Phylogenetics of the *Malacothamnus* alliance (Malvaceae): Assessing the role of hybridization and molecular and morphological variation in species delineation

Tracey Ann Bodo Slotta

Dissertationsubmitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Biology

Approved by:

Duncan M. Porter

Robert H. Jones Brent D. Opell

Randall L. Small Brenda Winkel

June 8, 2004
Blacksburg, Virginia

Keywords: Malvaceae, *Iliamna, Malacothamnus, Phymosia*, ITS, *trnL-F, rpL16*, GBSSI-1, hybridization, ISSR, and taxonomic revision

Phylogenetics of the *Malacothamnus* alliance (Malvaceae): Assessing the role of hybridization and molecular and morphological variation in species delineation

Tracey Ann Bodo Slotta

Abstract

The Malacothamnus alliance consists of three genera, *Iliamna*, *Malacothamnus*, and *Phymosia*. The genera are considered taxonomically complex since hybridization freely occurs, polyploidy levels vary, and there is a lack of distinct morphological characters to delineate taxa. Several taxonomic treatments have been prepared for each genus, but relationships within the genera and the relationship of the Malacothamnus alliance to others in the Malvaceae remains unknown. This multifaceted study aimed to (a) examine the monophyly of the Malacothamnus alliance and its position in the Malvaceae, (b) determine the relationships between genera in the alliance, (c) compare variation of nuclear and chloroplast genes in the alliance, (d) prepare revised taxonomic treatments for *Iliamna* and *Malacothamnus*, and (e) examine the probability of successful hybridization in *Iliamna*.

The monophyly of the Malacothamnus alliance was not confirmed using DNA sequences of both nuclear and chloroplast regions. In *Iliamna*, little sequence variation was detected among taxa in the Rocky Mountains; however, the nuclear and chloroplast regions conflicted with regard to the relationships of the western and eastern taxa. An ancestral copy of the chloroplast genome is shared between the two eastern U.S. *Iliamna* species and *Phymosia* (Bahamas and Mexico). The nuclear ITS sequences indicated the western U.S. *Iliamna* species were more closely related to *Phymosia* and *Malacothamnus* than to other

species in *Iliamna*. Neither data set provided sufficient variation to resolve relationships of species in *Malacothamnus*.

Genetic variation and the feasibility of hybridization in *Iliamna* supported the results of the broader phylogenetic studies. *Iliamna corei* and *I. remota* are recently derived from *I. rivularis*. Hybrid offspring of *I. corei* and *I. remota* had higher viability and fecundity than did hybrids between crosses of either species and *I. rivularis*. The Virginia populations of *I. corei* and *I. remota* are more genetically similar than either is to Illinois populations of *I. remota*. However, the species are morphologically distinct and can easily be distinguished from others in the genus. Revised taxonomic treatments for *Iliamna* and Malacothamnus based on surveys of herbarium material are presented. Taxonomic revisions include the new combinations of *Iliamna grandiflora* subsp. *grandiflora* and *I. grandiflora* supsp. *crandallii* and the resurrection of *Malacothamnus hallii* and *M. orbiculatus*.

Acknowledgements

I could not have completed this work without the assistance of numerous people. First, I express my sincere gratitude to my advisor, Dr. Duncan Porter, who introduced to me *Iliamna*. Dr. Porter has always had an open door policy and a willingness to listen to me go on at length, even when I interrupted other work. Through Dr. Porter's encouragement, I applied for, and received, numerous grants to fund my laboratory research and plant collecting adventures. Secondly, my graduate committee, Drs. Bob Jones, Brent Opell, Randy Small, and Brenda Winkel, have been an invaluable source of inspiration and advice over the years. In particular, Dr. Winkel provided training and bench space for the molecular analyses conducted for the project. Tom Wieboldt, an extraordinary botanist, has enriched my graduate studies with his helpful discussions, guidance on specimen preparation, and directions to those off-the-beaten-path locations to find beautiful plants. Deborah Wiley was a great help in turning my thumb green; Chapter 7 would not have happened without Debbie's expertise and patience and the assistance of Kelly Sloan and Kendra Pope. To the remaining individuals in the Virginia Tech Biology Department, too many to mention, that have known where to order unusual supplies, when to turn in forms, distributed the wealth, and provided a friendly ear, I sincerely thank you.

Dr. Lawrence Alice has served as a mentor to me throughout graduate school. Dr. Alice had taught me the majority of my lab techniques, is a great person to review drafts of manuscripts and grant proposals, and always finds the Thai restaurant closest to the conference center. In addition to Dr. Alice, Dr. Small and his lab have made scientific meetings a joy to attend with discussions of the days' seminars. I appreciate the technical advice and lively discussion of botany and teaching biology I have had with Dr. M. Catherine Aime, Dr. Mary Lipscomb, Dr. Mary Schaeffer, and Rhonda Edwards. Without Dr. M. Kowaleweski's instruction, I would never have jumped into SAS and discovered the wonders of statistical programming. Drs. Jason Bond, Jennifer Clevinger, Jennifer Tate, and Andrea Wolfe were gracious in providing suggestions for data analysis. I cannot forget my botany family, Drs. Beth Painter and Dieter Wilken who led me through the backcountry of California in search of *Malacothamnus*. With our "*Malacothamnus*-eyes" and their vast

knowledge of the California flora, we found more species in one week than I had imagined I would find in a month. Individuals at the following institutions are appreciated for granting access to herbarium collections: California Academy of Science, Gray Herbarium, Jepson Herbarium, Missouri Botanical Garden, New York Botanical Garden, Rocky Mountain Herbarium, Santa Ana Botanical Garden, Santa Barbara Botanical Garden, United States National Herbarium, Universidad Nacional Autonoma de Mexico, University of Colorado-Boulder, University of New Mexico, and University of Texas.

Of course, if I thank my botany family, I must thank my own family as well. First, my husband, Douglas Slotta, who has withstood my bad research days along with the good, must be mentioned. As in our wedding vows, no plant, animal, or computer has separated us since he enjoys touring the States on our "Wild West Wesearch Expeditions" and maintained a website during our travels. My dad, Richard Bodo, has been supportive throughout our graduate school years and an excellent worker as we rebuilt our house during this time. And the remaining family (Bodo's, Brady, Goodwin's, and Slotta's), to answer that question asked at every family function, I am finally graduating. And, the most important members of my immediate family, or so they think, Carbon and Nitrogen showed purrfect support by guarding the computer by sleeping on the keyboard.

Lastly, I thank the other group of people, my friends. Becky and Steve Abler kept lab work entertaining. Chris Dana helped out immeasurably with BGSA and can make a mean white chocolate mousse cheesecake to cheer you up. Rob Hunter helped with rebuilding Marshmallow. I think that covers everyone, if not, thank you.

Iliamna bakeri

Malacothamnus palmeri

Table of Figures

Table of Tables

Chapter 1

Literature Review

Introduction to the Malvaceae: A Review of Taxonomic Classification

The number of genera that have been placed in the order Malvales has varied greatly (see Table 1.1). Carl Linneaus (1753) classified members of the Malvaceae as "Monadelphia Polyandria" and included the following genera: *Alcea, Althaea, Camellia, Gossypium, Hibiscus, Lavatera, Malope, Malva, Napaea, Pentapetes, Sida,* and *Stewartia*. The genera were grouped together based upon flower traits such as the stamen arrangement and polypetaly. Three of these genera, *Camellia*, *Pentapetes*, and *Stewartia*, are not placed in the family by others (see below). Taxa now known as the Malvaceae were described by Bernard de Jussieu in 1759 as Les Malves.

In Michel Adanson's system, the family Malvaceae was described as being annual shrubs, trees, or herbs with abundant mucilage, having four types of trichomes (conical, spindle, tuft, and stellate), stipules being present, and the stamens forming a cylinder that is attached to the calyx under the ovary (Stafleu 1963). Adanson described the anthers as being 2-celled (2-locular or bithecate). This is in contrast to other classifications that consider the anthers in Malvaceae as having a single locule. Adanson thought that the baobabs (*Adansonia*, Bombacaceae) were closely related to his true mallows, suggesting the classification of the future order Malvales. Adanson thought that the Malvae were

intermediate to the Gerania (Geraniaceae) and Capprides (Capparaceae), a point of view not expressed by others and probably based on the fusion of stamens (Stafleu 1963).

In Hutchinson (1973), the Malvales were described, as herbs to softly woody plants that are morphologically more or less like those in Tiliales. His separation of the Malvales is also based on taxa having stamens in a monadelphous arrangement, and the anthers having one locule. The Malvales, in this case, consists of only one family, the Malvaceae, with 17 genera. Taxa are as described above, with valvate sepals and flowers occasionally subtended by an epicalyx, or an involucre of bracteoles. Other families traditionally placed in the Malvales (Tiliaceae, Sterculiaceae, and Bombacaceae) are placed in Hutchinson's Tiliales along with the Dirachmaceae, Scytopetalaceae, and Peridiscaceae. He does note that Bombacaceae is the only member in the Tiliales with 1-locular anthers; the other families have 2-locular anthers.

Hutchinson (1969) earlier elaborated on his designation of Malvaceae as the only member in the classic "core" Malvales, in order to emphasize its derived nature. The soft-bodied habit of the Malvales, according to Hutchinson, is derived from the woody and less advanced habit seen in the Tiliales. He recognizes a superficial resemblance of the Malvaceae to the Geraniaceae. His tribe Malopeae may be the most primitive in the family because its members have gynoecia with numerous single-seeded carpels, lending support that the Malopeae were probably derived from the Ranales. The most advanced tribe, Hutchinson says, would be the Ureneae, with its reduced carpel number and staminal column lacking trichomes. *Nototriche* is an additional genus he considered to be advanced, because of its

mostly acaulescent habit and high altitude habitat in South America. In this system, the Malvales do not represent a terminal lineage; Hutchinson placed Euphorbiales as derived from malvaceous origins.

According to Cronquist (1988), the Malvales is in the subclass Dilleniidae and consists of five families (from primitive to advanced Elaeocarpaceae, Sterculiaceae, Tiliaceae, Bombacaceae, and Malvaceae) with 3000 to 3500 species. The order is characterized as being polypetalous with valvate sepals and cells with mucilage, and commonly has seeds with cyclopropenyl fatty acids. The Elaeocarpaceae belong in the order, although there are some differences and some of its taxa were occasionally placed in the Tiliaceae. However, the Elaeocarpaceae lack stellate trichomes and specialized nectaries, and have differences in general floral morphology (Watson and Dallwitz, 1992 onwards), all suggesting the family may not belong in the Malvales. Some members of the Elaeocarpaceae are similar to the Flacourtiaceae and may be more closely related to that family in the order Violales. The families Sarcolaenaceae (Chalanaceae) and Sphaerosepalaceae (Rhopalocarpaceae), occasionally put into the Malvales, are placed in the Theales by Cronquist (1988). These two families do exhibit stratified phloem as in Malvales, but have no other obvious affinities to the order. Since members in the Theales are rather heterogeneous, Cronquist places these families there rather than in the homogeneous Malvales. Because of anatomical similarities, Cronquist indicates that the Malvales are probably derived from the less modified members of the Theales. The Violales, Cronquist states, is the only other order with obvious relationship to the Malvales. Cronquist believes that both orders, Malvales and Violales, have descended from a common ancestor in the Theales.

In Takhtajan (1997), the Malvales includes twelve families. He includes Tiliaceae, Dirachmaceae (placed in Geraniaceae by Cronquist 1988), Monotaceae, Dipterocarpaceae, Sarcolaenaceae, Plagiopteraceae, Huaceae, Sterculiaceae, Diegodendraceae, Sphaerosepalaceae, Bombacaceae, and Malvaceae. Many of the families in Takhtajan's Malvales do not differ from those included in Cronquist's classification. Takhtajan has the superorder Malvanae consisting of the Cistales, Elaeocarpales, and Malvales. Malvales is characterized as having stellate trichomes or peltate scales and mucilage cells, cavities, or canals in the parenchyma. The phloem in most members of the Malvales is stratified, the exceptions being the Plagiopteraceae and Huaceae. The sepals in the Malvales are valvate, with the exception of the Monotaceae, which has imbricate sepals. The anthers in the Malvales usually open longitudinally or, less often, by apical pores, the exception being Plagiopteraceae where they open transversely (Watson and Dallwitz 1992 onwards). The Plagiopteraceae is the exception again, when an endothelium is present. The Plagiopteraceae is monotypic, with plants being lianas, similar to the Malpighiaceae in habit. It is a family of uncertain placement (Stevens 2001 onwards). Takhtajan states that the Malvales are closely related to the Cistales. Both the Cistales and the Malvales have many features in common with the Flacourtiaceae and Elaeocarpaceae and archaic members of the Theales. Anatomical evidence of vegetative organs, palynological data, and chemotaxonomical data indicate the Malvales and Cistales to be derived from the Flacourtiaceae through the intermediate group Scolopiaeae, *sensu lato*. The placement of families in Thorne's system (Thorne 1992) does not differ from Takhtajan's in many members. However, Thorne reduces several families to subfamilies in his classification (summarized in Table 1.2).

Chase et al. (1993) published the first revolutionary molecular analysis of angiosperm families. The study compiled data from numerous authors on the rbcL chloroplast gene. The product of this gene, ribulose-1,5-bisphosphate carboxylase, is crucial to carbon fixation in photosynthesis. It was shown to be a slowly evolving gene. The results of the project indicated many new relationships and confirmed the relationships of other families. This gene phylogeny was the first of its kind and, as such, did not have a large representation of the Malvales. However, the results indicated that the Malvales are in the Rosidae clade Rosid II. Families shown to be sister to the Malvaceae in the Malvales include Sterculiaceae, Tiliaceae, Bombacaceae, and Dipterocarpaceae. The Malvales clade was sister to a clade with members of the Sapindales (e.g., Sapindaceae, Anacardiaceae, Rutaceae, etc.).

In a review of morphological, anatomical, palynological, and chemical data, Judd and Manchester (1997) concluded that systems such as Cronquist's are artificial classifications of the Malvales. Their results indicate that Tiliaceae, Sterculiaceae, and Bombacaceae are paraphyletic. Of the four families, the Tiliaceae is thought to be the most primitive, with 2-locular anthers and stamens that are not in a monadelphous arrangement. Members of the Bombacaceae were found to be sister to members of the monophyletic Malvaceae. As indicated above, both the Bombacaceae and Malvaceae have unilocular anthers. To reflect their findings, Judd and Manchester (1997) suggest elevating the familial designation Malvaceae to include the four "core" Malvales. This was, in fact, originally suggested by Brown (1818), who thought that the families in the Malvales were synonomous to tribes in the Rosaceae.

Further support of this arrangement was obtained from analysis of additional rbcL sequences of members in the Malvales (Alverson et al. 1998). In their analysis, only the Malvaceae *sensu stricto* was found to be monophyletic. The expansion of the order Malvales was suggested to include Thymeleaeaceae, Elaeocarpaceae, Cistaceae, Dipterocarpaceae, Sarcolaenaceae, Sphaerosepalaceae, Bixaceae, Sterculiaceae, Tiliaceae, Bombacaceae, and the Malvaceae. The Thymeleaceae represented the most primitive member of the order and Malvaceae the most derived. In contrast to Cronquist's placement of the Malvales in the Dilleniidae, the rbcL data indicated that the order belongs in the Rosidae (Chase et al. 1993).

The monophyly of the "core" Malvales was confirmed in an analysis of another chloroplast sequence, the ndhF gene (Alverson et al. 1999). In examining phylogenetic relationships with this gene, the Tiliaceae, Sterculiaceae, and Bombacaceae were found not to be monophyletic. The results are based upon an alignment of 2226 basepairs (bp) from 70 taxa. A clade referred to as the /Malvatheca contains the traditional Malvaceae, Bombacaceae (excluding some members of tribe Durioneae), *Fremontodendron,* and *Chiranthodendron*. One of the surprising results from the study was the affinity of *Durio* and its relatives to *Helictreres* and *Reevesia*, taxa traditionally placed in the Sterculiaceae, not the Bombacaceae, of which *Durio* had been considered a member. Another surprise was the placement of *Fremotodendron* as sister to the traditional Malvaceae. This is an unusual finding since *Fremontodendron* has bithecate, tetrasporangiate anthers and apetalous flowers with a showy calyx. The authors concluded that there is extensive homoplasy in characters that previously have been used to delineate major groups in the core Malvales. Furthermore, the

synapomorphy of monothecate anthers uniting Bombacaceae and Malvaceae is incorrect. The /Malvatheca are actually united by dithecate, transversely septate anthers, which are found in basal members of both "families." The monothecate condition has arisen on more than one occasion. The traditional Malvaceae family was reduced to the subclade /Malvoideae in the /Malvadendrina clade, which is sister to six other subclades.

In a combined analysis of two chloroplast regions, rbcL and atpB, the "core" Malvales was found to be monophyletic (Bayer et al. 1999). Bayer et al.'s analyses support an expanded order Malvales with the addition of Bixaceae, Diegodendraceae, Cochlospermaceae, Cistaceae, Dipterocarpaceae, Sarcolaenaceae, Neuradaceae, Sphaerosepalaceae, Thymeleaceae, and Mutingiaceae. In this study, the Malvales are found to be sister to the Sapindales. The new taxonomic group Malvaceae *sensu lato* includes nine suprageneric groups (in order of ancentral to derived): *Leptonychia*, Byttnerioideae, Grewioideae, Tilioideae, Helicteroideae, Brownlowioideae, Sterculioideae, Dombeyoideae, Bombacoideae, and Malvoideae. Morphological features characterizing the Malvales include palmate leaves, stellate trichomes, mucilage, stratified phloem, and numerous stamens. As the authors pointed out, however, these features are not necessarily unique to this taxonomic group. There are a few apomorphic characters for the Malvaceae *sensu stricto,* including the bicolor floral unit in the inflorescence, trichomatous floral nectaries usually localized on the adaxial side of the perianth, and the valvate calyx. Cyclopropenyl fatty acids are no longer restricted to the "core" Malvales, as suggested in historical classifications. Such compounds have been found in some members of the Sarcolaenaceae and Thymeleaceae, and in the even

more distantly related families, Boraginaceae, Elaeocarpaceae, Fabaceae, Rhamnaceae, and Sapotaceae.

In the rbcL and atpB study (Bayer et al. 1999), representatives of the traditional Tiliaceae and Sterculiaceae were found scattered throughout the core Malvales. The Tilioideae is represented by a single taxon, *Tilia*, in the revised system. However, the authors did not propose any immediate action to revise the classification. With the phylogeny produced, an expansion of the Malvaceae to include Bombacaceae and an expansion of Tiliaceae to include all members of Sterculiaceae was suggested. This revision, in their opinion, would be inappropriate since a limited sampling was included. A second option proposed is to revise the Malvales to include numerous small families containing representatives of the former Tiliaceae and Sterculiaceae. This too was seen as impractical. Therefore, they retained their proposal of the Malvaceae *s.l.* as supported by the plastid gene sequence divergence and morphological variation. The variation found within the Malvaceae *s.l.* would not be greater than that found in other large families. The suprageneric, or subfamilial, classification proposed is similar to that of previous classifications with subfamilies (Malvoideae, Bombacoideae, and Durionoideae).

In a more recent angiosperm phylogeny produced using atpB and rbcL, the Malvales were again found to be sister to the Sapindales and also the Brassicales (Savolainen et al., 2000). In this broadscale plastid phylogeny, the Malvaceae is broadened to include members of the Bombacaceae, Sterculiaceae, Tiliaceae, and Malvaceae *s.s.* In this study, the Malvales included (ancestral to derived): Thymeleaceae, Mutingiaceae, Cistaceae, Sarcolaenaceae,

Dipterocarpaceae, Bixaceae, and Malvaceae. In comparison to the Chase et al. (1993) rbcL phylogeny, taxon representation of families was improved in the combined plastid analysis, but still lacked many malvaceous members.

Stevens (2001 onwards) placed eleven families in the order Malvales: Neuradaceae, Thymeleaceae, Sphaerosepalaceae, Cochlospermaceae, Bixaceae, Diegodendraceae, Cistaceae, Sarcolaenaceae, Dipterocarpaceae, Mutingiaceae, and Malvaceae. He stated that pulvinate leaves that are often palmately veined, stellate or fasciculate trichomes, fibrous bark, contorted corolla, and the stamens usually being numerous can be used to recognize members in the order. The Elaeocarpaceae, which has been placed in the Malvales by Cronquist (1988) and Bayer et al. (1999) to name two recent systems, is placed in the Oxalidales in this classification. The basal clade of the Malvales, with branches leading to Neuradaceae to Sarcolaenaceae, is well supported either with molecular or morphological evidence. Relationships within the Dipterocarpaceae are proposed but exhibit less than 80% support for the three subfamilies. Relationships within the Malvaceae *s.l.* are shown to be unresolved. The only clade with 50-80% support is one in which Malvoideae and Bombacoideae are found. The remaining subfamilies, which have less than 50% bootstrap support, are those discussed above.

The value of molecular data is further exemplified in the finding of *Fremontodendron* as the most primitive member of the subfamily Malvoideae (Alverson et al. 1999, Bayer et al. 1999, Pfeil et al. 2002). Previously, *Fremontodendron* was placed in the Sterculiaceae given its reduced flowers. However, in this revised classification *Fremontodendron* is situated

between the Malvoideae and Bombacoideae. With the collapse of the former Sterculiaceae and the placement of its genera interspersed with those of the Tiliaceae, it is not surprising to see how taxa in the family Sterculiaceae may be more closely related to other families (or subfamilies Malvoideae/Bombacoideae) in the order (or family Malvaceae *s. l.*).

The most recent treatment of the members in the Malvaceae *s. l.* described the genera as typically having alternately arranged simple leaves that are palmately lobed and subtended by stipules (Bayer and Kubitzki 2003). Their treatment of the Malvaceae included nine subfamilies: Byttnerioideae, Grewioideae, Tilioideae, Helicteroideae, Brownlowioideae, Sterculioideae, Dombeyoideae, Bombacoideae, and Malvoideae. This system reflects the previous studies using rbcL, ndhF, and atpB genes (Alverson et al. 1998, Alverson et al. 1999, Bayer et al. 1999). Characterizing the monophyletic Malvaceae is the occurrence of tile cells, valvate sepals, and trichomatous nectaries (Bayer and Kubitzki 2003). The calyx is composed of five lobes that are valvate in bud. Typically, there are five petals that may or may not be fused to the stamens at the base. The five to numerous stamens may be united into an androgynophore that often forms a staminal column.

Members in Malvaceae *s. l.* are found worldwide, with the family containing 243 genera and 4300 or more species (Bayer and Kubitzki 2003). Within the expanded Malvaceae, the subfamily Malvoideae consists of the former Malvaceae *s. s.* The stamens in the Malvoideae are united into a staminal column that is fused to the bases of the petals, and the fruits may be loculicidal capsules or schizocarps. Malvoideae is further subdivided into tribes. In tribe Malveae, the genera are characterized by schizocarps, a staminal tube apex

with anthers protruding, and the number of stylar branches equalling the number of locules in the ovary (Fryxell 1988, 1997; Bayer and Kubitzki 2003).

