........
M.guttatus          .......... ........T. .....G.... ........-. ..........
M.alsinoides        .....T.... .......-.A. .......... ........-. ..........
M.primuloides       ....G....T ........-. .......... ....-..... ..........
M.lewisii           .........T .......-.TA .......... ........-. ..........
M.arenarius         .........T .......-.TA .......TGA ........-. ..........
M.mephiticus        .......... GC..-.-.TA .......... ........-G. ...A..A...
M.whitneyi          .......... ACA.---.TA .......... ........-G. ...A..A...

                    351        361        371        381        391
M.ampliatus         TCCTTCTTGT TT-----ATC GAATAGAAGA AGAAAATCAA GAGCTTCGAG
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.breviflorus       .........A .........A .......... .T........ ..........
M.evanescens        .........A .........A .......... .T........ ..........
M.moschatus         .........A .........A .......... .......... ..........
M.floribundus       .........A .........A .......... .......... ..........
M.norisii           .........A .........A .......... .......... ..........
M.dudleyi           .........A .........A .......... .......... ..........
M.pulsiferae        .........A ..TATTT..A .......... .......... ..........
M.jungermannioides  .........A .........A .......... .......... ..........
M.washingtonensis   .........A .........A .......... .......... ..........
M.latidens          .........A .........A .......... .......... ..........
M.dentatus          .........A .........A .......... .......... ..........
M.guttatus          .........A .........A .......... ....C..... ..........
M.alsinoides        .........-- --......-A. TT...A.... .......... ..........
M.primuloides       ......---- --......-.A .....----- -A........ ..........
M.lewisii           .........-- --......A. TT......T. .A........ ..........
M.arenarius         .........-- --......A. TT......T. .A........ ..........
M.mephiticus        .........AC .......--- -......... .....C.... ......G...
M.whitneyi          .........AC G......--- --......... .....C.... ......G...
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                        401         411         421         431         441
M.ampliatus             CCAATAAAGA CTAAGAAAA- TTGACTCAAG AATAAATTGA TTATAAGCTC
M.patulus               .......... .......... .......... .......... ..........
M.hymenophyllus         .......... .......... .......... .......... ..........
M.breviflorus           .......... .........TG .......... .......... ..........
M.evanescens            .......... .........TG .......... .......... ..........
M.moschatus             .......... .........-G .......... .......... ..........
M.floribundus           .......... .........-G .......... .......... ..........
M.norisii               .......... .........-G .......... .......... ..........
M.dudleyi               .......... .........-G .......... .......... ..........
M.pulsiferae            .......... .........-G .......... .......... ..........
M.jungermannioides      .......... .........-G .......... .......... ..........
M.washingtonensis       .......... .........-G .......... .......... ..........
M.latidens              .......... .........-G .......... .......... ..........
M.dentatus              .......... .........-G .......... .......... ..........
M.guttatus              .......... .........-G .......... .......... ..........
M.alsinoides            .......... .........-G .......... .......... ..........
M.primuloides           .......... .........-G .......... .C........ ..........
M.lewisii               .......... .........-G .......... .......... ..........
M.arenarius             .......... .........-G .......... .......... ..........
M.mephiticus            .......... .........-G .......... .......... ..........
M.whitneyi              .......... .........-G .......... .......... ..........