The *Malacothamnus* alliance

Within the Malveae, the genera have been subdivided into twelve alliances. In Fryxell's *Malvaceae of Mexico*, *Malacothamnus*, *Neobrittonia,* and *Phymosia* are grouped into the *Phymosia* alliance based upon morphological similarities (Fryxell 1988). Bates (1968) places *Iliamna, Malacothamnus*, and *Phymosia* into the *Malacothamnus* alliance based upon chromosome numbers (Table 1.3). *Malacothamnus* and *Phymosia* have a base chromosome number of x=17, while *Iliamna* has x=33 and may be an allotetraploid derivative of one (or more) of the other genera that had subsequently lost a chromosome (Bates and Blanchard 1970). *Neobrittonia* was not included in the *Malacothamnus* alliance by Bates and Blanchard (1970) since it has a chromosome number of x=16. Instead, they placed *Neobrittonia* in the *Abutilon* alliance, which has genera with a wide range of chromosome numbers.

Research by LaDuke and Doebley (1995) using chloroplast restriction site data suggested an affinity of *Iliamna* and *Malacothamnus* within the tribe Malveae. However, *Phymosia* was not included in their study, and *Iliamna* and *Malacothamnus* were in a polytomy with members of the *Malva* and *Sphaeralcea* Alliances. The lack of resolution and the absence of *Phymosia* indicate the need for further examination of these genera to establish their relationships.

The goals of this project were to (1) evaluate the monophyly of the *Malacothamnus* alliance, (2) examine the relationships of and evaluate the monophyly of the genera within the alliance, and (3) delineate species within the genera. There has been no comprehensive study of the *Malacothamnus* alliance, and taxonomic studies of the included genera are rare (e.g., Wiggins 1936, Bates 1963, Fryxell 1971).

Iliamna

Iliamna Greene is in need of revision because (1) the classification of species within has been questioned; (2) hybridization between currently accepted species is believed to occur (Harrington 1964); and (3) all of the species are classified as rare or endangered. Species in *Iliamna* are distributed throughout the contiguous United States in small disjunct populations (Figure 1.1). The genus was first described by E. L. Greene in a brief publication and included five species (Greene 1906). Wiggins (1936) revised the genus and included seven species with two varieties. In the interim, Greene's *Iliamna* species were placed into either *Phymosia* or *Sphaeralcea* based upon morphology (Rydberg 1904, 1906, 1913, 1932). Since Wiggins' revision an additional species of *Iliamna* (*I. corei)* was described, and the varieties were reduced to synonomy. There is limited morphological variation in *Iliamna*, with few characters providing sufficient variation to easily delineate the species (Table 1.4).

The species with the largest distribution in the genus is *Iliamna rivularis*, which is found throughout the Rocky Mountains. Its distribution overlaps geographically with that of three other species, *I. crandallii* (restricted to northern Colorado), *I. grandiflora* (New Mexico, Arizona, and southern Colorado), and *I. longisepala* (eastern Washington).

Historically, *I. rivularis* has been found as far north as British Columbia and west to California. *Iliamna grandiflora* has been described as a large-flowered variety of *I. rivularis* in Colorado, and the two names have been used for the same populations (Harrington 1964). It is possible that these are two distinct entities that have hybridized, but this hypothesis has not been explored (Weber 1987). All four are state listed as rare as are the two Northern Californian species, *I. bakeri* and *I. latibracteata* (the latter also found in Oregon).

Much controversy surrounds the two eastern species, *I. corei* and *I. remota*. When *I. corei* was first described, it was as a variety of *I. remota* (Sherff 1946). However, it was later elevated to a species based on leaf characteristics, habitat, and the lack of floral scent (Sherff 1949). Presently, *I. corei* is listed as federally endangered and known from only one population in southwestern Virginia (Stewart and Porter 1995). *Iliamna remota* is found in several disjunct populations in Illinois, Indiana, and central Virginia. The two taxa currently do not overlap, and it has been hypothesized that the Virginia *I. remota* populations are the result of anthropogenic introductions. In an analysis using RAPD markers, these two taxa were distinct, but it was unclear if they warranted status as separate species or as subspecies (Stewart and Porter 1995, Stewart et al. 1996). An extensive discussion on the taxonomic history of *Iliamna* is included in Slotta (2000).

Malacothamnus

Taxa in *Malacothamnus* have undergone a similar treatment as those of *Iliamna*. Within *Malacothamnus* there is a great deal of morphological variation (Bates 1963) (Table

1.5), which has led to the description of numerous species and varieties that may or may not be valid (Eastwood 1936, Kearney 1951, Bates 1963, Fryxell 1988). With the numerous revisions in this genus, it is not surprising that 75% of the described taxa are referred to as taxonomically uncertain (Skinner et al. 1995). Hybridization is reported to occur freely among the species when their populations overlap and has resulted in the synonomy of many varieties (Bates 1993). Habitat destruction through fire suppression, urban sprawl, and agriculture have diminished plant population sizes and reduced the historical ranges of many taxa.

Malacothamnus species are much more noticeable in the landscape than those of *Iliamna* and consequently have been collected more frequently. Two taxa are listed as endangered in California (*M. clementinus* and *M. fasciculatus var. nesioticus*), ten others as rare (some of which are not widely accepted entities), and two are believed extinct (*M. mendocinensis* and *M. parishii)* (Thomas 2000). The most widely distributed species, *M. fasciculatus*, exhibits the greatest degree of morphological variation and has had several varieties described (*M. fasciculatus var. catalinensis, M. fasciculatus* var. *fasciculatus, M. fasciculatus* var**.** *laxiflorus, M. fasciculatus* var. *nesioticus,* and *M. fasciculatus* var. *nuttallii*).

The varieties of *M. fasciculatus* have been examined for genetic variation using isozyme and RAPD analysis (Swenson et al. 1995). Two populations of *M. fasculatus* var. *nesioticus* were surveyed with ten RAPD primers. One genotype was found in each population, suggesting that the populations are clonal. Analysis of isozymes supported this finding. The results also indicated *M. fasciculatus var. nesioticus* to be a unique variety,

genetically distinct from the others. However, *M. fasciculatus* var. *catalinensis*, *M. fasciculatus* var. *fasciculatus*, *M. fasciculatus* var. *laxiflorus*, and *M. fasciculatus* var. *nuttallii* had less than 15% genetic dissimilarity among the populations based on RAPDs and only three unique isozymes were identified.

In another study examining morphological variation between five taxa from Southern California (*M. clementinus, M. davidsonii, M. densiflorus, M. fasciculatus,* and *M. fremontii),* little distinction between taxa was found (Benesh and Elisens 1999). This reflected a limited number of characters with taxonomic utility in the phenetic analysis. The data were analyzed by cluster analyses, principal component analyses (PCA), and discriminant analyses. The PCA revealed loosely clustered groups of species with much overlap. This finding reflects the frequency at which taxa in *Malacothamnus* hybridize, given that the populations operate in a mixed mating system dominated by outcrossing. The 26 morphological characters chosen (11 quantitative, 15 qualitative) indicated floral traits to be the most consistent among the 46 specimens examined. However, many characters used to distinguish species in *Malacothamnus* are qualitative (e.g., degree of pubescence) and could benefit from quantitative analysis (e.g., number of trichomes per cm^2) and the use of stastical analyses. A phylogenetic study of the entire genus is needed in order to determine taxonomic entities in *Malacothamnus*.

Review of the Taxonomic History of Malacothamnus

In the publication in which *Iliamna* was first described, Greene (1906) also provided a list of eight species he considered as *Malacothamnus*, six of which were transferred from

Malavastrum. The eight species were *M. aboriginum, M. arcuatus, M. davidsonii, M. densiflorus, M. fasciculatus, M. fremontii, M. marrubioides,* and *M. orbiculatus*. A type species for the genus was not indicated. Greene's classification was recognized by Abrams (1910) and Kearney (1951), whereas others considered the species to be members of *Malvastrum* (Estes 1925, Eastwood 1939). A summary of characters defining species in *Malacothamnus* and the author of each species are listed in Table 1.5 and the range of the distribution is shown in Figure 1.1.

In his treatment of the shrubby *Malvastrum* taxa of Southern California, Estes (1925) recognized eight species and three varieties later transferred by Kearney (1951) to *Malacothamnus*. Estes selected the group for review since it had been identified as a group of "considerable difficulty to local botanists" (p. 81), with a lack of adequate keys and descriptions. Characters used in his key included pubescence of the calyx, texture of the leaves, length of the leaves and calyx, and characters of the inflorescence. Estes noted in his descriptions of the species that several appear to be intermediate. *Malvastrum nesioticum*, based on Estes' description, may be interpreted as intermediate between *M. nutallii* and *M. fasciculatum* or *M. fasciculatum* var. *laxiflorum*. The varieties newly described in this revision included: *M. fasciculatum* var. *typicum, M. densiflorum* var. *typicum,* and *M. densiflorum* var. *viscidum*. The last variety had been previously described as *Malvastrum viscidum* by Abrams (1910) and noted as a close relative of *M. densiflorum,* but with an increased density of glandular trichomes on its leaves. Both taxa also have shallowly lobed leaves.

Eastwood (1936) also referred to *Malacothamnus* taxa as the shrubby *Malvastrums*. She divided the genus into two groups. Within these groups, inflorescence type, calyx size and pubescence, thickness of leaves, leaf pubescence, and shape of the leaf base distinguished taxa. The first group contained taxa similar to *Malvastrum fasciculatum* and included *M. aboriginum, M. arcuatum, M. davidsonii, M. densiflorum, M. fasciculatum, M. gabrielense, M. involucratum, M. nesioticum, M. orbiculatum, M. palmeri,* and *M. splendidum*. Also in this group were six new species, *M. abbottii, M. catalinensis, M. hallii, M. laxiflorum, M. nuttallii,* and *M. parishii*. Characterizing the *M. fasciculatum* group is a rather short pubescence covering the carpels.

The second group was comprised of taxa similar to *Malvastrum fremontii* (Eastwood 1936). This included three previously described species, *M. clementinum, M. fremontii,* and *M. jonesii*. In addition to these, six new taxa were included in this group, *M. dudleyi, M. fragrans, M. gracile, M. helleri, M. howellii,* and *M. howellii* var. *cordatum*. Members of this second group were characterized as having no trichomes on the fruit and pubescence in the flower consisting of long white stellate trichomes.

Eastwood (1936) recognized groups of taxa with morphological affinities, such as *Malvastrum jonesii* with *M. dudleyi* and *M. fragrans*, and *M. fremontii* with *M. helleri* and *M. howellii*. Taxa in Baja California, Mexico, were noted but not included in her treatment since collections were few and little was known then of their relationships to the California species. The 27 species listed and described by Eastwood (1936) occurred in widely distributed,

isolated populations. The frequency of occurrence of populations led Eastwood to conclude that the species were relics of an older and declining genus (Eastwood 1939).

The next examination of *Malacothamnus* was conducted by Kearney (1951), who resurrected Greene's designation of the genus. Kearney included 21 species and 6 varieties of *Malacothamnus*. The taxa ranged from Mendocino and Tehama counties in northern California to northern Baja California and from the Pacific coast to the Sierra Nevada. In contrast to Eastwood's view of a declining group (Eastwood 1939), Kearney (1951) thought the opposite. The distribution of several species in small, isolated populations (e.g., *M. fremontii*) suggested to Kearney a waning species; however, other species were considered common (e.g., *M. fasciculatus* and *M. jonesii)*. Furthermore, the numerous varieties in *M. fasciculatus* had been reported to be freely hybridizing. This, in addition to the relatively few morphological characters having sufficient variation as to delineate species, led Kearney to conclude that *Malacothamnus* was a recently evolved group. He placed the genus in the subtribe Abutilinae with other uniovulate representatives of the tribe Malveae such as *Malvastrum* (*sensu stricto*), *Nototriche, Tarasa,* and *Sidopsis*. The common ancestor of this group, Kearney believed, was probably extinct.

In Kearney's treatment of *Malacothamnus*, he too recognized two groups, one with affinities to *M. fremontii* and one with *M. fasciculatus* (Kearney 1951). He characterized the *M. fremontii* group as having shallowly indented carpels and a densely woolly calyx. The group was comprised of *M. fremontii, M. helleri, M. howellii, M. marrubioides, M. niveus,* and *M. orbiculatus*. The *M. fasciculatus* group was characterized as often having deeply

incised carpels, with the calyx being covered in short, many-rayed stellate trichomes. Within the second group were *M. arcuatus, M. fasciculatus, M. hallii, M. mendocinensis,* and *M. parishii*. The remaining ten species were not allied to either of Kearney's groups and included *M. abbottii, M. aboriginum, M. clementinus, M. davidsonii, M. densiflorus, M. foliosus, M. jonesii, M. orbiculatus, M. palmeri,* and *M. paniculatus*. The varieties named as new combinations were *M. densiflorus* var. *viscidus, M. fasciculatus* var. *catalinensis, M. fasciculatus* var. *laxiflorus, M. fasciculatus* var. *nesioticus, M. fasciculatus* var. *nuttallii, and M. palmeri* var. *involucratus*. The taxonomic key provided by Kearney for *Malacothamnus* used leaf and calyx pubescence, shape and texture of leaves, and size and shape of bractlets and calyx. Characters of the fruits were not used by Kearney since insects often parasitize the fruits, and consequently mature fruits are infrequently found on herbarium specimens.

In 1963, David Bates completed his dissertation on *Malacothamnus,* in which he studied the distribution, ecology, population structure, breeding, and hybrid potential of the genus in order to provide a "different orientation for their classification" (Bates 1963, p. 4). The classification included two species, *M. chilensis* and *M. fasciculatus*. In *M. fasciculatus*, eleven subspecies from California were described. These were found in well-drained soils in chaparral or in the ecotone between chaparral and foothill woodlands, coastal sage, or oak woodlands. The subspecies of *M. fasciculatus* included subsp. *abbottii*, subsp. *aboriginum*, subsp. *clementinus*, subsp. *davidsonii*, subsp. *densiflours*, subsp. *exfibulosus*, subsp. *fasciculatus*, subsp. *foliosus*, subsp. *jonesii*, subsp. *marrubioides*, and subsp. *palmeri*. These subspecies could be defined by geographical location, suggesting an ecological (e.g., climate, elevation, habitat) influence on the morphological variation seen.

Many of the taxa previously described by Eastwood (1936) and Kearney (1951) were treated as synonymous in Bates' new combinations. For example, *M. fasciculatus* subsp. *jonesii* was based on *Malvastrum jonesii* Munz (Munz and Johnston 1925), *Malvastrum dudleyi* Eastwood (1936), *Malvastrum fragrans* (Eastwood) Kearney (1951), *Malvastrum gracile* Eastwood (1936), and *Malacothamnus fragrans*. Bates (1963) described *Malacothamnus fasciculatus* subsp. *jonesii* as heterogeneous with an overall continuity in morphology and geography. He further noted that this subspecies was similar to *M. fasciculatus* subsp. *exfibulosus* in the short-stalked pubescence, but that *M. fasciculatus* subsp. *jonesii* was more delicate in habit, similar to *M. fasciculatus* subsp. *fasciculatus*. Other characters used to distinguish between the subspecies included pubescence of the branches, leaves, and calyces and the sizes of the bracts and calyx. With *M. chilensis* and *M. fasciculatus*, the floral characters clearly delineated the species. Flowers that had the petal bases covered completely with trichomes and the pubescence continuing up the staminal column characterized *M. chilensis*; whereas, in *M. fasciculatus* the petal bases were not considered to be pubescent and the staminal column was glabrous or had a fine pubescence limited to its base.

In addition to taxonomic revision, Bates (1963) examined habitat requirements in *Malacothamnus*. He identified the genus as a fire-follower that could regenerate from root sprouts in mild fires or by seedling germination in areas cleared by fire. In a study of seed viability, seeds from herbarium specimens were found to be viable and germinate as long as fifty years after collection. Bates concluded from this, that a large number of *Malacothamnus*

seeds persist in the soil bank until favorable growing conditions occur. The persistence of seeds and ecological changes (e.g., climate) since the late Pliocene resulted in the isolation of populations with intermittent, recurring hybridizing zones. Hybridization, Bates concluded, readily occurs between taxa and has resulted in the morphological gradations observed today.

The Jepson Manual of Higher Plants of California (Hickman 1993) includes eleven species of *Malacothamnus* distributed from California to northwestern Mexico (Figure 1.1). In this treatment (Bates 1993), the genus is described as shrubby with varying degrees of pubescence that could be stellate, simple, or glandular. The flowers are described as being arranged in head-like to panicle-like inflorescences. The species are described as interfertile, and within populations variation of the pubescence and inflorescence structure can be great. Characters of significance in the key refer to the ratio of bractlet length to calyx length, flower arrangement in inflorescences, pubescence of the calyx, and petal color. Included in this treatment are notations on the frequency of species and potential threats to them. For example, *M. clementinus* is listed as endangered with populations threatened by military activity and feral animals. Synonymous taxa are indicated for their frequency of occurrence, as well. For example, *M. mendocinensis* is presumed extinct and *M. fasciculatus* var. *nesioticus* as endangered; both are considered synonymous with *M. fasciculatus* in the treatment. In addition to species descriptions, the geographical subdivisions of California where plants may be found are listed. *Malacothamnus aboriginum* is listed as occurring in the SCoRI division, which includes the Inner South Coast Ranges of Fresno, Kings, Merced, Monterey, and San Benito Counties. The key, descriptions, and illustrations provided are a practical alternative to previous treatments and keys of the genus.

Recently, *Malacothamnus chilensis* has been transferred to a new genus, *Andeimalva,* which includes four species in South America (Tate 2003). This revision was based, in part, on chromosome number; *M. chilensis* was observed to be x=6, whereas the North American *Malacothamnus* species are x=17 (Bates and Blanchard 1970). Morphologically, *M. chilensis* can be grouped with *Tarasa geranioides* and *T. meyeri,* since the three have smooth-walled and completely dehiscent mericarps. In phylogenetic analysis of ITS sequences, *M. chilensis* is located in a well-supported clade (100% bootstrap support) with *T. mandonii, T. machupicchensis,* and *T. spiciformis* (Tate 2003). The genus *Andeimalva* is described as a group of small trees to shrubs with a dense pubescence, simple leaves that have an acute apex, a calyx with five triangular lobes, mauve petals that are notched, and uniovulate carpels containing smooth single-seeded mericarps upon maturity. The species designated in the genus are *Andeimalva chilensis, A. machupicchenis, A. mandonii,* and *A. spiciformis.*

For the purpose of the present study, the species of *Malacothamnus* recognized by Bates (1993) will be referred to unless otherwise stated. It was a goal of this study to further elucidate relationships and taxonomic boundaries in this taxonomically difficult genus.

Review of Phymosia

Taxa in *Phymosia* are large shrubs or small trees with palmately lobed leaves (Kearney 1951). The type species of the genus, *P. abutiloides* (L.) Desv., is known from the Bahamas and Haiti (Figure 1.1). The specimen from which the type illustration was drawn probably originated with seeds grown by Mark Catesby in his greenhouse, the mature plants

of which were the subject of an illustration by Dillenius in *Hortus Elthamensis* in 1732 (Fryxell 1971). Linneaus observed this illustration, which typified his *Malva abutiloides*, which in turn is the nomenclatural type of *Phymosia* Desv. ex Hamilton (Hamilton 1825).

In Kearney's (1951) treatment of the American Malvaceae, *Phymosia* was classified as a member of Tribe Malveae, subtribe Abutilinae with *Iliamna*, *Malacothamnus*, and 14 additional genera. The Abutilinae is characterized as having apical stigmas, pluriovulate carpels, and usually involucrate. As with *Iliamna*, *Phymosia* is distinct from *Sphaeralcea* in that the carpels of *Phymosia* and *Iliamna* have a single compartment that dehisces along the outside margin. The stigmas in *Phymosia* are obliquely truncate and slightly decurrent. Kearney (1951) included three species in *Phymosia*, *P. abutiloides*, *P. rosea* (DC.) Kearney, and *P. umbellata* (Cav.) Kearney, the latter two occurring in Mexico and Guatemala. *Phymosia abutiloides* differs from the Mexican species in that it has smaller flowers that are pink, not red, and hasa deciduous involucre. Kearney also mentioned that *Sphaeralcea crenulata* Brandeg., *S. floribunda* (Schlecht) Walkp., *S. nutans* Scheidw., and *S. schenckii* Ulbr. may belong in *Phymosia*, but there was insufficient material for examination. *Phymosia* was allied with *Iliamna* and *Malacothamnus* in the *Malacothamnus* alliance by Bates (1968) and Bates and Blanchard (1970) based on chromosome number data. Bates (1968) noted that *Phymosia* was probably the most primitive member of the alliance with its woody habit and tropical montane distribution.

While the recognized species of *Phymosia* remained popular as cultivars in conservatories and greenhouses, taxonomic study of the species was not undertaken until

Fryxell revised the genus in 1971. Typically, species in *Phymosia* are found at elevations near 2000-3000m and the leaves, stems, petioles, and peduncles are densely covered with stipitate stellate trichomes (Fryxell 1971). Phyllotaxy of *Phymosia* resembles that of *Iliamna* in form with 3-7-lobed leaves with an elongated terminal lobe. The involucre in *Phymosia* is made of three distinct bracts, each as wide or wider than the sepals. The calyx is comprised of 5 lobes that are plicate in bud and are densely pubescent. The flowers are either solitary or occur in axillary inflorescences. The flowers in *Phymosia* are protandrous with stigmas being receptive only after the anthers dehisce. Fryxell included eight species: *P. abutiloides, P. anomola*, *P. crenulata*, *P. floribunda*, *P. pauciflora*, *P. rosea*, *P. rzedowskii*, and *P. umbellata*. Morphological variation in *Phymosia* is summarized in Table 1.6.

Project Summary

The *Malacothamnus* alliance (Malvaceae) includes the genera *Iliamna, Malacothamnus,* and *Phymosia* (Kearney 1951). The study described herein concentrates on assessing the taxonomic and evolutionary relationships within the alliance. Through examination of molecular and morphological data, several questions were addressed. First, are the genera closely related? Cytological evidence indicated they are (Bates and Blanchard 1970). *Malacothamnus* and *Phymosia* have a base chromosome number of x=17, whereas *Iliamna* has x=33. Based on this, it was believed that *Iliamna* was a polyploid derivative of one, or both, of those genera or a shared ancestor. Morphological evidence also supported this relationship. Members of the three genera have schizocarp fruits with mericarps that have a single compartment, unlike other members of the tribe Malveae (Fryxell 1988). The stellate trichomes among members of the *Malacothamnus* alliance are often stipitate or stalked. The

species range in habit from herbaceous (*Iliamna*) to shrubby (*Malacothamnus* and *Phymosia*) with flowers found in paniculate, racemose, or umbellate inflorescences.

The second question addresses the evolution and diversification of species within *Iliamna* and *Malacothamnus*. Will the establishment of the *Malacothamnus* alliance be confirmed and relationships within the genera clarified? To complement the results of the molecular data, morphological characters were examined. This investigation aimed to develop a taxonomic key and descriptions of species within the two genera by examining herbarium specimens and measuring numerous characters. The characters chosen were based on those previously used for the taxa in field guides, the original descriptions, and additional characters. The majority of features in this study characterize the flowers, fruits, and pubescence. The resulting molecular phylogenetic trees, taxonomic keys and descriptions were used to establish species boundaries within the two genera.