                        451         461         471         481         491
M.ampliatus             CGTTGTAGAA TTCTGACCTA ACCATTAAAT ACGAAGCGGT GGGAACGATG
M.patulus               .......... .......... .......... .......... ..........
M.hymenophyllus         .......... .......... .......... .......... ..........
M.breviflorus           .......... .......... .......... ....G..... ..........
M.evanescens            .......... .......... .......... ....G..... ..........
M.moschatus             .......... .......... .......... .......... ..........
M.floribundus           .......... .......... .......... .......... ..........
M.norisii               .......... .......... .......... .......... ..........
M.dudleyi               .......... .......... .......... .......... ..........
M.pulsiferae            .......... .......... .......... .......... ..........
M.jungermannioides      .......... .......... .......... ....G..... ..........
M.washingtonensis       .......... .......... .......... ....G..... ..........
M.latidens              .....C.A.. ..A....... .......... ........A. ..........
M.dentatus              .......... .......... .......... .......... ..........
M.guttatus              .......... .......... .......... .......... ..........
M.alsinoides            .......... .......... .....A.... .......... ..........
M.primuloides           .A........ .......... .......... .T........ ..........
M.lewisii               .A........ .......... .......... .......... ..........
M.arenarius             .A........ .......... .......... .......... ..........
M.mephiticus            .......A.. .......... .T........ .....A..A. ..........
M.whitneyi              .......A.. .......... .T........ .....A..A. ..........
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                        501         511         521         531         541
M.ampliatus             AAACCTGTGA ATGCAAAAGA TTTTTTT-GA ACAAATGAAT CTTGTTGATT
M.patulus               .......... .......... .......... .......... ..........
M.hymenophyllus         .......... .......... .......... .......... ..........
M.breviflorus           .......... .......... .......... .......... ...A......
M.evanescens            .......... .......... .......... .......... ...A......
M.moschatus             .......... .......... .......... .......... ...A......
M.floribundus           .......... .......... .......T.. .......... ...A......
M.norisii               .......... .......... .......T.. .......... ...A......
M.dudleyi               .......... .......... .......T.. .......... ...A......
M.pulsiferae            .......... .......... .......... .......... ...A......
M.jungermannioides      .......... .......... .......... .......... ...A......
M.washingtonensis       .......... .......... .......... .......... ...A......
M.latidens              .......... .......-.. .......... .......... ...A......
M.dentatus              .......... .......... .......... .......... ...A......
M.guttatus              .......... ..A....... ..A....... .......... ...A..A...
M.alsinoides            .......... .......... ....A..... .......... ...AC.A...
M.primuloides           .......... .......... ...C...T-- C......... ...AC.....
M.lewisii               .......... .......... ...A...T-- C......... ...AC.....
M.arenarius             .......... .......... ...A...T-- C......... ...AC.....
M.mephiticus            ...G...... .......... ...GA...A. .......... ...AC.....
M.whitneyi              ...G...... .......... ...GA...A. .......... ...AC.....


                        551         561         571         581         591
M.ampliatus             CACTAGTCGG GATGGCGAAA TGAACCGGAA ATCAATTCCT CTATTCTAAG
M.patulus               .......... .......... .......... .......... .......G..
M.hymenophyllus         .......... .......... .......... .......... ..........
M.breviflorus           .......... .......... .......... .......... .......G..
M.evanescens            .......... .......... .......... .......... .......G..
M.moschatus             .......... .......... .......... .......... .......G..
M.floribundus           .......... .......... .......... .......... .......G..
M.norisii               .......... .......... .......... .......... .......G..
M.dudleyi               .......... .......... .......... .......... .......G..
M.pulsiferae            .......... .......... .......... .......... .......G..
M.jungermannioides      .......... .......... .......... ..A....... .......G..
M.washingtonensis       .......... .......... .......... .......... .......G..
M.latidens              .......... .......... .......... .......... .......G..
M.dentatus              .......... .......... .......... .......... .......G..
M.guttatus              ........A. .......... .......... .......... .......G..
M.alsinoides            .......T.. .......... .......... .....C.... ....C..G..
M.primuloides           .....A.... .......... .......... .......... .......G..
M.lewisii               .......... .........C .......... ..A....... .......G..
M.arenarius             .......... .........C .......... ..A....... .......G..
M.mephiticus            .......T.. .......... ......A... C.A....AA. .....T.G..
M.whitneyi              .......T.. .......... ......A... ..A....AA. .......G..
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                        601         611         621         631         641
M.ampliatus             AAGTCAGGAA GAAGCGCTAC GACTGAAATA GAGATTG-CA AGAGTAAA-T
M.patulus               .......... .......... .......... .........M ..........
M.hymenophyllus         .......... .......... .......... .........M ..........
M.breviflorus           .......... .......... .G........ .......... ..........
M.evanescens            .......... .......... .G........ .......... ..........
M.moschatus             .......... .......... .......... .......G.. ........A.
M.floribundus           .......... .......... .......... .......... ..........
M.norisii               .......... .......... .......... .......... ..........
M.dudleyi               ....A..... .......... .......... .......... ..........
M.pulsiferae            .......... .......... .G........ .......... ..........
M.jungermannioides      .......... .......... .......... .......... ..........
M.washingtonensis       .......... .......... .......... .......... ..........
M.latidens              .......... .......... .......... .......... ..........
M.dentatus              .......... .......... .......... .......... ..........
M.guttatus              .......... ......A... ....C..... .......... ..........
M.alsinoides            ......C... .......... .......... .......... ..........
M.primuloides           ......C... ......T..T .......... .......... ..........
M.lewisii               ......C... N.....T..T ...--..... .......... ..........
M.arenarius             ......C... ......T..T .......... .......... ..........
M.mephiticus            ......C... .......... ....C..... .......... ..........
M.whitneyi              ......C... .......... ....C..... .......... ..........