The final question addressed here involves the classification of *Iliamna corei* and *Iliamna remota*. Are they separate taxa, or not? *Iliamna corei* is found in tree-filtered sunlight on a rocky outcrop on Peters Mountain in Giles County, Virginia. The population size is small (less than 50 plants) and this is its only known naturally occurring locality. *Iliamna remota* is found in disturbed sites along railways, rivers, and abandoned pastures. *Iliamna remota* is known from several populations in Illinois, Indiana, and Virginia. There has been doubt in the past that these two species are separate entities (Sherff 1946; Sherff 1949). To investigate the genetic similarity of the two species, inter-simple sequence repeats were examined. These are dominant genetic markers that have been used to assess hybridization

and speciation in natural and cultivated populations (Wolfe and Liston 1998). Several unique loci were detected in *I. corei* and *I. remota,* identifying population and geographical differences. In examining these differences, the degree of divergence between *I. corei* and *I. remota* was assessed.

To test the reliability of morphological differences between *I. corei, I. remota*, and *I. rivularis,* individuals from each species were grown in the greenhouse and then planted in a common garden to determine if the described traits were a reflection of habitat rather than genotype. While the plants were in the greenhouse, flowers were emasculated and pollinated from a selected donor. Germination rates of the resulting seeds were calculated and individuals were grown to the flowering stage to assess whether or not the generated hybrids exhibited intermediate phenotypes. Reciprocal crosses were also conducted with *I. rivularis.*

Literature Cited

Abrams, L. 1910. A phytogeographic and taxonomic study of the Southern California trees and shrubs. *Journal of the New York Botanical Garden* 6: 416-419.

Adanson, M. 1963. *Familles des plantes* 2 Vol. Paris.

Alverson, W.S., K.G. Karol, D.A. Baum, M.W. Chase, S.M. Swensen, R. McCourt, and K.J. Sytsma. 1998. Circumstription of the Malvales and their relationships to other Rosidae: Evidence from rbcL sequence data. *American Journal of Botany* 85: 876-887.

Alverson, W.S., B.A. Whitlock, R. Nyffeler, C. Bayer, and D.A. Baum. 1999. Phylogeny of the core Malvales: Evidence from ndhF sequence data. *American Journal of Botany* 86: 1474-1486.

Bates, D.M. 1963. *The Genus* Malacothamnus. Ph.D. Dissertation, University of California, Los Angeles.

Bates, D.M. 1968. Generic relationships in the Malvaceae, tribe Malveae. *Gentes Herbarium* 10: 117-135.

Bates, D. M. 1993. *Malacothamnus*. In Hickman, J.C., ed. *The Jepson Manual: Higher plants of California*. Berkeley and Los Angeles: University of California Press. Pp. 747-753.

Bates, D.M. and O.J. Blanchard, Jr. 1970. Chromosome numbers in the Malvales II: New or otherwise noteworthy counts relevant to classification in the Malvaceae tribe Malveae. *American Journal of Botany* 57: 927-934.

Bayer, C., M.F. Fay, A.Y. DeFruijn, V. Savolainen, C.M. Morton, K. Kubitzki, W.S. Alverson, and M.W. Chase. 1999. Support for an expanded family concept of Malvaceae within a recircumsbribed order Malvales: a combined analysis of plastid atpB and rbcL DNA sequences. *Botanical Journal of the Linnean Society* 129: 267-303.

Bayer, C. and K. Kubitzki. 2003. Malvaceae. In K.Kubitzki and C. Bayer, eds. *The families and genera of vascular plants: Volume V. Flowering plants. Dicotyledons: Malvales, Capparales and Non-betalain Caryophyllales*. Berlin: Springer-Verlag. Pp. 225-311.

Benesh, D.L. and W.J. Elisens. 1999. Morphological variation in *Malacothamnus fasciculatus* (Torrey and A. Gray) E. Greene (Malvaceae) and related species. *Madroño* 46: 142-152.

Brown, R. 1818. Observations, systematical and geographical, on the herbarium collected by Professor Christian Smith, in the vicinity of the Congo, during the expedition to explore that river under the command of Captain Tuckey, in the year 1816. In Bennett, J.J. ed. 1866. *The Miscellaneous Botanical Works of Robert Brown. Vol. 1. Containing I. Geographico-Botanical, and II. Structural and Physiological Memoirs.* London: The Ray Society. Pp. 97-173.

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

Cronquist, A. 1988. *The Evolution and Classification of Flowering Plants*, 2nd ed. New York: New York Botanical Garden. Pp. 332-336.

Eastwood, A. 1936. The shrubby Malvastrums of California with descriptions of new species and a key to the known species. *Leaflets of Western Botany* 1: 213-220.

Eastwood, A. 1939. New California Plants. *Leaflets of Western Botany* 2: 186-188.

Engler, A. and K. Prantl. 1891. *Die Natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Aretn*. Nachträge zum II.-IV. Teil. Leipzig: Verlag von Wilhelm Engelmann. III (6): 231-242.

Estes, F. 1925. The Shrubby *Malvastrums* of Southern California. *Bulletin of the Southern California Academy of Science* 24: 81-87.

Fryxell, P. A. 1971. A revision of *Phymosia* (Malvaceae). *Madroño* 21: 153-174.

Fryxell, P.A. 1988. Malvaceae of Mexico. *Systematic Botany Monographs*. 25.

Fryxell, P.A. 1997. The American genera of Malvaceae II. *Brittonia* 49: 204-269.

Greene, E. L. 1906. Certain malvaceous types. *Leaflets of Botanical Observation and Criticism*. Pp. 205-209.

Hamilton, W. 1825. *Prodromus plantarum Indiae Occidentalis*. London. P. 49.

Harrington, H. D. 1964. *Manual of the plants of Colorado*. Chicago: Swallow Press. P. 373.

Hickman, J.C., ed. 1993. *The Jepson Manual: Higher plants of California*. Berkeley and Los Angelos: University of California Press. Pp. 747-753.

Hutchinson, J. 1969. *Evolution and phylogeny of flowering plants: Dicotyledons*. London: Academic Press. Pp. 253-7.

Hutchinson, J. 1973. *The families of flowering plants, volume I Dicotyledons,* 3rd ed. Oxford: Clarendon Press.

Judd, W. and Manchester, S. 1997. Circumscription of Malvaceae (Malvales) as determined by a preliminary cladistic analysis of morphological, anatomical, palynological, and chemical characters. *Brittonia* 49: 384-405.

Jussieu, A. -L. de. 1789 *Genera plnatarum*. Paris.

Jussieu, B. de. 1759. Ordines naturals in Ludovici XV horto Trianonensi dispositi, anno 1759. In A. –L. de Jussieu, *Genera plantarum 1789*. Paris. Pp. lxiii-lxx.

Kearney, T.H. 1951. The American genera of Malvaceae. *American Midland Naturalist* 46: 93-131.

LaDuke, J. C. and J. Doebley. 1995. A chloroplast DNA based phylogeny of the Malvaceae. *Systematic Botany* 20: 259-271.

Linneaus, C. 1753. *Species Plantarum*. London. 2: 683-698.

Linneaus, C. 1764. *Genera Plantarum*. London. Pp. 751

Munz, P.A. and I. Johnston. 1925. *Malvastrum gabrielense* sp. nov. *Bulletin of the Torrey Club* 52: 223-224.

Pfeil, B., C. L. Brubaker, L. A. Craven, and M. D. Crisp. 2002. Phylogeny of *Hibiscus* and the tribe Hibisceae (Malvaceae) using chloroplast DNA sequences of *ndh*F and the *rpl*16 intron. *Systematic Botany* 27: 333-350.

Rydberg, P. A. 1904. Rocky Mountain Flora. *Bulletin of the Torrey Botanical Club* 31: 564-565.

Rydberg, P. A. 1906. Flora of Colorado. *The Agricultural Experiment Station of the Colorado Agricultural College, Bulletin* 100: 231.

Rydberg, P. A. 1913. Studies on the Rocky Mountain Flora, Contribution 156. *Bulletin of the Torrey Botanical Club* 40: 60.

Rydberg, P.A. 1932. *Flora of the Prairies and Plains of Central North America*. New York: New York Botanical Garden. P. 541.

Savolainen, V., M.W. Chase, S.B. Hoot, C.M. Morton, D.E. Soltis, C. Bayer, M.F. Fay, A.Y. De Bruijn, S. Sullivan, and Yin-Long Qiu. 2000. Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences. *Systematic Biology* 49: 306-362.

Sherff, E.E. 1946. Notes on certain plants in the Gray's Manual Range. *Rhodora* 48: 89-96.

Sherff, E.E. 1949. Further notes on *Iliamna. American Journal of Botany* 36: 502-503.

Skinner, M., D. Tibor, R. Bittman, B. Ertter, T. Ross, S. Boyd, A. Sanders, J. Shevock, and D. Taylor. 1995. Research needs for conserving California's rare plants. *Madroño* 42: 211-241.

Slotta, T.A.B. 2000. M.S. Thesis. Phylogenetic analysis of *Iliamna* (Malvaceae) using the internal transcribed spacer. Virginia Polytechnic Institute and State University.

Stafleu, F. A. 1963. Adanson and the "Familles des plantes." In *The Bicentennial of Michel Adanson's "Families des plantes."* 1: 123-264. Pittsburgh: The Hunt Botanical Library.

Stevens, P.F. (2001 onwards). Angiosperm Phylogeny Website. Version 3, May 2002 [but more or less continuously updated since] http://www.mobot.org/MOBOT/research/APweb/.

Stewart, C.N. and D.M. Porter. 1995. RAPD Profiling in Biological Conservation: An Application to estimating Clonal Variation in Rare and Endangered *Iliamna* in Virginia. *Biological Conservation* 74: 135-142.

Stewart, C.N., G. Rossen, B.W. Shirley, and D.M. Porter. 1996. Population genetic variation in rare and endangered *Iliamna* (Malvaceae) in Virginia. *Biological Journal of the Linnean Society* 58: 357-369.

Swenson. S., G. Allan, M. Howe, W. Elisens, and L. Riesberg. 1995. Genetic analysis of *Malacothamnus fasciculatus* (Nutt.) Greene var. *nesioticus* (Rob.) Kearn. *Conservation Biology* 9: 404-415.

Takhtajan, A. 1997. *Diversity and Classification of Flowering Plants.* New York: Columbia University Press. Pp. 227-236.

Tate, J. 2003. *Andeimalva*, A new genus of Malvaceae from Andean South America. *Lundella* 6: 10-18.

Thomas, T. 2000. *Thirteen Plant Taxa from the Northen Channel Islands Recovery Plan* Report to Region 1, U.S. Fish and Wildlife Service, Portland, Oregon. Pp. 43-46.

Thorne, R. F. 1992. Classification and geography of the flowering plants. *Botanical Review* 58: 225-348.

Watson, L. and Dallwitz, M. J. 1992 onwards. The Families of Flowering Plants: Descriptions, Illustrations, Identification, and Information Retrieval. Version: 14th December 2000. http://biodiversity.uno.edu/delta/

Weber, W.A. 1987. *Colorado Flora: Western Slope*. Boulder: Colorado Associated University Press. Pp. 330-331.

Wiggins, I.L. 1936. A resurrection and revision of the genus *Iliamna* Greene. *Contributions from the Dudley Herbarium, Stanford University* 1: 213-229

Wolfe, A. D. and A. Liston. 1998. Contributions of the polymerase chain reaction to plant systematics and evolutionary biology. In: Soltis, D. E.., P. S.. Soltis and J. J. Doyle (eds.): *Molecular Systematics of Plants II*. Boston: Kluwer. Pp. 43-86.

Table 1.1. Historical overview of the Classification of Mallows (after Stafleu 1963).

Author	**Year**	**Taxonomy**	**Rank**	**No. of Genera**
Linnaeus	1753	Columniferi	family	12
B. de Jussieu	1759	Malvae	family	19
Adanson	1763	Malvae	family	22
Linnaeus	1764	Columniferi	family	33
A. –L. de Jussieu	1789	Malvaceae	family	39
Engler and Prantl	1891	Malvineae	sub-order	48

Table 1.2. Thorne's classification of the Malvales - class Malvanae (Thorne 1992).

Sub-order	**Families**	**No. Genera**
Sterculiineae	Sterculiaceae	151
	Huaceae	2
	Elaeocarpaceae	9
	Plagiopteraceae	1
	Tiliaceae	49
	Monotaceae	3
	Dipterocarpaceae	16
	Sarcolaenaceae	8
	Sphaerosepalaceae	3
Malvineae	Bombacaceae	20
	Malvaceae	75

Table 1.3. Genera included in the *Malacothamnus* alliance.

	Described	Year Revised	Number of Species	Distribution	Habit
Iliamna	Greene, 1906	Wiggins, 1936	7 - 8	United States	Herbaceous perennials
Malacothamnus	Greene, 1906	Bates, 1963	2-27	California, Mexico	Perennial shrubs
Phymosia	Desvaux ex Hamilton, 1825	Fryxell, 1971	8	Mexico, Bahamas, Guatemala, Haiti	Small trees (up to 10 m)

Table 1.4. Morphological Variation in *Iliamna*

Taxa	Authority	Type Location	Height	Stem Pubescence	Leaf Length	Leaf Width	Leaf base	No. of Lobes	Leaf Sinus	Leaf Pubescence
Iliamna bakeri	(Jepson) Wiggins	Fall River Valley, Shasta Co. CA	0.3 to 0.7m	puberulent	1.5 to 4.5cm	2 to 5cm	tapered to truncate	3 to 5	shallow	puberulent
Iliamna corei	(Sherff) Sherff	Narrows, Giles Co. VA	1m	tomentose	8 to 15cm	10 to 15cm	cordate to tapered	5 to 7	deep	hirsute
Iliamna crandallii	(Rydb.) Wiggins	Steamboat Springs, Routt Co. CO	0.5m	sparingly stellate	2 to 5cm	5 to 10cm	cordate	5	deep	sparse
Iliamna grandiflora	(Rydb.) Wiggins	Mesa Verde, Montezuma Co. CO	1 to 2m	sparingly stellate	3 to 5cm	5 to 8cm	truncate to cordate	5 to 7	deep	sparse
Iliamna latibracteata	Wiggins	Prairie Creek, Humboldt Co. CA	1 to 2m	finely stellate	10 to 15cm	10 to 15cm	truncate to cordate	5 to 7	deep	upper leaves glabrous, lower leaves canescent
Iliamna longisepala	(Torr.) Wiggins	Upper Columbia, Washington Territory	1.2 to 2m	sparingly stellate	5 to 8cm	5 to 10cm	cordate to orbicular	5 to 7	shallow	dense
Iliamna remota	Greene	Kankakee River, Altorf, Kankakee Co. IL	2m	hirsute	15 to 20cm	15 to 20cm	cordate	5 to 7	shallow	dense
Iliamna rivularis	(Douglas ex Hooker) Greene	North-West America	0.5 to 2m	sparingly stellate	5 to 15cm	5 to 15cm	cordate to reniform	5 to 7	varies	puberulent

Taxa	Petiole Length	Leaf Margin	Inflorescence Type	Flower Diameter	Pedicel Length	Bractlet Length	Calyx Pubescence	Sepal Length	Sepal Width	Seeds per Mericarp	Seed Pubescence
I. bakeri	2 to 5cm	mucronate to crenate	axillary clusters	3 to 6cm	0.5cm	5 to 8cm	puberulent	9 to 12mm	1 to 2mm	3 to 4	fine
I. corei	1 to 3cm	serrate	raceme	4 to 6cm	0.5cm	<10mm	tomentose	10 to 15mm	5 to 10mm	2 to 3	fine
I. crandallii	1 to 3cm	coarse	raceme	4 to 6cm	1 to 3cm	10mm	sparse to fine	10 mm	5mm	1 to 2	glabrose
I. grandiflora	5 to 10cm	broadly triangular	crowded raceme	4 to 8cm	3 to 5cm	5 to 10mm	densely hirsute	8 to 15mm	5 to 10mm	2 to 3	puberulent
I. latibracteata	5 to 14cm	broadly serrate	crowded raceme	4 to 6cm	<0.5cm	10 to 14mm	hirsute	8 to 10mm	5mm	2 to3	fine
I. longisepala	2.5 to 4cm	coarsely serrated	axilary clusters	2 to 4cm	2.5cm	8 to 10mm	sparse	15 to 20mm	narrow	3	glabrose
I. remota	5 to 10cm	mucronate	axilary clusters	4 to 6cm	1 to 3cm	5 to 7.5mm	pillose	10 to 15mm	5mm	2 to 3	dense
I. rivularis	1 to 5cm	coarsely serrated	crowded raceme	3 to 8cm	1.5 to 4cm	2 to 4mm	pillose	5 to 8mm	3 to 5mm	2 to 4	sparse

Table 1.5. Morphological Variation in *Malacothamnus*.

Taxa	Authority	Type Location	Height	Stem Pubescence	Leaf Length	Leaf Width	Leaf base	No. of Lobes	Leaf Pubescence	Petiole Length	Leaf Margin
Malacothamnus abbottii	Eastwood	Salinas River, Monterey Co. CA	<1.5m	dense	<6.5cm	<6.5cm	truncate	0 to 3	tomentose	1 cm	crenate
Malacothamnus aboriginum	(Robins.) Greene	Indian Valley, Monterey Co. CA	0.75m	tawny, dense	3 to 6cm	3 to 6cm	cordate	3	tomentose	1 cm	crenate to dentate
Malacothamnus arcuatus	Greene	Coast Range, Belmont, San Mateo Co., CA	2m	tomentose, shaggy	6cm	<6cm	subcuneate	3 to 5	sparse	0.5 to 2cm	crenate to dentate
Malacothamnus clementinus	(Munz and Johnston) Kearney	San Clemente Island, Santa Barbara Co., CA	0.4 to 1m	gray, fine	<0.5cm	<0.5cm	deeply cordate	3 to 5	upper glabrous, soft below	1 to 1.5cm	rounded
Malacothamnus davidsonii	(Robins.) Greene	San Fernando Valley, Los Angeles Co, CA	3 to 5m	tawny, dense, glandular	5 to 11cm	5 to 11cm	round cordate	3 to 7	dense	1.5 to 3cm	wavy
Malacothamnus densiflorus var. *densiflorus*	(S. Wats.) Greene	Santa Ana Mountains, Riverside Co. CA	1m to 2m	tawny, shaggy to tomentose	2 to 5cm	2 to 5cm	truncate to cordate	0 to 5	sparse	0.5 to 2cm	rounded
Malacothamnus densiflorus var. *viscidus*	(Abrams) Kearney	El Nido, San Diego Co. CA	0.5 to 1m	tawny, dense, viscid - glandular	2 to 4cm	2 to 4cm	cordate	0 to 5	rugose	1.5 to 2cm	wavy
Malacothamnus fasciculatus var. *fasciculatus*	(Nutt. ex. Torrey and Gray) Greene	Santa Barbara Co. CA	1 to 5m	canescent to scurfy	2 to 8cm	2 to 8cm	truncate to shallowly cordate	2 to 4	copious	0.5 to 1cm	crenate to dentate
Malacothamnus fasciculatus var. *catalinensis*	(Eastw.) Kearney	Santa Catalina Island, Santa Barbara Co., CA	?	sparse	8cm	8cm	truncate to cordate	3 to 5	glabrous above, canescent below	?	crenate
Malacothamnus fasciculatus var. *nesioticus*	(Nutt. ex. Torrey and Gray) Greene	Santa Cruz Island, Los Angeles Co. CA	1 to 2m	tomentose	2 to 4cm	2 to 4cm	deeply cordate	3 to 5	glabrous above, fine below	?	crenate to subentire
Malacothamnus fasciculatus var. *nuttallii*	(Abrams) Greene	Casitas Pass, Ventura Co. CA	2 to 3m	canescent	2 to 3.5cm	1.5 to 3.5cm	?	5	canescent	1.5 to 2cm	crenate to serrate
Malacothamnus fremontii	(Torr. ex Gray) Torr. ex Greene	Interior CA, West Sierra Nevada	1 to 2m	tomentose	3 to 10cm	4 to 15cm	somewhat cordate	5 to 7	tomentose	1 to 3.5cm	crenate
Malacothamnus gracilis	(Eastw.) Kearney	Arroyo Grande, San Luis Obispo Co CA	1 to 2m	short and dense	2.5cm	2 to 5cm	truncate	3 to 5	pale tomentose	0.5 to 2cm	crenate
Malacothamnus hallii	(Eastw.) Kearney	Mt. Diablo, Contra Costa Co. CA	1 to 2m	canescent	6cm	6cm	cordate	3 to 5	densely tomentose	4cm	crenate
Malacothamnus helleri	(Eastw.) Kearney	Ladoga, Colusa Co. CA	1m	dense	2 to 5cm	2 to 5cm	truncate	0	closely tomentose	1 to 4cm	crenate
Malacothamnus howellii	(Eastw.) Kearney	Nortonville, Contra Costa Co. CA	?	dense to woolly	4cm	3 to 5cm	truncate	3 to 5	dense	1 to 2cm	crenate to dentate
Malacothamnus jonesii	(Munz) Kearney	Paso Robles, San Luis Obispo Co. CA	<2.5m	tomentose	2.5 to 4cm	4.5cm	truncate to cordate	3 to 5	tomentose	1 to 2cm	crenate
Malacothamnus marrubioides	(Durand and Hilg.) Greene	Ft. Miller, Modera or Fresno Co. CA	0.6 to 2m	tawny tomentose	2 to 5cm	1.5 to 4.5cm	truncate to cordate	0	tawny, dense	1 to 2.5cm	dentate
Malacothamnus mendocinensis	(Eastw.) Kearney	Ukiah, Mendocino Co. CA	2m	white tomentose	5cm	5cm	cordate	3 to 5	copious to minute	2 to 3cm	crenate
Malacothamnus niveus	(Eastw.) Kearney	El Dorado School, San Luis Obispo Co CA	<1.5m	dense	4cm	4cm	truncate to subcuneate	3	tomentose	0.7 to 1.4cm	crenate to dentate
Malacothamnus orbiculatus	Greene	Tehachapi, Kern Co. CA	0.5 to 2m	dense	1 to 2cm	1 to 2cm	subcordate	3 to 5	dense	1 to 2cm	coarsely crenate
Malacothamnus palmeri var. *palmeri*	(S. Wats.) Greene	Cambria, Monterey Co. CA	2.5m	dense	2 to 5cm	2 to 5cm	round	ca. 3	pubescent	1 to 2.5cm	crenate to dentate
Malacothamnus palmeri var. *involucratus*	(Robins.) Kearney	Jolon, Monterey Co. CA	<2.5m	shaggy	2 to 5cm	2 to 5cm	cordate	ca. 3	glabrous above, pubescent below	1 to 2.5cm	crenate to dentate
Malacothamnus palmeri var. *lucianus*	Kearney	Escondido Camp, Monterey Co. CA	?	copious	2 to 5cm	2 to 5cm	cordate	3 to 5	glabrous above, pubescent below	1 to 2.5cm	crenate to dentate
Malacothamnus paniculatus	(Gray) Kearney	Ensenada, Baja California, Mexico	1 to 2.5m	tomentose, tawny	2 to 5cm	1.5 to 4cm	cuneate	3	rugose to tomentose	0.4 to 1cm	irregular to coarsely dentate
Malacothamnus parishii	Eastwood	San Bernardino Co. CA	?	tawny, dense	5cm	4cm	cuneate	5 to 7	glabrous above, tomentose below	1cm	crenate

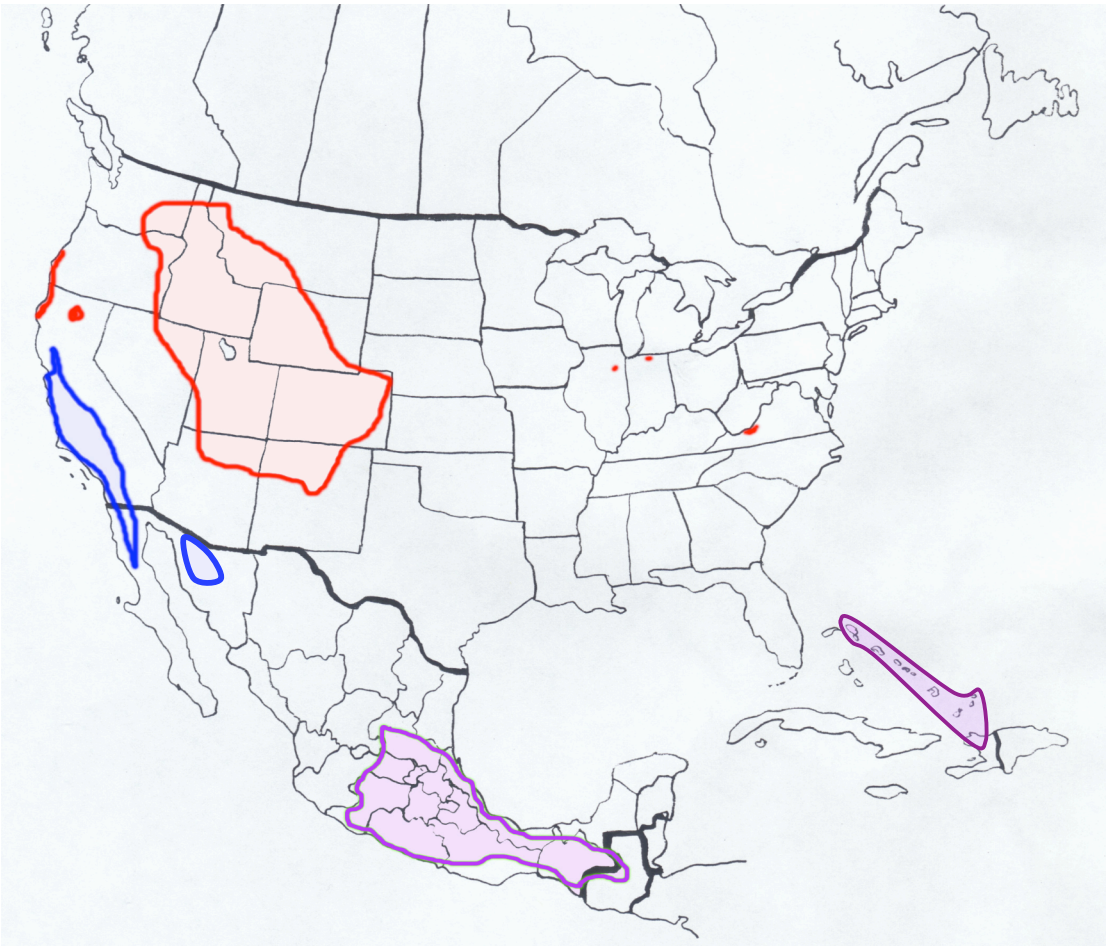
Table 1.5, continued.