                        651         661         671         681         691
M.ampliatus             ATTCGCCTGC GAAAACTTTC TTTTTTT--- -ATTGGTAAA CTTGTAGAAA
M.patulus               .......... .......... N......... ..W...K... ..........
M.hymenophyllus         .......... .......... .......... ......K... ..........
M.breviflorus           .......... .......... ......-... .......... A.........
M.evanescens            .......... .......... .......... .......... A.........
M.moschatus             .......... .......... .......... .......... ..........
M.floribundus           .......... .......... .......... .......... ..........
M.norisii               .......... .......... .......... .......... ..........
M.dudleyi               .......... .......... .......... .......... ..........
M.pulsiferae            .......... .......... .......... .......... ..........
M.jungermannioides      .......... .......... .......... .......... ..........
M.washingtonensis       .......... .......... .......... .......... ..........
M.latidens              .......... .........G .......... .......... ....G.....
M.dentatus              .......... .......... .......T.. .......... ....G.....
M.guttatus              .......... .......... .......TTT .......... ....G....C
M.alsinoides            .......... .......... .......... .......... ....G.A...
M.primuloides           .......... .........A .....A.TTT T......... ....G.A...
M.lewisii               .......... .......... .......... .......... ....G.A...
M.arenarius             .......... .......... .......... .......... ....G.A...
M.mephiticus            .......C.. .........T ...----... .......... ......T...
M.whitneyi              .......C.. ......C..T .....--... .......... ......T...
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                        701         711         721         731         741
M.ampliatus             GGACAAAAGA  AAAAAAAA--  GATTTATT-A  GAATAACTAT  T-----ATTT
M.patulus               ....M.....  ........AA  ..........  ..........  ..........
M.hymenophyllus         ..........  ........A.  ..........  ..........  ..........
M.breviflorus           ....M....R  ........AA  ..........  ..........  ..........
M.evanescens            ....M.....  ........AA  ..........  ..........  ..........
M.moschatus             ..........  .......-..  ..........  ..........  ..........
M.floribundus           ..........  .......-..  ..........  ..........  ..........
M.norisii               ........T.  .......-..  ..........  ..........  ..........
M.dudleyi               ..........  ..........  ..........  ..........  ..........
M.pulsiferae            ..........  ......--..  ..........  ..........  ..........
M.jungermannioides      ..........  ........M.  ..........  ..........  ..........
M.washingtonensis       ..........  .......-..  ..........  ..........  ..........
M.latidens              ..........  ...T...-..  ..........  ..........  ..........
M.dentatus              ..........  ...T...-..  ..........  ..........  ........--
M.guttatus              ..........  ...T...-..  ..........  ..........  ..........
M.alsinoides            ..........  ...T...-..  ....G.....  ..........  .CAATT....
M.primuloides           ..........  ..CT...-..  ..........  .........A  .CTATTC...
M.lewisii               ..........  ..CT...-..  ..........  .........G  CCTATTG...
M.arenarius             ..........  ..CT...-..  ..........  .........G  CCTATTG...
M.mephiticus            ..........  ...T...-..  ........T.  .....G-...  .CTATTT...
M.whitneyi              ..........  ...T...-..  ..........  .....G-...  .CTATT....


                        751         761         771         781         791
M.ampliatus             A---------  -------TAA  AATTTTTT-A  GAAAACTGTT  TTAATATCTA
M.patulus               ..........  ..........  ..........  ..........  ..........
M.hymenophyllus         ..........  ..........  ..........  ..........  ..........
M.breviflorus           C.........  ..........  ..........  ..........  C......G..
M.evanescens            C.........  ..........  ..........  ..........  C......G..
M.moschatus             ..........  ..........  ........T.  ..........  C.........
M.floribundus           ..........  ..........  ..........  T.........  C.........
M.norisii               ..........  ..........  ..........  ..........  C.........
M.dudleyi               ..........  ..........  ..........  ..........  C.........
M.pulsiferae            ..........  ..........  ..........  T.........  C.........
M.jungermannioides      C.........  ..........  ..........  ..........  C......G..
M.washingtonensis       C.........  ..........  ..........  ..........  C......G..
M.latidens              ..........  ..........  ..........  T.........  C.........
M.dentatus              .T....TATT  TA........  ..........  T.........  C.....C...
M.guttatus              ..........  ..........  ..........  T....T....  C.........
M.alsinoides            .TAAAATATT  TA........  ..........  .....A.C..  C.........
M.primuloides           .TAAAA....  ..........  ..........  .....A..G.  C.........
M.lewisii               .TAAAATGTT  TATAAAA...  ..........  .....AG.G.  C.........
M.arenarius             .TAAAA....  ..........  ..........  .....AG.G.  ..........
M.mephiticus            .T........  .......-..  ..........  A...TA----  -.........
M.whitneyi              .T....TATT  TA........  ..........  A...CA....  A.........
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                    801        811        821        831        841
M.ampliatus         TTCAAATTAA TTT------- ---------- ------AAAT TCCATTTTGA
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.breviflorus       .......... .......... .......... .......... ..........
M.evanescens        .......... .......... .......... .......... ..........
M.moschatus         .......... .......... .......... .......... ..........
M.floribundus       .......... .......... .......... .......... ..........
M.norisii           .......... .......... .......... .......... ..........
M.dudleyi           .......... .......... .......... .......... ..........
M.pulsiferae        .......... .......... .......... .......... ..........
M.jungermannioides  .......... .......... .......... .......... ..........
M.washingtonensis   .......... .......... .......... .......... ..........
M.latidens          .......... .......... .......... .......... ..........
M.dentatus          .......... .......... .......... .......... ..........
M.guttatus          ........C. .......... .......... .......... .T........
M.alsinoides        .......... ..-....... .......... .....G.... ..........
M.primuloides       .........C ---....... ......AAAT TAATTG.... ..........
M.lewisii           .......... .--....... .......... ....GG.... ..........
M.arenarius         .......... .--....... .......... ....GG.... ..........
M.mephiticus        .......... ..-....... .......... .....G.... ..T.C...AT
M.whitneyi          .......... ..GAAATTAT CTATTCAAAT TAATTG.... ..T.C...-T