Taxa	Leaf Sinus	Calyx Pubescence	Inflorescence Type	Flower Diameter	Pedicel Length	Bractlet Length	Bractlet Width	Sepal Length	Sepal Width	Mericarp Length	Seed Pubescence and Color
Malacothamnus abbottii	shallow	finely tomentose	panicle-like	3cm	1.5cm	1 to 9mm	5 to 8mm	9 to 11mm	6 to 7.5mm	?	?, ?
Malacothamnus aboriginum	sharp	?	naked spike	1 to 2.2cm	0cm	6 to 15mm	3 to 9mm	8 to 17mm	5 to 11mm	2.5 to 3mm	fine, dark
Malacothamnus arcuatus	shallow	tomentose to sparse	thyrse	3 to 4cm	0cm	3 to 9mm	?	5 to 9mm	5 to 7mm	3mm	rugose, dark
Malacothamnus clementinus	sharp	loosely tomentose	spike-like	1 to 1.3cm	0 to <0.5cm	3 to 9mm	<1mm	5 to 7mm	3.5 to 6.5mm	2mm	villose, dark
Malacothamnus davidsonii	generally closed	densely tomentose	spike	2.4 to 3cm	0 to <0.5cm	3mm	?	5 to 8mm	2 to 4mm	3mm	fine, dark
Malacothamnus densiflorus var. *densiflorus*	shallow	dense	interupted spike	2 to 3cm	0 to <0.5cm	7 to 16mm	<1mm	10 to 14mm	5 to 12mm	2.2 to 2.8mm	sparse, dark
Malacothamnus densiflorus var. *viscidus*	shallow	dense	interupted spike	2 to 3cm	0 to <0.5cm	4 to 6mm	<1mm	7 to 10mm	3 to 7mm	2.2 to 2.8mm	sparse, dark
Malacothamnus fasciculatus var. *fasciculatus*	shallow to acute	tomentose	open panicle	>4cm	1cm	2 to 4 mm	?	4 to 5mm	2 to 3mm	2 to 3mm	minute glandular, dark
Malacothamnus fasciculatus var. *catalinensis*	acute	tomentose to subglabrate	interupted panicle	3cm	?	4 to 5mm	?	7 to 8mm	4mm	3.2 to 3.8mm	?, ?
Malacothamnus fasciculatus var. *nesioticus*	shallow	canescent	loose panicle	2.2 to 3cm	1 to 2cm	1 to 2mm	?	5 to 8mm	3 to 5mm	0.8mm	fine, brown
Malacothamnus fasciculatus var. *nuttallii*	acute	canescent	loose panicle	4cm	1 to 2cm	1 to 2mm	<0.5mm	6 to 8mm	2 to 3mm	4 to 5mm	stellate on summit, dark
Malacothamnus fremontii	shallow	soft hairy	thyrse-like	2.5 to 4cm	<1cm	3 to 5mm	<0.5mm	7mm	7mm	1.5mm	glabrous, gray
Malacothamnus gracilis	shallow	pillose	panicle	2 to 5 cm	0cm	3 to 4mm	?	7mm	2.5mm	3mm	patchy, ?
Malacothamnus hallii	shallow	dense	terminal panicle	2 to 5cm	<1cm	2mm	?	5mm	5mm	2 to 3mm	pubescent,?
Malacothamnus helleri	shallow	tomentose	interupted spike	1 to 2cm	<1cm	3 to 5mm	?	5 to 7mm	5mm	2.5mm	obscurely stellate, brown
Malacothamnus howellii	moderate	dense	panicle	3cm	1 to 3cm	1cm	?	10mm	5mm	?	?, ?
Malacothamnus jonesii	shallow	pillose	raceme	1 to 2cm	1 to 2cm	2.5 to 8mm	<1mm	5mm	5mm	3mm	pubescent,?
Malacothamnus marrubioides	none	densely tomentose	spike	3 to 4cm	0 to <0.5cm	7 to 12mm	?	7 to 15mm	4 to 12mm	2.5 to 3.5mm	pubescent,?
Malacothamnus mendocinensis	shallow	dense	paniculate	1 to 2cm	?	1 to 2mm	?	5 to 6mm	?	2.2mm	sparse, ?
Malacothamnus niveus	shallow	woolly (tomentose)	paniculate	3 to 4cm	1 to 4cm	4 to 6mm	?	8 to 10mm	2 to 4mm	2.5 to 3mm	pubescent, brown
Malacothamnus orbiculatus	shallow	hispid to dense	thyrse	2.5 to 3cm	<0.5cm	4 to 6mm	?	3 to 7mm	1.5 to 3mm	2mm	?, red-brown
Malacothamnus palmeri var. *palmeri*	shallow	dense	capitate	4 to 8cm	0cm	8 to 16mm	1 to 8mm	12 to 14mm	5 to 15mm	2 to 5mm	brown
Malacothamnus palmeri var. *involucratus*	shallow	dense	capitate	3 to 4cm	0cm	10 to 14mm	?	10 to 14mm	5 to 15mm	1 to 3mm	?, ?
Malacothamnus palmeri var. *lucianus*	shallow	dense	capitate	?	0cm	2.5mm	5 to 10mm	?	5 to 15mm	?	?, ?
Malacothamnus paniculatus	obscurely to distinct	hirsute	panicle	2 to 3cm	3 to 4cm	3 to 4mm	?	6 to 15mm	5 to 8mm	2 to 5mm	patchy puberulent, brown
Malacothamnus parishii	shallow	sublepidote	paniculate	2.5cm	1.5cm	2mm	?	7 to 8mm	?	3mm	pubescent,?

Table 1.6. Morphological Variation in *Phymosia* (after Fryxell 1971)

Taxa	Authority	Type Location	Leaf Length	Stipule Length	No. of Lobes	Leaf Pubescence	Leaf Margin	Inflorescence Type	Petal Color	Flower Diameter	Bract Length	Bract Width	Sepal Length	Mericarp Length
Phymosia abutiloides	(L.) Desvaux ex Hamilton	Bahamas	13 cm	5 to 10 mm	7 to 9	dense below, moderate above	dentate	axillary panicle	lavender	3.6 to 4.4 cm	6 to 7 mm	1 mm	8 to 10 mm	8 to 11 mm
Phymosia anomala	Fryxell	Real del Monte, Hidalgo, Mexico	4 cm	4 mm	3 to 5	tomentose below, sparse above	crenate	solitary	rose	6 to 7 cm	10 to 13 mm	9 to 12 mm	16 to 20 mm	?
Phymosia crenulata	(Brandg.) Fryxell	Barranca Cerro de Paxtle, Puebla, Mexico	5 cm	4 to 9 mm	3 to 5	moderate below, sparse above	crenate	solitary in leaf axils	lavender	4 to 6 cm	10 to 14 mm	6 to 8 mm	10 to 12 mm	?
Phymosia floribunda	(Schlecht.) Fryxell	Yavesia, Oxaca, Mexico	9 cm	6 to 9 mm	3 to 5	dense below, sparse above	crenate	terminal panicle	lavender	3.6 to 4 cm	4 to 5 mm	1.5 to 2 mm	7 to 10 mm	8 mm
Phymosia pauciflora	(Bak. f.) Fryxell	Zimapan, Hidalgo, Mexico	4 to 7 cm	4 to 8 mm	5	dense	crenate	axillary umbel	lavender	4 to 6 cm	6 to 11mm	1 to 4 mm	10 to 14 mm	8 to 17 mm
Phymosia rosea	(DC.) Kearney	Calque des Dessins de la Flore, Mocino, Mexico	14 cm	6 to 13 mm	3 to 7	dense below, sparse above	dentate	axillary umbel	rose or white	7 to 14 cm	15 to 25 mm	10 to 20 mm	25 to 40 mm	15 to 32 mm
Phymosia rzedowski	Fryxell	San Pablo Ixayoc, Texcoco, Mexico	12 cm	10 mm	5	moderate below, sparse above	crenate	axillary umbel	burgundy	10 to 12 cm	20 to 30 mm	4 to 11 mm	25 to 35 mm	25 to 30 mm
Phymosia unbellata	(Cav.) Kearney	Veracruz, Mexico	9 cm	4 to 7 mm	5	moderate below, sparse to grabrous above	subentire	axillary umbel	burgundy	4 to 5 cm	10 to 20 cm	4 to 8 mm	1.5 to 2 cm	8 to 17 mm

Figure 1.1. Distribution of genera in the *Malacothamnus* alliance. *Iliamna* is indicated by the red outline, *Malacothamnus* by the blue outline in California and Baja California, Mexico, and *Phymosia* by the purple outline in Mexico, Guatemala the Bahaman Islands and Haiti.



Chapter 2

Molecular phylogenetics of the *Malacothamnus* alliance using the ITS, *rpL16*, and *trnL-F* regions

Abstract

This study constructs a phylogeny for the *Malacothamnus* alliance (Malvaceae: tribe Malveae) and examines its relationships to other genera within the tribe based on molecular data. Sequences from the nuclear ITS region and the plastid *rpL*16 intron, *trn*L intron, and the *trn*L-*trn*F intergenic spacer were used. Included taxa represent all currently recognized species of *Iliamna* and *Malacothamnus*, and four of eight species in *Phymosia*, along with representatives of nine additional genera from the Malveae. Parsimony analysis of each data set did not support the monophyly of *Iliamna* as currently circumscribed. In the ITS analysis, two species (*I. bakeri* and *I. latibracteata*) formed a clade sister to other genera in the alliance. The monophyly of *Malacothamnus* and *Phymosia* was supported by ITS, although relationships within the genera were not resolved. In the plastid phylogenetic analyses, *I. corei* and *I. remota* were in a weakly supported clade with representatives of *Phymosia*, while *Malacothamnus* was monophyletic. The ITS data provided the phylogeny with the highest statistical support and resolution of the data generated.

Introduction

Within the Malvoideae (Malvaceae) four tribes are described, Kydieae, Hibisceae, Gossypieae, and Malveae (Bayer and Kubitzki 2003). Tribe Malveae is comprised of fifteen

alliances that informally subdivide the tribe based on morphological affinities and gametophytic chromosome numbers. The focus of this study was the *Malacothamnus* alliance, which includes the genera *Iliamna*, *Malacothamnus*, and *Phymosia* (Fig. 2.1) (Bates 1968, Bates and Blanchard 1970). A fourth genus, *Neobrittonia*, has been included in the *Malacothamnus* or *Phymosia* alliance on occasion (Fryxell 1997, Bayer and Kubitzki 2003). *Iliamna*, *Malacothamnus*, and *Phymosia* are united based on chromosome number and morphological synapomorphies. However, in a study that used data from restriction enzyme digestion of the chloroplast genome, the relationship between *Iliamna* and *Malacothamnus* was unresolved (LaDuke and Doebley 1995).

Malacothamnus and *Phymosia* have a basic chromosome number of x=17 and *Iliamna* has x=33 (Bayer and Kubitzki 2003). It is conceivable that *Iliamna* is an allotetraploid or autotetraploid derivative of *Malacothamnus* or *Phymosia* that has lost a pair of chromosomes (Bates and Blanchard 1970). *Neobrittonia*, on the other hand, has a basic chromosome number of x=16, which is also true of members of the *Abutilon, Batesimalva, Gaya,* and *Kearnemalvastrum* alliances. Bates and Blanchard (1970) placed *Neobrittonia* in the *Abutilon* alliance, which has genera with a wide range of chromosome numbers. *Neobrittonia* lacks the distinctly 3-parted epicalyx that *Iliamna*, *Malacothamnus*, and *Phymosia* share. Additional characters that unite *Neobrittonia* with the *Abutilon* or *Batesimalva* alliances include the presence of basal spines on dehiscent mericarps, rough or warty seeds, and a pubescent staminal column, all of which are lacking in *Iliamna*, *Malacothamnus*, or *Phymosia*.

Morphological synapomorphies in the *Malacothamnus* alliance include numerous stylar branches, capitate stigmas that are truncate or decurrent, the calyx being plicate in bud, and mericarps with a single compartment containing two to four reniform seeds (Bayer and Kubitzki 2003). The genera range in habit from small trees (*Phymosia*), to shrubs (*Malacothamnus*), to herbaceous perennials (*Iliamna*). *Phymosia* consists of eight species from Mexico, Guatemala, Haiti, and the Bahamas (Fryxell 1971) (Fig. 2.1). *Malacothamnus* is endemic to California and Baja California and Sonora, Mexico and has from two to 27 described species (Greene 1906, Eastwood 1936, Kearney 1951, Bates 1963, 1993). Members of *Malacothamnus* were once considered to be the shrubby Malvastrums of California (Estes 1925, Eastwood 1936). *Iliamna*, with seven to eight species, occurs from northern California to Washington, throughout the Rocky Mountains, and in disjunct populations in Illinois, Indiana, and Virginia (Wiggins 1936, Sherff 1949). In the past, species in *Iliamna* have been placed in *Phymosia* and *Sphaeralcea*, but are now considered to constitute a distince genus (Rydberg 1932).

As a group, the *Malacothamnus* alliance is taxonomically complex. The numerous species described in *Malacothamnus* are reported to frequently hybridize (Bates 1993). The species are morphologically very similar and intermediate forms have been collected. Taxa in *Malacothamnus* may be found as far north as Mendocino County, California and south to Baja California Norte, Mexico. All but one species (*M. orbiculatus*, synonymous with *M. fasciculatus*) are found to the west of the Sierra Nevada. The largest number of populations and the greatest diversity of *Malacothamnus* taxa is centered in Monterey and San Luis Obispo Counties, with eight of the eleven species overlapping in distribution (Bates 1993).

In Eastwood's (1936) classification, two groups were identified, those with morphological affinities with *M. fasciculatus* or to *M. fremontii.* Inflorescence structure varies between the two groups and in a third group not described by Eastwood. In the *M. fasciculatus* group, the inflorescence resembles an open panicle with long peduncles. In the *M. fremontii* group, the inflorescence is much more compact, with greatly reduced pedicels, resembling a thyrse; the pubescence is much denser, with the stellate trichomes having much shorter arms. The genus, as a whole, was last studied using morphological characters in 1963, at which time the currently recognized eleven species were described (Bates 1963). Two taxa are listed as endangered in California (*M. clementinus* and *M. fasciculatus* var. *nesioticus*) (Thomas 2000), ten others as rare (some of which are not widely accepted entities), and two, which are no longer recognized, are believed to be extinct (Bates 1993).

Patterns of gene flow for some members of *Malacothamnus* have been explored (Swensen et al. 1995, Benesh and Elisens 1999). Two populations of *M.* fasculatus var. *nesioticus* were surveyed with ten RAPD primers (Swensen et al. 1995). One genotype was found in each population, suggesting that the populations are clonal. Analysis of isozymes supported these findings. The results indicated that *M. fasciculatus* var. *nesioticus* is a unique variety, genetically distinct from the others. However, *M. fasciculatus* var. *laxiflorus*, *M. fasciculatus* var. *catalinensis*, *M. fasciculatus* var. *fasciculatus*, and *M. fasciculatus* var. *nuttallii* had less than 15% genetic dissimilarity among the populations based on RAPDs, and only three different isozymes were identified. This indicates that these are not four unique varieties. In examination of 26 morphological characters, five varieties of *M. fasciculatus* and four additional species (*M. clementinus*, *M. davidsonii*, *M. densiflorus*, and *M. fremontii*) could not be

distinguished (Benesh and Elisens 1999). Only *M*. clementinus could be distinguished morphologically from the other taxa examined. Of the 46 specimens examined in a discriminant function analysis, only 43% of taxa were correctly classified, based on identification by collectors of the herbarium specimens. The remaining species in *Malacothamnus* have not been studied using morphometric or molecular techniques.

Identification of species in *Iliamna* is taxonomically challenging as well. E. L. Greene first described the genus in 1906 in a brief publication that included five species (Greene 1906). In 1936, *I*. L. Wiggins revised the genus and included seven species with two varieties (Wiggins 1936). In the interim, taxa in Greene's *Iliamna* were placed into either *Phymosia* or *Sphaeralcea* based upon morphology (Rydberg 1906, 1932). After Wiggins' revision, an additional species of *Iliamna* (*I*. *corei* (Sherff) Sherff) was described and *I*. *rivularis* var. *rivularis* and var. *diversa* were reduced to synonomy (Sherff 1946, 1949). The species with the widest distribution in the genus is *Iliamna rivularis*, which is found throughout the Rocky Mountains (Wiggins 1936). Its distribution overlaps geographically with that of three other species: *I*. *crandallii* (restricted to northern Colorado), *I*. *grandiflora* (New Mexico, Arizona, and southern Colorado) (Weber and Whittman 1991), and *I*. *longisepala* (eastern Washington) (Wiggins 1936). All four species are state listed as rare, as are the two Northern Californian species, *I*. *bakeri* and *I*. *latibracteata* (the latter also found in Oregon).

The two eastern species, *I*. *corei* and *I*. *remota*, were the focus of a previous study (Stewart and Porter 1995). When *I*. *corei* was first described, it was as a variety of *I*. *remota* (Sherff 1946). However, later it was renamed as a separate species, based on vegetative

characters, habitat, and the lack of floral scent (Sherff 1949). Presently, *I. corei* is listed as federally endangered and is known from only one population in southwestern Virginia (Stewart and Porter 1995). *Iliamna remota* is found in several disjunct populations in Illinois, Indiana, and west-central Virginia. Results from the survey of RAPD markers indicated that *I. corei* and *I. remota* are genetically distinct, yet it remains unclear if they warrant species or varietal status (Stewart and Porter 1995, Stewart et al. 1996).

In *Phymosia*, the eight recognized species were examined in a taxonomic revision that used morphological characters (Fryxell 1971). The genus is morphologically distinct, with the leaves, stems, petioles, and peduncles densely covered with stipitate stellate trichomes and fruits and flowers larger than those in *Iliamna* or *Malacothamnus*. The involucre in *Phymosia* is made of three distinct bracts, each as wide or wider than the sepals. Typically, species in *Phymosia* are found at elevations between 2000-3000m. *Phymosia rosea* was once a popular conservatory plant and may still be found in the horticulture industry or in botanical gardens featuring plants of Mexico. The genus has not been examined for genetic variation, or comprehensively revised. Taxa in *Phymosia* were once placed in *Sphaeralcea*, but have been separated because taxa in *Sphaeralcea* have two-chambered mericarps that do not completely dehisce and that are often reticulate (Kearney 1951).

In addressing relationships in the *Malacothamnus* alliance, one nuclear (ITS) and two chloroplast (*rpL*16 and *trn*L-*trn*F) regions were examined. This study is the first use of sequence data to develop phylogenetic hypotheses for members in the *Malacothamnus* alliance. In order to establish effective conservation and management strategies for the rare and endangered taxa in

the alliance, an understanding of relationships with the genera and how the genera relate to others in the tribe Malveae are necessary. By examining bi-parental (nuclear) and maternal (chloroplast) lineages, the biogeography of the *Malacothamnus* alliance can be further examined.

The Internal Transcribed Spacer

The internal transcribed spacer (ITS) region has been used in numerous systematic studies at the generic and specific levels (Baldwin et al. 1995). The ITS region is located between genes encoding the ribosomal RNA subunits. Several factors make the ITS region valuable for use in phylogenetic analyses (Baldwin et al. 1995). The ITS region is highly repeated in plant nuclear genomes along with other components of the nrDNA multigene family. The entire nuclear ribosomal DNA (nrDNA) repeat may be copied thousands of times and arranged in tandem repeats. The high copy number increases the probability of detection, amplification, and sequencing of the nrDNA. The two internal spacers, ITS-1 and ITS-2, are divided by the 5.8S nrDNA coding region (Fig 2.1 A). ITS-1 and ITS-2 are each around 300 bp in length and the 5.8s subunit is almost invariant in length (163-164bp), making the entire ITS region less than 700 bp in length.

The nrDNA multigene family may undergo rapid concerted evolution by unequal crossing over and gene conversion (Baldwin et al. 1995). In concerted evolution, members of a repetitive gene family do not evolve independently of each other, and as a result greater sequence similarity is found within a species than between species (Liao 1999). This property of the ITS

region is the most important from a phylogenetic standpoint and promotes accurate reconstruction of species relationships most of the time.

Even though ITS is part of the ribosomal transcriptional unit, it is not incorporated into mature ribosomes. The two ITS subunits do, however, appear to function in the maturation of nuclear ribosomal RNAs. Certain deletions or point mutations in ITS-1 can inhibit production of mature large and small subunit rRNAs, and deletions or point mutations in ITS-2 prevent or reduce processing of large subunit rRNAs.

Lastly, as mentioned above, the ITS region is relatively small (<700 bp) and is flanked by highly conserved sequences, the 18S and 26S nrDNA (Baldwin et al. 1995). Because of this, universal primers may be used to amplify and sequence the ITS region. Primers were originally designed for amplification of the regions in fungi and were derived from sequences of fungi (*Sacchomycetes*), animals, and plants (*Oryza sativa* and *Hordeum vulgaris*) (White et al. 1990). These primers have been used successfully with members of the plant families Liliaceae, Asteraceae, Rosaceae, Araliaceae, and other plant families and with such animals as fruit flies (*Drosophila melagonaster*).

Phylogenetically, ITS-1 is more informative than ITS-2 by an average of 29% more variable nucleotides than ITS-2 (Baldwin 1992). ITS-1 is also longer than ITS-2 in most angiosperms and the percentage of potentially informative and alignable nucleotide sites is greater in ITS-1 (Baldwin et al. 1995). Resolution of phylogenic trees based on ITS-1 or ITS-2 alone is comparable to the extent of variation in each spacer, with ITS-1 data providing more

complete phylogenetic resolution. Trees based on either region alone indicate relationships that are weakly supported or unresolved by the other spacer. By combining data sets from ITS-1 and ITS-2, more robust trees result than in those based on either spacer alone. Therefore, the ITS-1 and ITS-2 regions were used together in this study in order to provide maximal phylogenetic resolution and support.

An example of the utility of ITS is with *Rubus* (Rosaceae) (Alice and Campbell 1999). The entire ITS region was used (ITS-1, 5.8S rDNA, and ITS-2) to construct a phylogeny of *Rubus*. Fifty-seven taxa were used, including representatives of the subgenus Rubus, the eleven remaining subgenera, and a closely related species in another subgenus. Length and GC content of *Rubus* ITS were consistent with those reported in other angiosperms. The ITS phylogeny was generally consistent with biogeography and ploidy level of *Rubus*, but, interestingly, traditional morphological characters used in *Rubus* were not consistent with the molecular phylogeny.

Information from ITS is often used in combination with sequences from additional regions. In assessing the phylogenetic position of the *Lampranthus* group in the family Aizoaceae, ITS, the *trn*L-*trn*F regions (chloroplast), and the 5S non-transcribed spacer (NTS) (nuclear) were used (Klak et al. 2003). The ITS and *trn*L-*trn*F regions lacked sufficient variation to resolve relationships at the specific and generic levels. The NTS region provided sufficient phylogenetic information to resolve relationships at the generic level but not within this large genus. Combining data from three regions resulted in a phylogeny that indicated that the *Lampranthus* group was polyphyletic. Within the genus, a core group of species formed a well-supported clade with 98% bootstrap support. Even though the three regions did not provide

phylogenetic resolution individually, the combined analysis provided greater support for several clades and information on the evolutionary history of the group. First, previous morphological work suggested that fruit type is a significant taxonomic character in the genus; however, molecular evidence indicated that morphology in fruits is homoplasious. Secondly, the rate of sequence evolution in the group indicated that *Lampranthus* was the result of a recent radiation of taxa. By using a combination of data sets, a more robust hypothesis of evolution in *Lampranthus* was obtained.

Background on *rpL*16

The *rpL*16 region consists of two exons and an intron of approximately 1000 to 1500 bp in length (Schnabel and Wendel 1998) (Fig 2.2 B). The 3' portion of the *rpL*16 intron was used in the present study. The *rpL*16 gene encodes an approximately 15 kD chloroplast ribosomal protein and is located in the large single copy region of the chloroplast genome (Posno et al. 1986). The intervening intron separates a short 5' exon from the larger 3' exon. Codon usage in the *rpL*16 gene is similar to those in other chloroplast genes such as *rbc*L, *psb*A, and *atp*B. As expected, the greatest variation in the region is found in the intron sequences (Schnabel and Wendel 1998). Primers for the region have been designed based on the conserved exon sequences. As with ITS, *rpL*16 data are often used in conjunction with sequence data from other regions in phylogenetic analyses

The *rpL*16 region has been used with *ndh*F, another chloroplast region, to infer phylogenetic relationships of Hibiscus and of tribe Hibisceae (Malvaceae) (Pfeil et al. 2002). The *ndh*F region contained 112 (8.9%) parsimony informative characters of the 1260 bp of sequence data. In *rpL*16, 150 of the aligned 1226 bp (12.2%) were phylogenetically informative.

The regions were used independently in parsimony analyses and the resulting phylogenetic trees were largely congruent. In both analyses, all representatives of Hibiscus were in a clade along with representatives of tribes Decaschistieae, Malvavisceae, and other members of the tribe Hibisceae. When the data sets were combined, a partition homogeneity test revealed no significance difference. Furthermore, the strict consensus tree in the combined *ndh*F and *rpL*16 analysis was consistent with the consensus trees of the individual analyses. In all three cases, Hibiscus was paraphyletic. This finding was further supported when morphological characters were examined. Among members in the Hibiscus clade, there were no clear morphological synapomorphies. In this example, *rpL*16 sequence data were consistent with a second chloroplast region. The study demonstrated that in the Malvaceae, *rpL*16 is capable of producing a well-resolved phylogeny with implications of generic and specific relationships.

In a biogeographical study in *Gleditsia* (Leguminosae), sequences from *rpL*16 were used in conjunction with sequences of *ndh*F (Schnabel and Wendel 1998). Both regions had low levels of intraspecific polymorphic nucleotides, 0.9% in ndhF and 2% in *rpL*16. Phylogenetic reconstructions based on the two regions were comparable and the combined analysis revealed greater confidence (bootstrap values) for nodes than the individual analyses. The results of the combined analysis provided insight as to the origin of the genus and biogeographic origins of species on several continents. Furthermore, hypotheses of molecular divergence and molecular clock hypotheses were applied to the results. The authors used an estimated rate of $1x10^{10}$ substitutions per year for *ndh*F based on other studies and calculated an estimate for the rate of evolution in *rpL*16 as 6×10^{10} substitutions per year. The value for *rpL*16 was calculated from an average substitution rate of $\sim 5 \times 10^{10}$ for a variety of chloroplast genes, an average of ~8 x

10^{10} substitutions per site per year for synonymous mutations in grasses and palms, and included consideration of the life history of *Gleditsia*, a long-lived woody perennial. Time estimates based on substitutions per site per year of the *ndh*F and *rpL*16 sequences suggest *Gleditsia* and *Gymnocladus* diverged from a common ancestor between 23 and 35 million years before present. As this study and the study of Hibiscus show, the *rpL*16 region had sufficient variation to produce phylogenetic hypotheses consistent with that was obtained using another chloroplast gene. In both examples, *rpL*16 had slightly greater phylogenetically informative sites than *ndh*F. Bootstrap values on the inner nodes in both studies were greater with *rpL*16 than they were with *ndh*F alone. Results from *rpL*16-based phylogenetics may also be used to infer a timeline of species divergence, as was applied in *Gleditsia*.

Background on *trn*L-*trn*F

The three non-coding spacers of the *trn*T, *trn*L, and *trn*F genes are commonly used to investigate interspecific relationships among plants, but the conserved nature of the chloroplast genome often limits its use at the intraspecific level (Taberlet et al. 1991). The intergenic spacers and intron regions of *trn*T, *trn*L, and *trn*F are useful in phylogenetic analyses of closely related genera and species for several reasons. First, the genes are in a large, single copy unit. Several hundred bases of non-coding region separate the *trn*A genes, and the regions frequently have informative insertions/deletions located throughout. The higher rate of mutations found within the non-coding regions relative to coding regions can increase the resolution of phylogenies and can even be useful at intrageneric and intraspecific levels (Gielly and Taberlet 1996). In the current study, the *trn*L intron and the *trn*L-*trn*F intergenic spacer regions were used (Fig 2.2 C).

The use of the non-coding *trn*L-*trn*F region for phylogenetic analysis continutes to increase. In a recent study of *Fragaria* (Rosaceae), sequences from the ITS region, the *trn*L intron, and the *trn*L-*trn*F intergenic region were used (Potter et al. 2000). The ITS region provided 14.5% variable and 6% parsimony informative characters. The chloroplast data provided 4% variable sites and 1.3% parsimony informative sites. Of the most parsimonious trees produced, those using the ITS data alone were more resolved than those using the *trn* data alone. Strict consensus trees produced with the separate data sets differed in the placement of only two taxa. By combining data from the two regions, each of which showed low variability, adequate resolution of interspecific relationships was obtained.

In an analysis of relationships in *Geum* (Rosaceae), the ITS region, the *trn*L intron, and the *trn*L to *trn*F intergenic spacer regions were used (Smedmark and Eriksson 2002) as were used in this study. The *trn*L-*trn*F regions (1252 bp aligned) included 156 (12.4%) parsimony informative and 979 invariable characters. The ITS region (678 bp aligned) produced 194 (28.6%) parsimony informative and 395 invariable characters. Topology of the most parsimonious trees produced from the individual regions was mostly congruent. Bootstrap and decay analysis values were greater in the combined data set, indicating stronger statistical support for inferred clades. Taxonomic implications from ITS and the *trn*L-*trn*F regions suggested that *Geum andicola* may be derived from hybrid origins or reticulate evolution, since there is a contradiction in the placement of this taxon in the independent phylogenies. By comparing the two regions, the authors were able to compare the nuclear-based phylogeny to a chloroplast phylogeny and propose origins of the taxa included. Such comparisons are

frequently used in order to address bi-parental inheritance (ITS) and maternal inheritance (chloroplast) (Schnabel and Wendel 1998, Potter et al. 2000, Pfeil et al. 2002, Smedmark and Eriksson 2002). In the present study, ITS, the *rpL*16 intron, the *trn*L-*trn*F intergenic spacer, and the *trn*L intron were used to reconstruct hypotheses of phylogenetic relationships in the *Malacothamnus* alliance.

Studies of Combined Data Sets

By comparing several genes we can obtain a more accurate depiction of the evolutionary process, not only in the taxa studied, but also in the genes themselves (Soltis et al. 1998). In the nuclear genome, genes are often found to be a part of a gene family (Small and Wendel 2000). Within a gene family, there may be a few to hundreds of copies of a gene, all with a similar function. The process controlling how the copies interact and evolve is poorly understood. The ITS region is often found to provide sufficient variation, but several problems have been encountered when using it. Its numerous copies undergo concerted evolution, a process that homogenizes the individual copies, producing identical sequences. However, the process may not be complete and variation among the individual copies may be misleading in phylogenetic reconstruction. In the case of polyploids, which occur in the *Malacothamnus* alliance, an ancestral copy of the nrDNA repeat may be retained. This was found in studying relationships between species of cotton (*Gossypium*) and resulted in a misleading phylogenetic hypothesis (Soltis et al. 1998). After comparing several regions, both nuclear and chloroplast, a well-supported tree resulted with little conflict for *Gossypium* was obtained (Cronn et al. 2002).

The objectives of the study were to (1) examine the monophyly of the *Malacothamnus* alliance, (2) determine relationships within *Iliamna* and *Malacothamnus*, and (3) address the placement of the *Malacothamnus* alliance in the tribe Malveae.

Materials and Methods

Taxon Sampling

Representatives from the eight species of *Iliamna*, eleven representatives of *Malacothamnus*, and four representatives of *Phymosia* were used in this study, as well as additional representatives of tribes Malveae, Gossypieae, and Hibisceae (Table 2.1). T.F. Wieboldt and N. Stewart obtained plant material from 1994 to 1995 for *I. latibracteata*, *I. longisepala*, *I. remota*, and *I. rivularis*. Material was collected for the remaining specimens of *Iliamna* and *Malacothamnus* by the author. Voucher specimens were placed in the Massey Herbarium at Virginia Tech (VPI).

DNA Isolation and PCR Amplification

Plant material was used to isolate total genomic DNA following the CTAB protocol (Doyle and Doyle 1987, Stewart and Porter 1995). Tissue collected by the author was dried in silica gel, at a ratio of 50 g of silica gel to no more than 5 g of leaf tissue (after Chase and Hills 1991). Material previously collected was stored at -80°C. During DNA isolation, mucilage high in polysaccharides prevented the precipitation of a high quality of DNA. To improve upon the procedure, 2% PVP (polyvinyl pyrolidone) was added to the 2% CTAB (hexadecyltrimethyl-

ammonium bromide), and a phenol: chloroform: iso-amyl alcohol (24:24:1) step was incorporated to aid in the removal of phenolics. The DNA was then resuspended in TE (10 mM Tris-Cl, pH7.4, 0.1 mM EDTA) for storage. Isolated genomic DNA was quantified on a 1% agarose TAE (Tris, Acetic Acid, and EDTA) gel containing 0.5 (g/L of ethidium bromide and visualized under ultra-violet light.

Total genomic DNA was used in the amplification reactions. The ITS4 and ITS5 primers, designed by White et al. (1990) (Table 2.2), were used in a polymerase chain reaction (PCR) consisting of 1 min at 95°C, 1 min at 48°C, 45 sec at 72°C adding 4 seconds per cycle to this extension step for 45 cycles, followed by a 7 min extension at 72°C (Baldwin et al. 1995). Each 25 μl amplification reaction contained approximately 20 to 60 ng of genomic DNA, 0.5 (M of each oligonucleotide primer (Gibco, BRL), 1.9 mM MgCl2, 200 (M of each deoxynucleotide triphosphate (Promega, Corp., Madison, WI), 10 mM Tris-HCl, 0.1% Triton X, 50 mM KCl, and 1 unit Taq Polymerase (Promega, Corp. Madison, WI).

The *trn*L-*trn*F region was amplified using the above conditions except that 1.5 mM MgCl2 was used in a program consisting of an initial denaturation of 95°C for 3 min. followed by 25 cycles of 1 min at 95°C, 1 min at 49°C, 2 min at 72°C, then a 7 min extension at 72°C. Primers within the *trn*L 3'exon and the *trn*F intron were used (Table 2.2). For *rpL*16, products were amplified with conditions as in *trn*L-*trn*F and a reaction consisting of an initial denaturation of 95°C for 5 min followed by 35 cycles of 1 min at 95°C, 1 min at 48°C with an increase of 0.6°C and 4 sec per cycle, 4 min at 65°C, and followed by a 10 min extension at 65°C. Primers used for the amplification of *rpL*16 were within the exon regions (Table 2.2). The amplified

products for all regions were purified by gel electrophoresis by excising the band from gel cleaning with the Qiagen Gel Extraction kit (Qiagen Corp., Valencia, CA) to remove excess template, primer-dimers, and oligonucleotides.

Cloning and Sequencing

Initially, sequences of ITS in *Iliamna* were determined directly from the gel-purified PCR products using the dideoxy chain termination method with AmpliTaq DNA Polymerase using an ABI PRISM Dye Terminating Cycle Sequencing Ready Reaction Kit (PE-Applied Biosystems, Foster City, CA) with 5 pmol of either the ITS4 or ITS5 primer and 100 to 200ng of DNA. The results of direct sequencing showed multiple overlapping sequences and single copies could not be congruently interpreted. This was not found in the remaining genera sampled.

First, samples were re-amplified using modified primers to add restriction enzyme recognition sites to the fragment, *Hind*III to the 5' end of ITS5 and *Eco*RI to the 3' end ITS4. Amplification followed the method above with approximately 20 ng of amplified DNA in place of the genomic DNA. To clone the ITS fragment into the plasmid pBluescript (Stratagene, La Jolla, CA), the PCR products and plasmid were digested with *Eco*RI and *Hind*III, T4 ligase (Promega Corp., Madison, WI) was used with a 5:1 insert to plasmid ratio. *Escherchia coli* strain DH10B were transformed by electroporation in reactions containing 2 μl of the ligation reaction and 40 μL of DH10B cells in 0.1 cm cuvettes, pulsed at 1.8 kV and 200 Ω (Dower 1989, Smith et al., 1990). Cells were then placed in 1 mL of SOC medium for 1 h at 37°C. Then, 200 μL of the culture was used to inoculate solid LB medium containing 100 mg/mL of Ampicillin

($LBAmp_{100}$) coated with 20 μL each of 20 mg/mL both of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-GAL) and 20 mg/mL isopropyl thio-β-D-galactoside (IPTG).

After overnight incubation at 37°C, blue/white screening was used to identify colonies likely to contain recombinant plasmids. Individual white colonies were used to inoculate 5 mL $LBAmp_{100}$ medium and incubated overnight at 37°C. Plasmid DNA was isolated following an alkaline lysase miniprep (Birnboim and Doly 1979). A 2 μL sample of the plasmid DNA was digested as above with *Eco*RI and *Hind*III in a 50 μL reaction to verify the presence of the ITS fragment insert. Digestion products were fractionated by agarose gel electrophoresis and quantified by comparison to a *Lambda Hind*III standard. Double stranded plasmid DNA was used in sequencing reactions with the PE-Applied Biosystems Big Dye Terminator kit (Foster City, CA). The two to four independent clones recovered for ITS of each species sample were sequenced. The ITS4 and ITS5 primers were used in sequencing at 5 pmol per reaction with 200 ng of plasmid DNA.

The *trn*L-F region was sequenced using the "c," "d," "e," and "f" primers (Taberlet et al. 1991). The *rpL*16 region was sequenced only with “R1516,” the “F71” primer was not consistent in sequencing reactions. The two chloroplast regions were sequenced directly from PCR fragments that were cleaned as described above. All three regions, samples were sequenced at either the University of Maine Sequencing Facility or at the Virginia Bioinformatics Institute at Virginia Tech. Both locations used an ABI 373A automated sequencer (Applied Biosystems, Inc., Foster City, CA). Each primer pair provided at least 75% overlap in sequence data. The derived sequences were deposited in GenBank.

Phylogenetic Analyses

Sequences were manually edited and contiguous sequences generated using the SeqMan program of DNA*Lasergene (Madison, WI) and aligned in MegAlign using the CLUSTAL W algorithm (Thompson et al. 1994). Boundaries for the ITS-1, ITS-2, and 5.8s rDNA regions were determined by comparing sequences available in GenBank (*Gossypium darwinii* U12716). For the chloroplast data, boundaries of the non-coding regions were determined using sequences from GenBank as well [*trn*L-*trn*F: *Gossypium darwinii* (AF031444); *rpL*16: *G. darwinii* (AF031456), and *Abutilon fraseri* (AF384562)].

For each data set, phylogenetic trees were generated using PAUP*4.0b10 (Swofford 2000). The RANDOM TREES option was used to infer the presence of phylogenetic signal (Hillis and Huelsenbeck 1992). The g1 value for the skewness of distribution for 100,000 random trees was calculated using the critical value (at α=0.05) for 250 variable characters and 25 taxa. A heuristic search was performed by RANDOM stepwise addition (10,000 replicates) with tree bisection-reconstruction (TBR) branch swapping in PAUP 4.0b10 (Swofford 2000). For each step, a maximum of 25 trees was retained. From the resulting trees, the majority rule consensus was calculated. Searches were conducted using the ITS spacer region, the *rpL*16 region, the *trn*L-*trn*F region, the combined chloroplast data set, and total genomic analysis. Gaps were treated as missing data. Robustness and topology of the trees were evaluated using the consistency index (CI) and retention index (RI). Robustness of the resulting phylogenies was measured by bootstrap (1,000 replicates with full heuristic searches) and jackknife (with 50%

character deletion and 1,000 replicates) analyses. The level of congruence between data sets was assessed by the incongruence length difference index (IMF) (Farris et al. 1994) by implementing the partition homogeneity test in PAUP 4.0b10. Using this test, the data sets were compared with 1,000 heuristic searches with TBR, keeping 25 trees within each replicate. This test compares differences of tree-length among the trees resulting from partitioned data sets (Zimmer et al. 2002). Significant incongruence among the data sets is indicated by $P<0.01$. The data sets were also compared for topological congruence using subtree agreement, which measures the number of taxa necessarily removed in order to transform discordant topologies into concordant topologies (Wendel and Doyle 1998). Differences in branch support of phylogenies were assessed by comparing bootstrap (bts) and jackknife (jk) support values for branches that were not consistent among the data sets.

Results

ITS

Sequences of *Gossypium* were used as an outgroup, since the tribe Gossypieae has been shown to be sister to the tribe Malveae (Cronn et al. 2002). For each of the eight species of *Iliamna*, sequences were obtained for two to four independent clones of the ITS region, and ITS sequences for the remaining genera were generated directly from cleaned PCR products. Within *Iliamna*, representatives from two populations were analyzed for each species, with the exception of *I. grandiflora* and *I. latibracteata*. Of the cloned sequences, only those for, *I. bakeri*, *I. corei*, and *I. latibracteata,* respectively, formed clades with strong support. In *Malacothamnus*, multiple populations were sampled as well; however, only one sample per taxon is shown (Fig.

2.3). The aligned length for the ITS data set was 724 characters, and after exclusion of the 5.8S nrDNA region 560 characters were used. In comparing members of the *Malacothamnus* alliance, 19.3% of characters were parsimony informative.