                    851        861        871        881        891
M.ampliatus         ATCC------ -----TTTTA TTCGCGAGGA GCTGGATGAG AAGAAACTCT
M.patulus           .......... .......... .......... .......... ..........
M.hymenophyllus     .......... .......... .......... .......... ..........
M.breviflorus       ..T....... .........A. .......... .......... ..........
M.evanescens        ..T....... .........A. .......... .......... ..........
M.moschatus         .......... .......... .......... .......... ..........
M.floribundus       .......... .........A. .......... .......... ..........
M.norisii           .......... .........A. .......... .......... ..........
M.dudleyi           .......... .........A. .......... .......... ..........
M.pulsiferae        .......... .........A. .......... .......... ..........
M.jungermannioides  ..T....... .........A. .......... .......... ..........
M.washingtonensis   ..T....... .........A. .......... .......... ..........
M.latidens          .......... .......... .......... .......... ..........
M.dentatus          ....TTTTTG AATCC..... .......... .......... ..........
M.guttatus          .......... .......... .......... .......... ..........
M.alsinoides        .G........ .......... .......... .......... ......T...
M.primuloides       ..T....... .......... .......... .......... ..........
M.lewisii           .......... .......... .......... .......... ..........
M.arenarius         .......... .......... .......... .......... ..........
M.mephiticus        .AT....... ....T..... ....T..... .......... ..........
M.whitneyi          .AT....... ....T..... ....T..... .......... ..........
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                        901        911        921        931        941
M.ampliatus             CACGTCCAGT TTTGTAGTAG AGATGGAATT CCGAAACAAC CATCAACTAT
M.patulus               .......... .......... .......... .......... ..........
M.hymenophyllus         .......... .......... .......... .......... ..........
M.breviflorus           .......... .......... .......... .......... ..........
M.evanescens            .......... .......... .......... .......... ..........
M.moschatus             .......... .......... .......... .......... ..........
M.floribundus           .......... .......... .......... .......... ..........
M.norisii               .......... .......... .......... .......... ..........
M.dudleyi               .......... .......... .......... .......... ..........
M.pulsiferae            .......... .......... .......... .......... ..........
M.jungermannioides      .......... .......... .......... .......... ..........
M.washingtonensis       .......... .......... .......... .......... ..........
M.latidens              .......... ......A... .......... .......... ..........
M.dentatus              ..T....... .......... .......... .......... ..........
M.guttatus              ..T....... .......... .......... A......... ..........
M.alsinoides            .......... .......... .......... ......G... ..........
M.primuloides           .......... .......... .......... GT........ ..........
M.lewisii               .......... .......... .......... .......... ..........
M.arenarius             .......... .......... .......... .......... ..........
M.mephiticus            .......... .......... .......... ..A....... ..........
M.whitneyi              .......... .......... .......... .......... ..........


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M.ampliatus             AACCCC
M.patulus               ......
M.hymenophyllus         ......
M.breviflorus           ......
M.evanescens            ......
M.moschatus             ......
M.floribundus           ......
M.norisii               ......
M.dudleyi               ......
M.pulsiferae            ......
M.jungermannioides      ......
M.washingtonensis       ......
M.latidens              ......
M.dentatus              ......
M.guttatus              ......
M.alsinoides            ......
M.primuloides           ......
M.lewisii               ......
M.arenarius             ......
M.mephiticus            ......
M.whitneyi              ......
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