Figure 2.3 shows the majority rule consensus tree of the 6,045 equally parsimonious trees (881 steps) recovered in the analysis. Within the Malveae, two clades are evident. One consists of representatives *of Abutilon, Anoda, Bakerdesia*, and *Neobrittonia* (95% bts and 95% jk). The other clade contains the remaining representatives of Malveae, including the *Malacothamnus* alliance (92% bts and 93% jk). *Malacothamnus* is strongly supported as monoplyletic with two main clades therein (100% bts and 100% jk). *Phymosia* is also strongly supported as monophyletic (100% bts and 100% jk), with P. umbellata as the most primitive member included here. The monophyly of *Iliamna*, however, is not supported. Two species, *I. bakeri* and *I. latibracteata*, are in a well-supported clade (73% bts and 73% jk), sister to *Modiola*, with *Callirhöe*, *Eremalche*, and *Sidalcea.* The remaining species of *Iliamna* are in a strongly supported clade (100% bts and 100% jajkckknife) sister to *Malacothamnus* and *Phymosia*. Within the core *Iliamna* clade, only the eastern taxa, *I. corei* and *I. remota*, are united in a strongly supported clade (100% bootstrap and 99% jackknife). Two clades result within *Malacothamnus*, with *M. fasciculatus* and *M. marrubioides* sister to *M. fasciculatus* var. *nuttallii* (77% bts and 69% jk) and separate from the remaining taxa in *Malacothamnus*. Matrix and tree statistics for ITS are summarized in Table 2.3.

rpL*16*

The *rpL*16 data set consisted of nine sequences of *Iliamna*, twelve of *Malacothamnus*, two of *Phymosia*, and eight from additional genera in Malveae. The analysis included sequences from the 3' region of *rpL*16 and resulted in an alignment of 965 bp. A portion (238 bp) from the first third of the sequenced region was excluded from the phylogenetic analyses because of alignment ambiguities. The data set contributed 7.01% (51 bp) parsimony informative characters to the analysis of the *Malacothamnus* alliance. Phylogenetic analysis resulted in 4,514 equally parsimonious trees, consisting of 354 evolutionary steps, from which the majority rule consensus was computed (Fig. 2.4). The analysis resulted in a strongly-supported Malveae clade (100% bts and 99% jk) with the formation of the same two major clades as in the ITS analysis. One clade contained *Abutilon*, *Anoda*, and *Neobrittonia* (100% bts and 98% jk), and the other combined the remaining representatives of the Malveae. As with the ITS results, there was poor resolution among taxa within the genera. *Malacothamnus* was supported as monophyletic (81% bootstrap and 71% jk), but relationships among the species lack resolution. In contrast, the monophyly of *Iliamna* and *Phymosia* did not have bootstrap or jackknife support above 50%. In the majority rule consensus (Fig. 2.4), *Phymosia* is paraphyletic and *Iliamna* was polyphyletic. Taxa in *Iliamna* were in clades sister to *Malacothamnus* (*I.* latibraceata) and *Phymosia*, *Modiola*, and *Andeimalva* (*I. corei* and *I. remota*). Data matrix and tree statistics are summarized in Table 2.3.

trnL-trnF

The *trn*L-*trn*F data set consisted of thirteen sequences of *Iliamna*, ten of *Malacothamnus*, four of *Phymosia*, and seven sequences from other genera in Malveae. The analysis included 1,029 aligned characters after 136 bp from the middle of the *trn*L intron were excluded because of ambiguous sequence alignment. The excluded portion encompassed an insertion shared by all

Iliamna samples except *I. corei* and *I. remota*, as well as, *M. clementinus*, *M. palmeri*, and *P. umbellata*. In the analysis, 10.2% of the characters were parsimony informative. A similar topology was obtained with the *trn*L-*trn*F majority rule tree (Fig. 2.5) as with the *rpL*16 data set. *Abutilon* and *Neobrittonia* formed a strongly supported clade (97% bts and 99% jk) sister to the remaining representatives of Malveae (98% bts and 96% jk). Support for internal nodes was greater with *trn*L-*trn*F, providing greater resolution within genera. As with *rpL*16, the results of *trn*L-*trn*F did not support the monophyly of *Iliamna* or *Phymosia*. A core *Iliamna* clade resulted, excluding *I. corei* and *I. remota*, with strong support (80% bts and 79% jk) but there was little resolution with the remaining species. Three sequences of *I. grandiflora* formed a weakly supported clade. A well-supported clade of *I. corei*, *I. remota*, and *Phymosia* (94% bts and 93% jk) resulted in the *trn*L-*trn*F analysis. Summarized in Table 2.3 are statistics from the data matrix and parsimony analysis.

Congruence of Data Sets

No significant incongruence (α=0.01, P<0.01) was found between data from the two plastid regions (P=0.10), therefore these data sets were treated as a unit (Fig. 2.6). However, the partition homogenetiy index revealed significant incongruence between the plastid and ITS data sets (P=0.001), therefore the three regions were not treated in a single analysis. The incongruence of multiple data sets does not mean that one set is more reliable than the other (Soltis et al. 1998, Alverez Fernandez et al. 2001). The separate data sets, plastid versus nuclear, may be indicative of independent biological processes in terms of character evolution. The combined chloroplast analysis resulted in 82 most parsimonious trees consisting of 1,371 steps.

As with the individual analyses, phylogenetic signal was significant (g1=-0.0.52403). The 303 (15.2%) parsimony informative characters resulted in phylogenies with a consistency index (excluding uninformative characters) of 0.6115 and retention index of 0.7467.

As with the independent data set analyses, *Iliamna* was not supported as monophyletic in the combined chloroplast analysis (Fig. 2.6). In *Iliamna*, *I. corei* and *I. remota* were placed in a weakly supported clade (56% bts and 57% jk) with *Callirhöe, Phymosia, Sidalcea,* and *Sphaeralcea*, while the remaining taxa in the *Iliamna* were in a weakly supported clade with *Malacothamnus* (<50% bts and 50% jk). The monophyly of the *Malacothamnus* alliance was not supported as well, since the three genera comprise an unresolved clade with *Callirhöe, Eremalche*, *Malva, Modiola, Sidalcea,* and *Sphaeralcea*. In the combined chloroplast analysis, *Neobrittonia* and *Abution* were strongly supported (100% bts/ 100% jk) as allies in a clade sister to the remaining representatives of Malveae (100% bts/ 100%jk).

Discussion

The monophyly of the *Malacothamnus* alliance is not supported by ITS, *rpL*16, or *trn*L-*trn*F. *Neobrittonia* allied with *Abutilon* with significant statistical support in all analyses conducted. Morphology and chromosome numbers support this finding. Within *Abutilon* a wide range of chromosome numbers have been detected, including n=16, as in *Neobrittonia* (Bates and Blanchard 1970). Numerous morphological characters unite *Neobrittonia* with the *Abutilon* alliance, such as the lack of an involucre and characteristics of fruits and seeds (Kearney 1951, Bayer and Kubitzki 2003). This relationship of *Neobrittonia* to *Batesimalva* or *Abutilon* also

resulted in a larger study examining phylogenetics of Malveae using ITS (J. Tate, Univ. of Florida, pers. comm.)

Of the three remaining members of the *Malacothamnus* alliance, only *Malacothamnus* was shown to be monophyletic in all analyses conducted. Generic boundaries in *Iliamna* and *Phymosia* lacked statistical support and the relationships of the species therein differed in the phylogenies of each analysis. Comparisons of the traditional classification of genera and the results of this molecular phylogenetic analysis are discussed below.

Iliamna

The ITS gene tree produced for *Iliamna* (Fig. 2.3) could be influenced by a number of factors, such as introgression and/or hybridization between species or non-coalescence or gene duplication in the ITS region (Alverez and Wendel 2003). Further complicating the ITS phylogeny of *Iliamna* is the lack of homogenization in the multiple copies of the ITS region, as is seen in the length variation of the region within a single specimen. The copies of ITS recovered in *Iliamna* were examined for the conservation of the ITS-1 and ITS-2 structural stem-loop sequences and found to be homologous. The copies recovered in *I. bakeri* and *I. latibracteata,* while homologous to the others recovered for *Iliamna*, represent an ancestral copy that has been maintained in the genome. The sequences of ITS in *I. bakeri* and *I. latibracteata* have a 4% to 5% pairwise sequence difference from others in the genus, suggesting an extended period of diversification from those recovered in the remaining members of the genus.

Among the other members of the genus, the eastern species, *I. corei* and *I. remota,* form a well-supported clade in the ITS data (the Eastern Clade), with 100% bts and 99% jk support separating them from the Rocky Mountain group (*I. crandallii*, *I. grandiflora*, *I. longisepala*, and *I. rivularis*). However, the plastid data sets place the eastern *Iliamna* species with *Phymosia*. The *trn*L-*trn*F data (95% bts, 94% jk) have greater support for this relationship than *rpL*16 (<50% bts and jk). Such findings imply that *Phymosia*, or a shared ancestor, was one of the maternal progenitors in the origins of *Iliamna*. In all analyses, *I. corei* and *I. remota* are distinct taxa with the Illinois/Virginia *I. remota* in a well-supported clade separate (*trn*L-*trn*F and *rpL*16) or sister to *I. corei* (ITS). Morphologically, *I. corei* and *I. remota* are distinct as well and are found in different habitats (Sherff 1949, Stewart and Porter 1995, Swinehart and Jacobs 1998).

None of the regions used provided sufficient variation to delineate taxa in the Rocky Mountain Group (RMG) (*I. crandallii*, *I. grandiflora*, *I. longisepala*, and *I. rivularis*). Harrington (1964) suggested that, since *I. grandiflora* and *I. crandallii* vary only in sizes of floral parts, they may be considered the same species. Rydberg (1904) also noted that *I. grandiflora* and *I. rivularis* were close allies when he first described the species. He based his separation of the two on the larger flowers, broader bractlets, long trichomes of the calyx, blunter leaves, and more rounded carpels of *I. grandiflora*. Rydberg's (1904) distinction of *I. crandallii* is even less supported as he states, "this resembles a small *S[phaeralcea] rivularis*, but differs in the long bractlets and the lanceolate sepals" (p. 564). Whether hybridization occurs between these taxa has not been determined. The RMG may be a case of "rapid radiation" in which a formerly widespread population (*I. rivularis*) has been fragmented into a series of geographically isolated populations, or rapid diversification of *I. rivularis* took place in response to novel ecological or

morphological adaptations. Either one of these situations is possible for *I. crandallii*, *I. grandiflora*, *I. longisepala*, and *I. rivularis*.

Malacothamnus

Relationships among the species in *Malacothamnus* are equally unresolved in the current study. The *rpL*16 data were unable to distinguish, with statistical support >50%, any taxonomic delineations in the genus. Results from ITS indicate that *M. fasciculatus* var. *fasciculatus*, *M. fasciculatus* var. *nuttallii*, and *M. marrubioides* are closely related, as well as *M. fasciculatus* var. *nesioticus* and *M. aboriginum*, and *M. davidsonii* and *M. gracilis*. While the genus is not statistically supported as monophyletic in the *trn*L-*trn*F analysis, two groups of taxa do form clades with greater than 50% bts and jk support. One group retains *M. fasciculatus* and its two varieties as a clade (87% bts and 85% jk). Nested with *M. fasciculatus* var. *fasciculatus* are *M. abbottii* and *M. marrubioides* although they are morphologically distinct (Bates 1993). Four taxa (*M. fasciculatus* var. *fasciculatus*, *M. fasciculatus* var. *nuttallii*, *M. abbottii*, and *M. marrubioides*) do overlap in distribution in Santa Barbara and Los Angeles Counties and they have the potential for gene flow. *Malacothamnus fasciculatus* var. *nesioticus* is an endemic on Santa Cruz Island that has been previously shown to be genetically distinct from the mainland taxa (Swensen et al 1995), and it does result as a separate entity from the other *M. fasciculatus* varieties. The second group allies *M. clementinus* (San Clemente Island, Los Angeles County) and *M. palmeri* (Monterey and San Luis Obispo Counties).

The relationships indicated in the unresolved clades of this molecular analysis of *Malacothamnus* are not consistent with prior studies based on morphological characters, other

than Benesh and Elisen's (1999) conclusion that *M. davidsonii*, *M. densiflorus*, *M. fasciculatus*, and *M. fremontii* cannot be distinguished. Taxa in Eastwood's (1936) groups do not form clades nor do they result in clades that would follow Kearney's (1951) classification.

Results of the combined chloroplast data yielded little contribution to classification of species within *Malacothamnus* as well. The genus was supported as monophyletic (91% bts and 90% jk) within a clade with *Iliamna bakeri, I. latibracteata,* and *I. longisepala*. *Malacothamnus palmeri* (Monterey and San Luis Obispo Counties) was weakly supported (69% bts and 70% jk) as the most primitive member of the genus. The taxa *M. abbottii*, *M. fasciculatus* var. *fasciculatus*, *M. fasciculatus* var. *nesioticus*, *M. fasciculatus* var. *nuttallii*, and *M. marrubioides* form a clade with 62% bts and 61%jk. The lack of statically supported clade formation within *Malacothamnus* and the degree of similarity of sequences within the genus support Kearney's supposition that *Malacothamnus* is a recently evolved group with continuous hybridization.

Phymosia

Although a limited sample of the genus is included in the current study (four of eight species), the role of *Phymosia* in the development of *Iliamna* can be inferred. *Phymosia umbellata* shares an approximately 90 bp indel in the *trn*L-*trn*F region with the western *Iliamna* species and with *M. clementinus* and *M. palmeri*. This region was not found in *P. pauciflora, P. roseae,* and *P. rzedowski*, or in the remaining *Malacothamnus* sequences, or *I. corei* and *I. remota*. The relationship of *Phymosia* to the eastern *Iliamna* species in the plastid phylogenies clearly indicates shared history that is not reflected in the ITS data. This suggests that *Iliamna*

corei and *I. remota* retained an ancient copy of the plastid genome while undergoing concerted evolution of the nuclear genome.

Whether or not *Phymosia* is the most primitive member of the alliance with *Iliamna* and *Malacothamnus* (Fryxell 1988) cannot be concluded from these results. The three representatives of *Phymosia* included in the plastid analyses form a weakly supported group, allied with the eastern *Iliamna* species. The ITS and combined analysis result in a strongly supported monophyletic *Phymosia* with *P. umbellata* as the most primitive member of the genus. It is likely that relationships within *Phymosia* could be clarified with the addition of information from the remaining four species of the genus, especially *P. abutiloides* from the Bahamas to determine biogeographic relationships in the genus.

Conclusions

The results described herein indicate the need for further research on relationships within the *Malacothamnus* alliance. The lack of genetic variation detected within the genera suggests they are recently diverged. The data, however, suggest that two distinct ancestors gave rise to *Iliamna*. The phylogenies based on *rpL*16, *trn*L-*trn*F, and the combined plastid data set suggest a Caribbean or southern Mexican ancestor contributed to an *Iliamna* progenitor from eastern North America. The nuclear phylogeny suggests a Californian or Mexican ancestor contributed to an *Iliamna* progenitor from western North America. Sampling with additional highly variable genetic markers may be able to resolve relationships within the genera. Examination of morphological characters is being conducted in an effort to determine species boundaries in the genera (see Chapters 4 and 5).

Literature Cited

Alice, L.A. and C.H. Campbell. 1999. Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. *American Journal of Botany* 86: 81-97.

Alverez, *I.* and J.F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417-434.

Alverez Fernandez, *I.* J. Fuertes Aguilar, J.L. Panero, and G. Nieto Feliner. 2001. A phylogenetic analysis of *Doronicum* (Asteraceae, Senecioneae) based on morphological, nuclear ribosomal (ITS), and chloroplast (*trnL-F)* evidence. *Molecular Phylogenetics and Evolution* 20: 41-64.

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3-16.

Baldwin, B.G., *M*.J. Sanderson, J.*M*. Porter, *M*.F. Wojciechowski, C.S. Campbell, and *M*.J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247-267.

Bates, D.*M*. 1963. *The Genus Malacothamnus*. Ph.D. Dissertation, University of California, Los Angeles.

Bates, D.*M*. 1968. Generic relationships in the Malvaceae, tribe Malveae. *Gentes Herbarium* 10: 117-135.

Bates, D. *M*. 1993. *Malacothamnus*. In Hickman, J.C., ed. *The Jepson Manual: Higher plants of California*. Berkeley and Los Angeles: University of California Press. Pp. 747-753.

Bates, D.*M*. and O.J. Blanchard, Jr. 1970. Chromosome numbers in the Malvales II: New or otherwise noteworthy counts relevant to classification in the Malvaceae tribe Malveae. *American Journal of Botany* 57: 927-934.

Bayer, C. and K. Kubitzk*I*. 2003. Malvaceae. In K.Kubitzki and C. Bayer, eds. *The families and genera of vascular plants: Volume V. Flowering plants. Dicotyledons: Malvales, Capparales and Non-betalain Caryophyllales*. Berlin: Springer-Verlag. Pp. 225-311.

Benesh, D.L. and W.J. Elisens. 1999. Morphological variation in *Malacothamnus fasciculatus* (Torrey and A. Gray) E. Greene (Malvaceae) and related species. *Madroño* 46: 142-152.

Birnboim, H.C. and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* 7: 1513-1523.

Chase, *M*. W. and H. H. Hills. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215-220.

Dower, W.J. 1989. Transformation of *E. coli* to extremely high efficiency by electroporation. *Molecular Biology Reports* 6: 3-4.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.

Eastwood, A. 1936. The shrubby Malvastrums of California with descriptions of new species and a key to the known species. *Leaflets of Western Botany* 1: 213-220.

Estes, F. 1925. The Shrubby *Malvastrums* of Southern California. *Bulletin of the Southern California Academy of Science* 24: 81-87.

Farris, J.S., *M*. Kallersjo, A.G. Kluge, and C. Bult. 1994. Testing significance of incongruence. *Cladistics* 10: 315-319.

Fryxell, P. A. 1971. A revision of *Phymosia* (Malvaceae). *Madroño* 21: 153-174.

Fryxell, P.A. 1988. Malvaceae of Mexico. *Systematic Botany Monographs*. 25.

Fryxell, P.A. 1997. The American genera of Malvaceae II. *Brittonia* 49: 204-269.

Gielly, L. and P. Taberlet. 1996. A phylogeny of the European gentians inferred from chloroplast *trnL* (UAA) intron sequences. *Botanical Journal of the Linnean Society* 120: 57-55.

Greene, E. L. 1906. Certain malvaceous types. *Leaflets of Botanical Observation and CriticisM*. Pp. 205-209.

Harrington, H. D. 1964. *Manual of the plants of Colorado*. Chicago: Swallow Press. P. 373.

Hillis, D. *M*. and J. P. Huelsenbeck. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189-195.

Kearney, T.H. 1951. The American genera of Malvaceae. *American Midland Naturalist* 46: 93-131.

Klak, C., Hedderson, T.A., and H.P. Linder. 2003. A molecular systematic study of the *Lampranthus* group (Aizoaceae) based on the chloroplast *trn*L-*trn*F and nuclear ITS and 5S NTS sequence data. *Systematic Botany* 28: 70-85.

LaDuke, J. C. and J. Doebley. 1995. A chloroplast DNA based phylogeny of the Malvaceae. *Systematic Botany* 20: 259-271.

Liao, D. 1999. Concerted Evolution: Molecular Mechanism and Biological Implications. *American Journal of Human Genetics* 64: 24-30.

Pfeil, B.E., C.L. Brubaker, L.A. Craven, and *M*.D. Crisp. 2002. Phylogeny of *Hibiscus* and the Tribe Hibisceae (Malvaceae) using chloroplast DNA sequences of *ndhF* and the *rpL16* intron. *Systematic Botany* 27: 333-350.

Posno, *M*., A. van Vliet, and G. S. P. Groot. 1986. The gene for *Spirodela oligorhiza* chloroplast protein homologous to *E. coli* ribosomal protin L16 is split by a large intron near its 5' end: structure and expression. *Nucleic Acids Research* 14: 3181-3195.

Potter, D., J.J. Luby, R.E. Harrison. 2000. Phylogenetic relationships among species of *Fragaria* (Rosaceae) inferred from non-coding nuclear and chloroplast DNA sequences. *Systematic Botany* 25: 337-348.

Rydberg, P. A. 1904. Rocky Mountain Flora. *Bulletin of the Torrey Botanical Club* 31: 564-565.

Rydberg, P. A. 1906. Flora of Colorado. *The Agricultural Experiment Station of the Colorado Agricultural College, Bulletin* 100: 231.

Rydberg, P.A. 1932. *Flora of the Prairies and Plains of Central North America*. New York: New York Botanical Garden. P. 541.

Schnabel, A. and J.F. Wendel. 1998. Cladistic biogeography of *Gleditsia* (Leguminosae) based on *ndhF* and *rpL16* chloroplast gene sequences. *American Journal of Botany* 85: 1753-1765.

Sherff, E.E. 1946. Notes on Certain Plants in the Gray's Manual Range. *Rhodora* 48: 89-96.

Sherff, E.E. 1949. Further notes on *Iliamna*. *American Journal of Botany* 36: 502-503.

Small, R.L. and J.F. Wendel. 2000. Phylogeny, duplication, and intraspecific variation of Adh sequences in New World diploid cottons (*Gossypium* L.). *Molecular Phylogenetics and Evolution* 16: 73-84

Smedmark, J.E.E. and T. Eriksson. 2002. Phylogenetic relationships of *Geum* (Rosaceae) and relatives inferred from the nrITS and *trnL-trnF* regions. *Systematic Botany* 27: 303-317.

Smith, *M*., J. Jessee, T. Landers and J. Jordan. 1990. High efficiency bacterial electroporation. *Focus* 12: 38-40

Soltis, D.E., P.E. Soltis, *M.* Mort, *M.*Chase, V. Savolainen, S. Hoot and C. Morton. 1998. Inferring complex phylogenies using parsimony: An emperical approach using three large DNA data sets for angiosperms. *Systematic Biology* 47: 32-42.

Stewart, C.N. and D.*M.* Porter. 1995. RAPD Profiling in Biological Conservation: An Application to estimating Clonal Variation in Rare and Endangered *Iliamna* in Virginia. *Biological Conservation* 74: 135-142.

Stewart, C.N., G. Rossen, B.W. Shirley, and D.*M.* Porter. 1996. Population genetic variation in rare and endangered *Iliamna* (Malvaceae) in Virginia. *Biological Journal of the Linnean Society* 58: 357-369.

Swenson. S., G. Allan, *M.* Howe, W. Elisens, and L. Riesberg. 1995. Genetic analysis of *Malacothamnus fasciculatus* (Nutt.) Greene var. *nesioticus* (Rob.) Kearn. *Conservation Biology* 9: 404-415.

Swinehart, A. L. and *M.* E. Jacobs. 1998. Rediscovery, status and preservation of the endangered Kankakee Globe Mallow (*Iliamna remota*) in Indiana. *Rhodora* 100: 82-87.

Swofford, D.L. 2000. *PAUP: phylogenetic analysis using parsimony*, version 4.0. Illinois Natural History Survey, Champaign, Ill.

Taberlet, P., L. Gielly, G. Pautou, J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105-1109.

Thomas, T. 2000. *Thirteen Plant Taxa from the Northern Channel Islands Recovery Plan.* Report to Region 1, U.S. Fish and Wildlife Service, Portland, Oregon. Pp. 43-46.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680.

Weber, W. A. and R. C. Wittman. 1991. *Catalog of the Colorado Flora: A biodiversity baseline.* Boulder, CO: University Press of Colorado. P. 215.

Wendel, J.F. and J.J. Doyle. 1998. Phylogenetic Inongruence: window into genome history and molecular evolution. In *Molecular systematics of plants II: DNA sequencing*, D. E. Soltis, P. S. Soltis and J.J. Doyle, eds. Boston: Kluwer Academic Press. Pp. 265-296.

White, TJ, T Bruns, S Lee and J Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protoocols: A guide to Methods and Applications,* D.G.*M.* Innis, J. Sninsky and T. White, eds. San Diego, CA: Academic Press. Pp. 315-322.

Wiggins, *I*.L. 1936. A resurrection and revision of the genus *Iliamna* Greene. *Contributions from the Dudley Herbarium, Stanford University* 1: 213-229

Zimmer, E.A., E.H. Roalson, L.E. Skog, J.K. Boggan, and A. IdnurM. 2002. Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA ITS and cpDNA trn L-F and trn E-T spacer region sequences. *American Journal of Botany* 89: 296-311.

Table 2.1. Taxa sampled, location of the population, abbreviation, and voucher specimens, which are deposited at VP*I*. Sequences are deposited in GenBank with the following accessions. Collections were made by RF=R. C. Friesner, PF=Paul Fryxell, KK=Kevin Kane, TBS=Tracey Slotta, DS=Douglas Soltis, VS=Veva Stansell, TFW=Tom Wieboldt.

Species	Location (and abbreviation)	Voucher Number
Iliamna bakeri	McCloud, CA (MC)	TBS 991
	Mt. Shasta, CA (MB)	TBS 992
I. crandallii	Buffalo Pass, Routt Co., CO (BP)	TBS 993
	Fish Creek Falls, Steamboat Springs, CO (FC)	TBS 994
I. corei	Research Station (VPI)	TFW 6761
I. grandiflora	Grand Canyon, AZ (AZ)	TBS 995
	Mesa Verde National Park (MV)	TBS 2001-3
I. latibracteata	Agness, OR	VSs.n. 7/6/1994
I. longisepala	Swakane Canyon, WA (S)	KKs.n. 6/1994
	Rocky Reach, WA (RR)	KKs.n. 7/19/1994
I. remota	Kankakee River, Altorf, IL (IL)	RFsn 7/14/1945
	James River, Amherst Co., VA (VA)	TFW 5577
I. rivularis	Clearwater NF, Idaho Co., ID (CL)	DS s.n. 7/1994
	Cache Co., UT (UT)	TFW 1704
Malacothamnus abbottii	Hesperia School, CA	TBS 2002-11A
M. aboriginum	Pacines, CA	TBS 2002-31
M. clementinus	San Clemente Is., CA	TBS 2000-15
M. davidsonii	Cosio Knob, CA	TBS 2002-15
M. densiflorus	UC-Berkeley Botanical Garden	Plant 99.0611
M. fasciculatus var. *fasciculatus*	Santa Barbara Co, CA	TBS 2000-14
M. fasciculatus var. *nesioticus*	Santa Cruz Is. CA	TBS 2000-11
M. fasciculatus var. *nuttallii*	Tidlen Park, Berkeley, CA	Malvaceae 88.135
M. fremontii	UC-Berkeley Herbarium (JEPS)	570,829
M. gracilis	Upper Lopez Canyon, CA	TBS 2002-7
M. jonesii	Camp Roberts, CA	TBS 2002-19
M. marrubioides	Castaic Lake, CA	TBS 2002-2
M. niveus	Calf Canyon, CA	TBS 2002-8
M. palmeri var. *palmeri*	Santa Barbara Botanical Garden	TBS 2000-10
M. palmeri var. *involucrata*	Jolon Grade, CA	TBS 2002-26
Phymosia pauciflora	UNAM (Mexico)	982,512
P. rosea	MO. Bot. Gard. (MO)	1,103,200
P. rzedowskii	UNAM (Mexico)	1.028,925
Phymosia umbellata	Tamaulipas, Mexico,	P.F. 4959
Callirhöe digitata	MO Bot. Gard. Living Collection	Garden 1,113
Eremalche exilis	MO. Bot. Gard. (MO)	4,000,372
Bakerdesia gaumeri	MO. Bot. Gard. (MO)	4,241,025
Modiola caroliensis	College of William&Mary	
Neobrittonia acerifolia	MO. Bot. Gard. (MO)	3,927,289
Sidalcea candida	Steamboat Springs, CO	TBS 99-12
Sphaeralcea incana	Painted Desert, AZ	TBS 99-13

Table 2.2. Primers used in amplification with sequence and references listed.

Primer	Sequence	Reference
ITS4	5'-TCCTCCGCTTATTGATATGC-3'	White et al. (1990)
ITS5	5'-GGAAGTAAAAGTCGTAACAAGG-3'	White et al. (1990)
"c"	5'-CGAAATCGGTAGACGCTACG-3'	Taberlet et al. (1991)
“d”	5’-GGGGATAGGGACTTGAAC-3’	Taberlet et al. (1991)
“e”	5’-GGTTCAAGTCCCTCTATCCC-3’	Taberlet et al. (1991)
"f"	5'-ATTGAACTGGTGACACGAG-3’	Taberlet et al. (1991)
“F71”	5’-GCTATGCTTAGTGTGTGACTCGTTG-3’	Small et al. (1998)
“R1516”	5’-CCCTTCATTCTTCCTCTATGTTG-3’	Small et al. (1998)

Table 2.3. Summary of features within the ITS, *rpL16*, and *trnL-F* regions

Region	Length (aligned)	% sites Parsimony Informative	Number of Trees	Tree Length	CI	RI	g1
ITS	724	19.3	6,045	881	0.5432	0.7264	-0.849
rpL16	965	7.01	4,514	354	0.6538	0.8034	-0.494
trnL-F	1,029	10.2	4,022	535	0.6389	0.7254	-0.730
Combined Plastid	1,994	15.2	82	1,371	0.6115	0.7467	-0.524

Figure 2.1. Distribution of taxa in the *Malacothamnus* alliance. *Iliamna* indicated in solid dark gray, *Malacothamnus* in solid black, and *Phymosia* by the gray outline and light gray center.

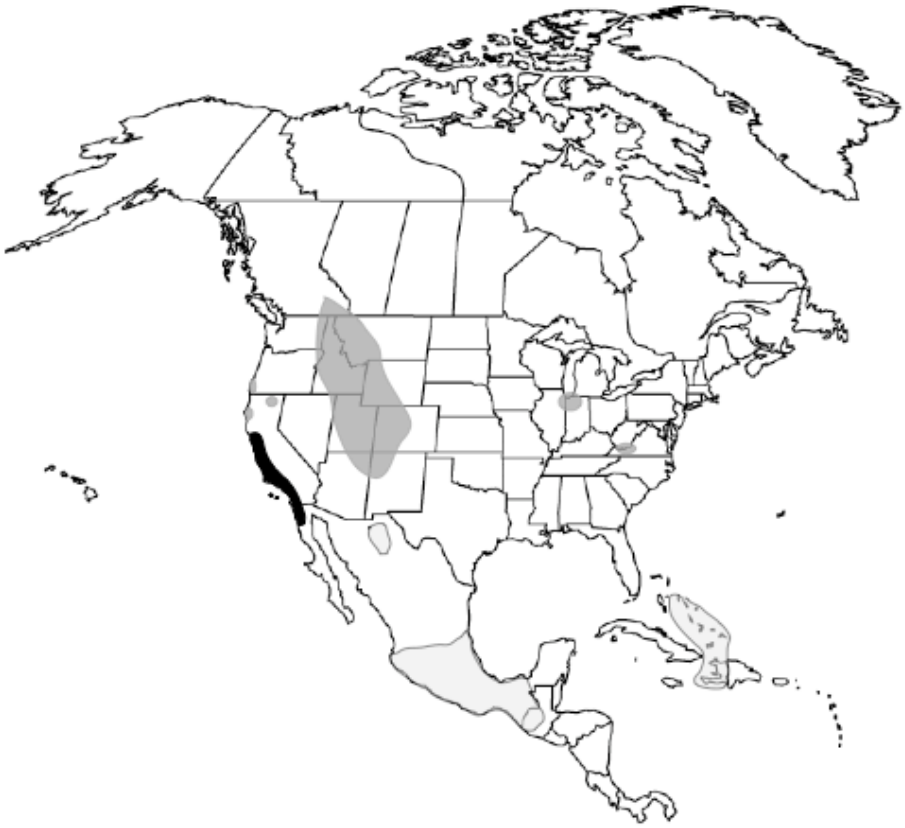


Figure 2.2. The three regions used in phylogenetic analyses of the *Malacothamnus* alliance. A: The nuclear ITS region, B: The *rpL16* plastid intron, C: The *trnL* intron, *trnL* 3'exon, and the *trnL-trnF* intergenic spacer. A scale bar of 100bp is provided for reference.

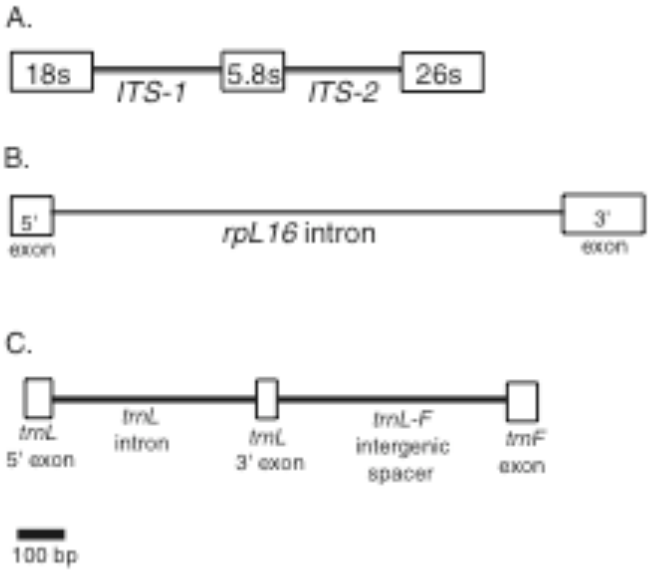


Figure 2.3. The majority rule consensus tree generated with ITS from 6,045 equally parsimonious trees showing relationships of the *Malacothamnus* alliance within tribe Malveae. The data set included 19.3% parsimony informative characters, which produced a tree of 881 steps with CI=0.5432, RI=0.7264, and g1=-0.849. Numbers above branches indicate the percentage of equally parsimonious trees that recovered the clade, numbers below branches are bootstrap and jackknife (bts/jk) values recovered that are greater than 50%. If a jackknife value greater than 50% was not recovered, a "-" is placed next to the bootstrap value. Labels for abbreviated genera are as follows *I=Iliamna, M=Malacothamnus,* and *P=Phymosia*.

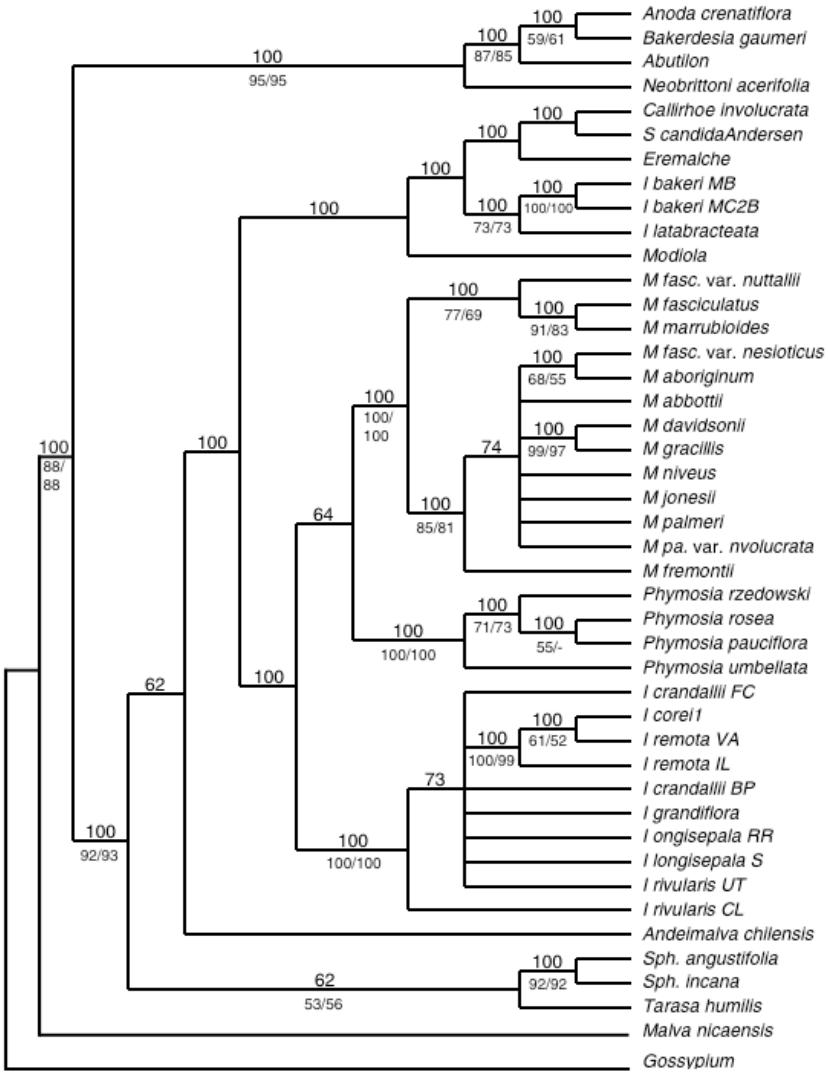


Figure 2.4. The majority rule consensus tree generated with *rpL16* from 4,514 equally parsimonious trees showing relationships of the *Malacothamnus* alliance within tribe Malveae. The data set included 7.01% parsimony informative characters, which produced a tree of 354 steps with CI=0.6538, RI=0.8034, and g1=-0.494. Numbers above branches indicate the percentage of equally parsimonious trees that recovered the clade, numbers below branches are bootstrap and jackknife (bts/jk) values recovered that are greater than 50%. If a jackknife value greater than 50% was not recovered, a "-" is placed next to the bootstrap value. Labels for abbreviated genera are as follows *I=Iliamna, M=Malacothamnus,* and *P=Phymosia*.

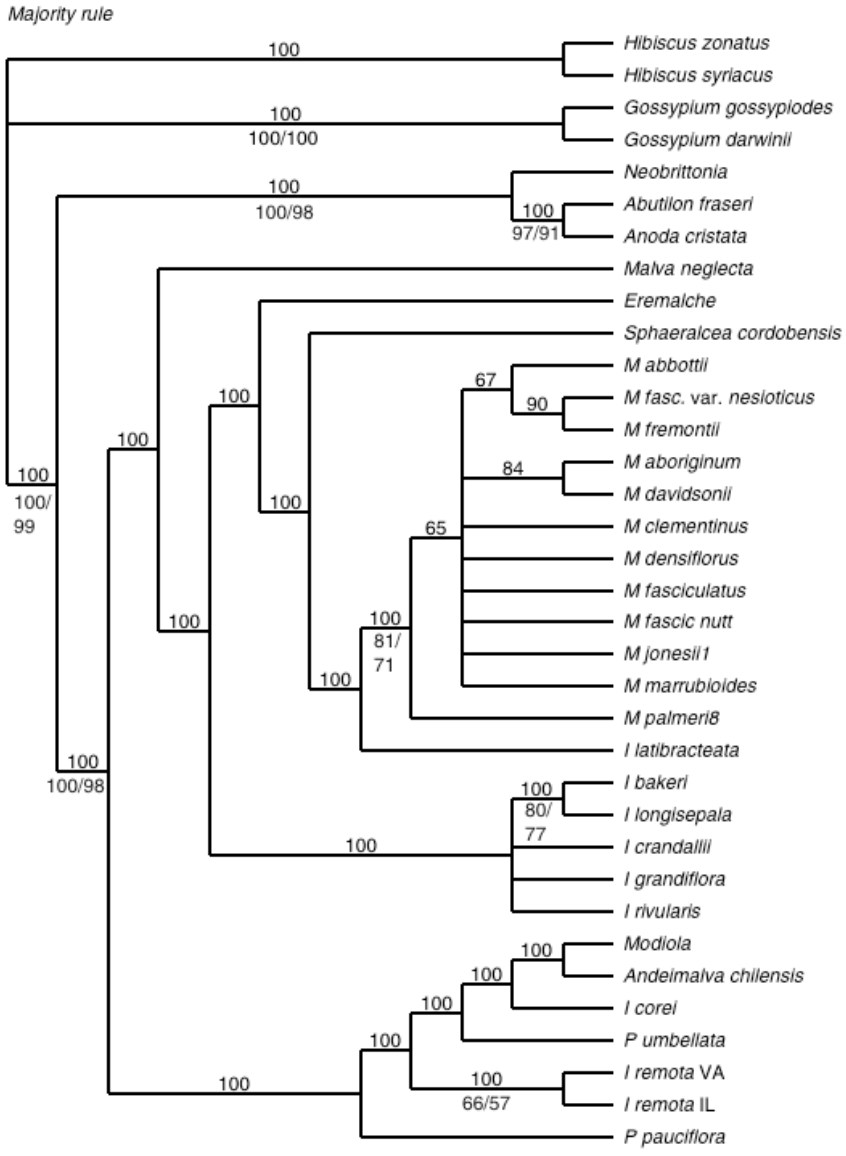


Figure 2.5. The majority rule consensus tree generated with *trnL-trnF* from 4,022 equally parsimonious trees showing relationships of the *Malacothamnus* alliance within tribe Malveae. The data set included 10.2% parsimony informative characters, which produced a tree of 535 steps with CI=0.6389, RI=0.7254, and g1=-0.730. Numbers above branches indicate the percentage of equally parsimonious trees that recovered the clade, numbers below branches are bootstrap and jackknife (bts/jk) values recovered that are greater than 50%. If a jackknife value greater than 50% was not recovered, a "-" is placed next to the bootstrap value. Labels for abbreviated genera are as follows *I=Iliamna, M=Malacothamnus,* and *P=Phymosia*.

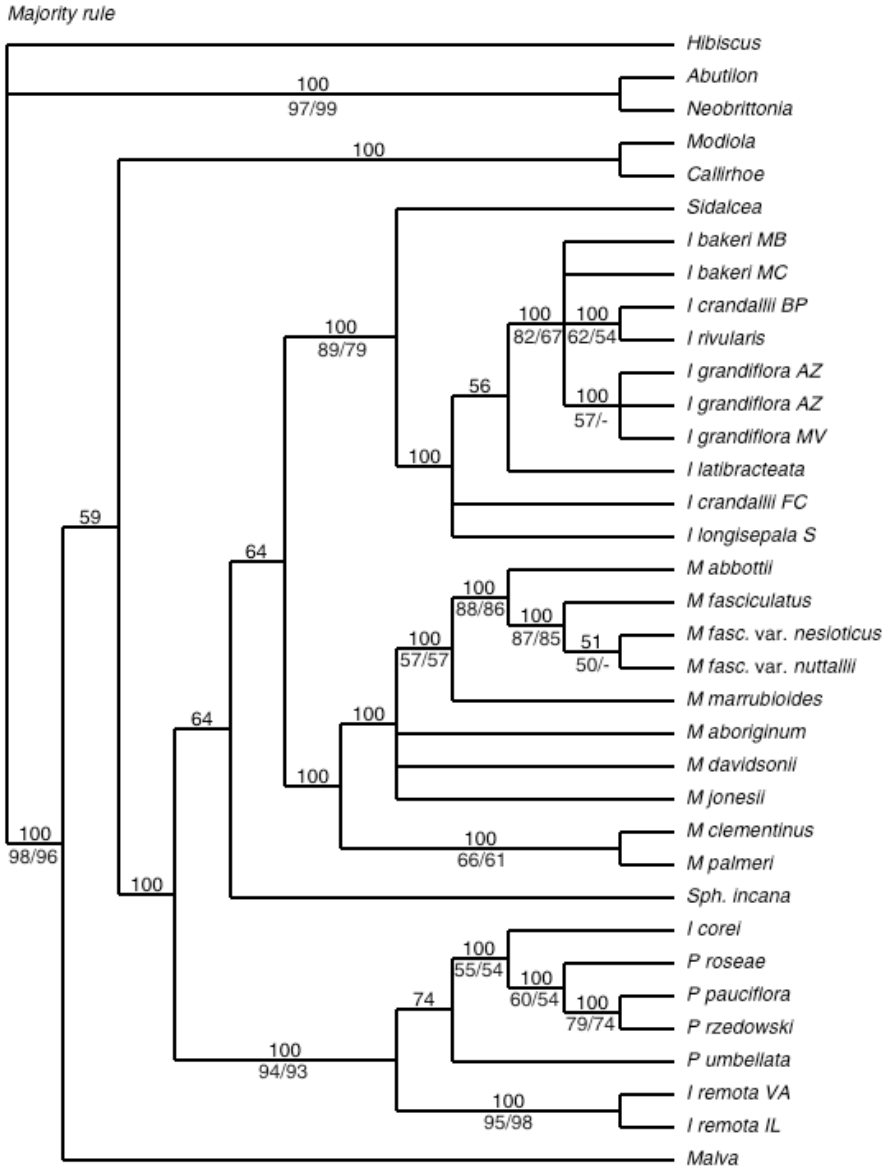
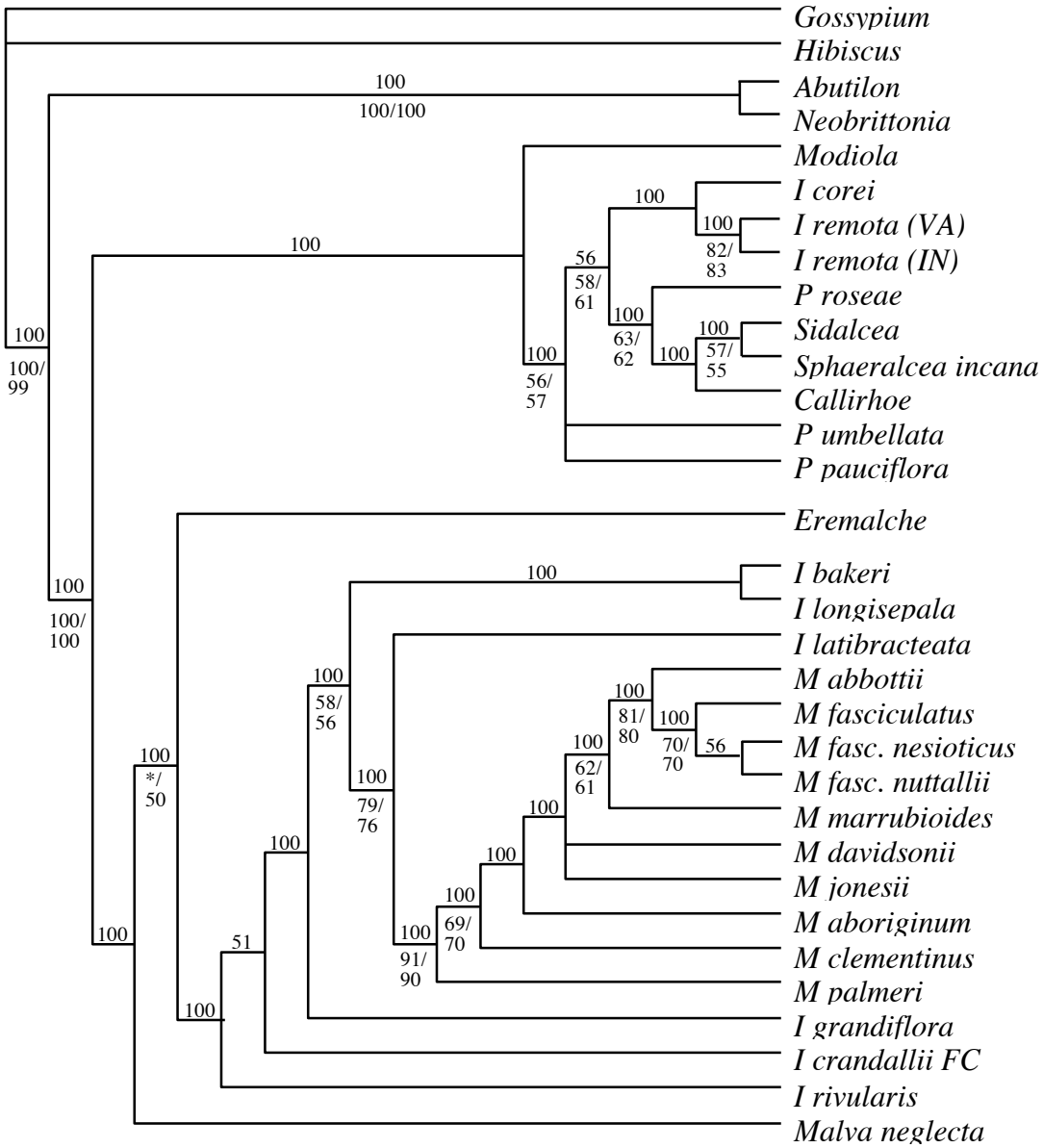


Figure 2.6. The majority rule consensus tree generated with the combined chloroplast data set from 82 equally parsimonious trees showing relationships of the *Malacothamnus* alliance within tribe Malveae. The data set included 15.2% parsimony informative characters, which produced a tree of 1,371 steps with CI=0.6115, RI=0.7467, and g1=-0.524. Numbers above branches indicate the percentage of equally parsimonious trees that recovered the clade, numbers below branches are bootstrap and jackknife (bts/jk) values recovered that are greater than 50%. If a bootstrap value greater than 50% was not recovered, a "-" is placed next to the jackknife value. Labels for abbreviated genera are as follows I=*Iliamna*, M=*Malacothamnus*, and P=*Phymosia*.



Chapter 3

The Evolution of GBSSI-1 in *Iliamna* and *Malacothamnus* and Implications for Phylogenetics

Abstract

A portion of the low copy-number granule bound starch synthase (GBSSI-1) nuclear gene was used to study polyploid origins and relationships within and between two genera. Genetic variation and number of paralogs and homeologs for GBSSI-1 were surveyed for *Iliamna* and *Malacothamnus*. The genera represent two-thirds of the *Malacothamnus* alliance of the tribe Malveae (Malvaceae). Both genera are polyploid (*Iliamna* n=33 and *Malacothamnus* n=17) but their origins are unknown. Taxa within the genera are reported to hybridize and many are classified as rare or endangered. Three copies of GBSSI-1 were detected in *Iliamna*. One of these is shared with *Malacothamnus*. Both genera have undergone independent duplication events and the phylogeny reveals a complex history of evolution in the alliance. The GBSSI-1 exons were able to resolve infrageneric relationships, but little resolution within the genera was obtained due to incomplete sequence representation.

Introduction

Prior evaluation of relationships within *Iliamna* and *Malacothamnus* using the ITS, *trn*L-F, and *rpL*16 regions revealed little phylogenetic resolution within the genera and topological incongruence between the data sets (Chapter 2). The genera in the *Malacothamnus* alliance,

Iliamna, *Malacothamnus*, and *Phymosia*, were chosen for study because the genera do not currently overlap in distribution, taxa within are morphologically similar, and several members of the genera are rare (LaDuke and Doebley 1995, Fryxell 1997). The majority of species in *Iliamna* (six of eight) are found from Northern California and Oregon east to Montana and south to Arizona. Two species, *I. corei* and *I. remota*, are found in Virginia (both species) and Illinois to Indiana (*I. remota*) (Stewart and Porter 1995). All eight species in *Iliamna* are classified as rare or endangered. Species in *Malacothamnus* are endemic to California and northern Baja California and Sonora, Mexico and two species are classified as endangered in California (*M. fasciculatus* var. *nesioticus* and *M. catalinense*) (Bates 1993). Lastly, *Phymosia* occurs in the Bahamas, Haiti, and from southern Mexico to Guatemala, and two species (*P. rzedowskii* and *P. rosea*) are listed as rare in Mexico (Fryxell 1988). Despite the broad distribution of taxa, the genera are allied through chromosome numbers (Bates and Blanchard 1970) and morphological synapomorphies (Fryxell 1997) (see Chapter 1 for a complete overview).

In a phylogenetic study using the nuclear ITS region, *Iliamna bakeri* and *I. latibracteata* were sister to *Sidalcea*, another member of the tribe Malveae. The remaining six species of *Iliamna* were in a clade sister to representatives of *Malacothamnus* (see Chapter 2). Information from two chloroplast regions (*trn*L-F and *rpL*16) provided a contrasting hypothesis. *Iliamna corei* and *I. remota* were sister to representatives of *Phymosia*. The remaining species of *Iliamna*, including *I. bakeri* and *I. latibracteata*, were in a clade sister to *Malacothamnus*. The contrasting topologies may coincide with the different lines of inheritance. The chloroplast-based phylogeny suggests *Phymosia*, or a common ancestor, as the maternal parent of *Iliamna* species in the Eastern United States. The nuclear-based phylogeny suggests a western ancestor, perhaps shared

with *Sidalcea*, as a second contributor to the *Iliamna* genome. To explore this possibility further, an additional nuclear region was chosen for evaluation of relationships in the *Malacothamnus* alliance. In this study, only representatives of *Iliamna* and *Malacothamnus* were included, with several additional genera as outgroups. *Phymosia* was not included because only material from herbarium specimens was available, which yielded DNA of poor quality that was not amenable to sequence

The objective of this study was to use a low copy nuclear gene to examine relationships in *Iliamna* and *Malacothamnus*. Numerous copies of GBSSI were sequenced for several taxa to study the number of copies present and the homology the sequenced copies. Based upon the alignment of exon sequences, homologous copies were identified. For each copy, both intron and exon sequences were aligned and analyzed in order to examine relationships within the genera.

Granule Bound Starch Synthase

The GBSSI gene is a low-copy nuclear gene containing 13 introns that encodes an essential enzyme in starch synthesis (Mason-Gamer et al. 1998, Peralta and Spooner 2001, Walsh and Hoot 2001). There are several advantages in using GBSSI in phylogenetic studies within families. First, the GBSSI introns exhibit a higher rate of change than do other nuclear regions, such as ITS, and GBSSI has been shown to differentiate between species in taxonomic studies (Mason-Gamer et al. 1998, Evans et al. 2000). Also, due to its size (approximately 2 kb)

GBSSI has the potential to provide a greater number of phylogenetically informative sites than the ITS region (approximately 700 bp).

The multiple copies of GBSSI may provide additional information on genome evolution when paralogous and orthologous copies are examined. In contrast to the detection of a single locus (GBSSI-1) in diploid taxa of Poaceae (Mason-Gamer et al. 1998) and *Ipomea* (Miller et al. 1999), Rosaceae appears to contain two loci (GBSSI-1 and BGSSI-2) (Evans et al. 2000). These vary with respect to, intron length and number and the rate of substitutions. The greatest variation of GBSSI-1 occurs within the introns. For example, the sub-family Maloideae was characterized as having a long first intron and no sixth intron. A portion of each locus was used as probes in Southern hybridization survey of the family. All of the genera surveyed contained GBSSI-1 and GBSSI-2. These results indicate that the duplication of GBSSI occurred before diversification within the Rosaceae. In phylogenetic analysis of the separate loci, two well-supported clades, each with 100% bootstrap support, emerged. Within the GBSSI-1 and GBSSI-2 clades, strong statistical support (based on bootstrap and decay analyses) was found for the majority of the interior nodes. In lower level analyses, GBSSI produced a phylogeny for five species of *Rubus* (Evans et al. 2000) that was consistent with another study that used ITS (Alice and Campbell 1999). Furthermore, in *Rubus*, pairwise sequence divergence values were greater in GBSSI than those of the ITS sequences.

In examining allopolyploidy in the tribe Geinae (Rosaceae), several copies of GBSSI-1 were recovered for *Geum* and related genera (Smedmark et al. 2003). Of the cloned sequences, several represented pseudogenes and were omitted from their analysis. In examination of the

copies, sequences of different species were more closely related to each other than to clones from the same species. The results of the phylogenetic analyses indicated reticulate evolution with homologs from different ancestors serving as donors to each line. Three topologically similar clades, representing the three copies of GBSSI-1, were recovered, with high bootstrap support for interior nodes of each clade. Prior parsimony analyses of the chloroplast *trn*L-F region were congruent with the Geinae GBSSI clade A. The authors concluded that clade A of GBSSI-1 represented the maternal history of polyploid formation and indicated *Geum heterocarpum* as the maternal donor to the polyploid line. The three paralogous copies of GBSSI-1 found in three clades may be the result of either allopolyploidy, or an ancenstral duplication event with lineage sorting, or a duplication event in an outgroup.

The phylogenetic utility of GBSSI gene sequences for systematics has been explored in the Malvaceae as well (Small 2002). Three copies of GBSSI appear in *Gossypium* and *Hibiscus*. The three Malvaceae copies are more similar to each other than to the Rosaceae loci, suggesting that duplication occurred indepently in the lineages. In *Hibiscus* sect. *Muenchhusia*, GBSSI-1 was used in a phylogenetic analysis along with ITS, *rpL*16, and *ndh*F (Small 2004). Sequence data from *ndh*F and *rpL*16 did not contribute any parsimony informative sites. In the ITS data, only 2 of 683 (0.3%) aligned bases were informative. However, information from GBSSI was able to resolve relationships resulting, in a robust phylogenetic tree for the five species of sect. *Muenchhusia*. Of the 1,972 aligned nucleotides in GBSSI, 28 (1.5%) were phylogenetically informative. Several informative sites were also polymorphic in two species, *Hibiscus dasycalyx* and *H. grandiflorus*, which confirmed that interspecific gene flow has occurred. Morphological

intermediates of *H. dasycalyx* and *H. grandiflorus* with *H. moscheutos* occur where the distributions of the species overlap, supporting hypotheses of interspecific gene flow.

Methods

Plant Materials

Taxa included in this analysis were collected from native populations and the material was dried in silica gel prior to DNA extraction (Table 3.1). The GBSSI region was successfully amplified (Table 3.2) and sequenced for six of eight species of *Iliamna* and eight of the eleven species of *Malacothamnus*. A representative of *Sphaeralcea* (S. incana) was included as an outgroup since species in *Iliamna* were once thought to be members of this genus (Rydberg 1904). Additional taxa from Malveae included in the study are *Callirhöe involucrata*, *Modiola caroliniana*, *Malva sylvestris*, and *Sidalcea candida*. In a phylogenetic examination of tribe Malveae with ITS, *Callirhöe, Modiola*, and *Sidalcea* were found to be sister to clades containing *Iliamna* and *Malacothamnus* (J. Tate, pers. comm., University of Florida).

DNA Extraction, PCR, Cloning, and Sequencing

Total genomic DNA was extracted for samples of *Iliamna* following Slotta (2000) and from *Malacothamnus* using a modified procedure of the Qiagen DNEasy kit (Qiagen Corp., Valencia, CA), which removes the high quantity of polysaccharides found in mallows. Amplification of GBSSI followed the procedure of Evans et al. (2000) using the Epicentre

FailSafe PCR PreMix (Madison, WI) and Taq polymerage. PreMixE was used to ensure consistency of amplification. The Rosaceae GBSSI-1 primers 1F and 9R were used to amplify an approximately 2.0 kb fragment from the 5í end of the GBSSI gene that encompassed portions of exons 1 and 9 and the intervening sequences (Fig. 3.1). A stepdown PCR procedure was employed, since a single annealing temperature was unsuccessful in amplification in *Iliamna* and *Malacothamnus*, as was found in Rosaceae (Evans et al. 2000). The reaction was as follows: 94˚C for 1 min. then 94˚C for 45 sec., 58˚C for 1 min. 40 sec., and 72˚C for 1.5 min., for 5 cycles then, 94˚C for 30 sec., 59˚C for 1 min., and 72˚C for 1 min., for 40 cycles, then 72˚C for 10 min. The amplified products were separated on a 1% agarose gel and purified using the Qiaquick Gel Extraction Kit (Valencia, CA). Several internal primers were used for DNA sequencing as well. In addition to these primers, modified 1F and 9R primers for Malvaceae were designed and are located approximately 50 bp downstream (upstream for 9R) from the Rosaceae primers (Table 3.2) in order to increase the probability of amplification.

The GBSSI-1 region was sub-cloned prior to sequencing using the pGem T vector (Promega, Madison, WI) according to the manufactures directions. In order to clone the fragment into the plasmid by T/A cloning, T4 ligase was used with a 2:1 insert to vector ratio (Promega Corp., Madison, WI). Electro-competent cells of E. coli strain DH10B were transformed by electroporation in reactions containing 2 □l of the ligation reaction and 40 □l of cells in 0.2 cm cuvettes, pulsed at 2.5 kV and 200 Ω (Dower 1989, Smith et al. 1990). The cells were then placed in 1 mL of SOC medium for 1 h. at 37˚C. Then, 200 □l of the culture was plated on LB medium containing 100 mg/mL of Ampicillin and coated with 40 μL each 20 mg/mL 5-bromo-4-chloro-3-indolyl-□-D-galactopyranoside (X-GAL) and 20 mg/mL isopropyl

thio- □ -D-galactoside (IPTG). The recombinants were digested with the SacI restriction enzyme to verify the presence of the GBSSI insert and determine the approximate size of the amplicon. Recombinant clones were sequenced using the PE-Applied Biosystems Big Dye Terminator Kit (La Jolla, CA) and electrophoresis was conducted at the Virginia Bioinformatics Institute Core Lab Sequencing Facility (Blacksburg, VA) on an ABI 373 automated sequencer. In addition to the 1F and 9R, primers 3F, 4F, 6R, and 8R (Table 3.2) were used for sequencing the GBSSI region.

Selection of Clones

Numerous colonies were screened for each template (Table 3.3). Several recalcitrant amplicons were not successfully cloned despite multiple attempts. To increase the probability of ligation into the plasmid, truncated portions of GBSSI were amplified using internal primers. Additional primers, T1F, T9R, and several unlisted primers, were used to increase the success of amplification. One sequence (*Iliamna grandiflora*) was generated with primers 4F and T9R, yielding a portion of the GBSSI region. The sequence for *Malacothamnus fremontii*-A is not complete as well; one-third of the sequence from the 5í region is not included because of ineffective sequencing reactions.

Phylogenetic Analyses

Sequences were manually edited and contiguous sequences were generated using the SeqMan program of DNA Star Lasergene (Madison, WI) and aligned in MegAlign using the

CLUSTAL W algorithm (Thompson et al. 1994). For identification of exon boundaries, sequences of Hibiscus coccineus (Genbank AY341421.1), Gossypium (unpublished data, R. Small, University of Tennessee), and Malva sylvestris (unpublished data, R. Small, University of Tennessee) were included. Phylogenetic analyses were first conducted on exon sequences with PAUP 4.0b10 using random stepwise addition using the tree bisection reconnection (TBR) method with 10,000 replicates, ACCTRAN optimization, and gaps treated as missing data (Swofford 2002). A consensus tree was generated using the Majority Rule option, which retains clades that are found in 50% or more of the generated trees. Support of inferred phylogenetic relationships was calculated with bootstrap indices (1,000 replicates, 10 TBR replicates per step). Clades resulting in the analysis of exons were used to identify paralogous copies of GBSSI-1. MacClade was used to translate the exons in order to identify mutations effecting protein formation, such as pre-mature stop codons (Maddison and Maddison 1992). For analysis of relationships within the copies of GBSSI-1, separate data matrices for each copy were prepared and included both exon and intron sequences. Data from the entire region from exons 1 to 9, including introns, were analyzed as described above.

Results

Sequence variation within GBSSI-1

Sequences of GBSSI-1 were generated for six taxa in *Iliamna*, eight taxa in *Malacothamnus*, and one representative each of *Sidalcea*, *Sphaeralcea*, *Callirhöe*, and *Modiola.* Up to four clones of each individual were sequenced in order to recover orthologous and

paralogous copies representing the diversity of GBSSI-1 within the taxa. Eight exons and seven introns were identified in the region of GBSSI sequenced based on alignments to *Hibiscus coccineus*. Exon sequences were easily aligned and the boundaries and sizes of introns identified (Table 3.4). The second intron identified here, Intron 3, contains the greatest length variation. Based on the alignment, four classes of GBSSI-1 can be distinguished (Table 3.5). Each class contains multiple sequences and three of the four classes are represented by multiple genera. No pseudogenes were apparent from the translation of coding regions.

The exon data set consisted of 892 aligned nucleotides from exons 2 to 8 of GBSSI-1. Within the ingroup, *Iliamna* and *Malacothamnus*, 148 characters (5.4%) were parsimony informative and 505 characters (57%) were constant. Based on the phylogeny produced in the analysis of the exon sequences, taxa were divided into four groups A-D (Fig. 3.2). Data analyzed from the four groups included sequences from both introns and exons. Within Group A, 2,866 aligned characters were analyzed of which 549 (19%) were parsimony informative, excluding Gossypium. Group A included representatives from five genera, Callirhˆe, *Iliamna*, *Malacothamnus*, *Sidalcea*, and *Sphaeralcea*. Sequences within Group A included those with the full-length Intron 3 (class A), partial sequence in the intron (class B), and the short intron (class D). In Group B, 1,978 characters were aligned, of which only 36 (1.8%) were parsimony informative. Group B included only four species of *Iliamna*, all of which are representative of Intron 3 class D. Group C included six species of *Malacothamnus* with 1,923 aligned characters with 47 (2.4%) being parsimony informative. This Group included representatives of Intron 3 classes C and D. Lastly, Group D from the exon-based phylogeny included representatives of *Callirhöe* ˆe, *Iliamna* (5 species), *Modiola*, and *Sidalcea*. The *Iliamna grandiflora*,

Malacothamnus fremontii-A, and *Sidalcea*-Little) sequences were omitted due to alignment difficulties of the intron sequences. In addition, 391 characters of Intron 3 were eliminated from the alignment leaving 1,698 aligned characters of which 236 (14%) were parsimony informative. Sequences in Group D were diverse with members of Intron 3 classes B, C, and D included.

Phylogenetic Analyses

Phylogenetic analyses of the exon sequences resulted in 598 equally parsimonious trees, the majority rule consensus of which is shown in Fig. 3.2. The results of this analysis were used to subdivide the data set into four presumptive copies of GBSSI-1, labeled as A-D. The groups did not have bootstrap values greater than 50%, but resulted in the majority rule consensus of trees generated in several replicate generations of the analysis. Although support for the division of the groups was low, the data contain limited homoplasy (RI=0.7149) and significant phylogenetic signal (g1= -0.55252) (Hillis and Huelsenbeck 1992). Groups A and D consisted of multiple genera, while Group B (*Iliamna*) and Group C (*Malacothamnus*) had representatives of single genera. The position of *I. grandiflora* and *M. fremontii* in the exon-only analysis may be misleading since these sequences are incomplete because of truncated PCR products (*I. grandiflora*) or failed sequencing attempts (*M. fremontii*).

In the phylogenetic analyses of the copies, Group A resulted in 3 equally parsimonious trees of 2234 steps with increased statistical support for interior nodes (Fig. 3.3-A). Group B under maximum parsimony produced 2 trees of 436 steps and increased statistical support for nodes (Fig. 3.3-B). However, the multiple sequences representing single taxa in Group B do not

result in clades. Group C, consisting of taxa in *Malacothamnus* plus the truncated sequence of *I. grandiflora*, resulted in 5 equally parsimonious trees (578 steps) with increased resolution (Fig. 3.3-C). Two taxa sampled, *M. aboriginum* and *M. clementinus*, have multiple sequences included and form well-supported clades, whereas multiple alleles of other taxa do not. In Group D, 5 equally parsimonious trees resulted (994 steps); again, support increased for interior nodes when intron sequences were included in the analysis (Fig. 3.3-D).

Discussion

Polyploidy and the Evolution of GBSSI-1

Polyploid origins for *Iliamna* and *Malacothamnus* are supported by the GBSSI-1 data. Clones from the same individual appear in different clades in the GBSSI-1 exon gene tree (Fig. 3.2). Among the clades, sequences of some species are more closely related to those of other species than they are to each other. Clones of *Iliamna longisepala* may be found in Groups A, B, and D (Fig. 3.3) and are sister to *I. crandallii* and *I. remota* (Group A), *I. remota* and *I. corei* (Group B), and *I. corei*, *I. crandallii*, *I. remota*, and *I. rivularis* (Group D). A similar arrangement of relationships among clones of single taxa results for *I. corei, I. crandallii, I. remota,* and *I. rivularis*. This finding suggests that the duplication event leading to three paralogous copies of GBSSI-1 occurred prior to the diversification of *Iliamna*, with taxa retaining earlier copies.

In *Malacothamnus*, only sequences of *M. clementinus* appear in multiple groups (Groups A and C, Fig. 3.3). However, *M. fasciculatus* var. *fasciculatus* (Group A) and *M. fasciculatus* var. *nesioticus* (Group C) have been considered synonymous due to morphological intergration and limited genetic variation (Swensen et al. 1995, Benesh and Elisens 1999). If this is factored into the present analysis of GBSSI-1, then a second genotype of *Malacothamnus* results in both Group A and Group C. At least one duplication event occurred prior to the separation of *Iliamna* and *Malacothamnus*. The two genera share a paralogous copy of GBSSI-1 as seen in Clade A of the exon phylogeny.

Genera related to the *Malacothamnus* alliance in tribe Malveae (e.g., *Callirhöe* and *Sidalcea*) are represented in Group A and Group D (Fig. 3.2). Group D represents one ancestral copy of GBSSI-1 and Group A, with a significantly larger third intron, a second ancestral copy of GBSSI-1. The presence of these two distinct copies suggests a duplication prior to the origin of the tribe Malveae. *Iliamna* and *Malacothamnus* have had an additional duplication of the GBSSI-1 gene prior to their diversification.

Phylogeny of the Malacothamnus alliance

Analysis of the GBSSI data does not provide sufficient evidence to place *Iliamna* and *Malacothamnus* into an alliance separate from other genera in Tribe Malveae. Clones from species of each genus are found in different clades in the analysis. Only in Group A are both reliable sequences for *Iliamna* and *Malacothamnus* represented. As mentioned above, *I. grandiflora* and *M. fremontii*-A are incomplete sequences and their placement in the exon-based

phylogeny may be misleading. Within Goup A, too few sequences of *Iliamna* and *Malacothamnus* were obtained in order to develop hypotheses of evolution of the *Malacothamnus* alliance. In the analysis of intron and exon sequences in this subset of samples, neither *Iliamna* nor *Malacothamnus* are monophyletic.

To examine relationships within the genera separately, portions of the phylogenetic tree for the Groups A, B, C and D are analyzed (Fig. 3.3). For *Iliamna*, it is clear that *I. corei* and *I. remota* are closely related (Group D with 83% bootstrap), a result that is supported by prior analyses of sequences of ITS, *trn*L-F, and *rpL*16 and data from RAPDs (Stewart and Porter 1995). Sequences from *I. remota* from Illinois (ROx samples) and *I. remota* from Virginia (5A, 6A, 6B, 6D) do not result in a single clade in the analysis of Group A nor in D. The limit of phylogenetic information in GBSSI-1 may have been reached in examining relationships between populations, and the genetic diversity therein.

Phylogenetic analyses of Group B (100% bootstrap) and Group D (75% bootstrap) clearly indicate *Iliamna* to be monophyletic. Any further taxonomic conclusions in *Iliamna* cannot be surmised here. Among the data sets, insufficient parsimony informative sites (B=1.8%, D=13%) and incomplete representation of species in each clade were obtained to provide robust phylogenetic hypotheses of relationships in *Iliamna*

Analysis of Group C confirms the monophyly of *Malacothamnus*, with 100% bootstrap support (Fig. 3.3 C). Within this reduced data set, sequences of clones for *M. aboriginum* and *M. clementinus* indicate the monophyly of GBSSI-1 for the two species, with 80% bootstrap support

for *M. aboriginum* and 94% for *M. clementinus*. Surprisingly little phylogenetic resolution and genetic divergence result among the taxa sampled despite morphological distinction. *Malacothamnus aboriginum*, *M. clementinus*, and *M. fremontii*, with thyrse-like inflorescences, *M. fasciculatus* and *M. jonesii* with raceme or panicle-like inflorescences, and *M. palmeri*, a capitulum inflorescence do not form distinct clades (Chapter 5). Neither can pubescence nor geography in *Malacothamnus* be mapped onto the GBSSI-1 gene phylogeny. The limited variation (Group C with 3.3% parsimony informative sites) and the influence of homoplasy (CI=0.6821) prevent the formation of well-resolved clades.

Future Directions

Analysis of GBSSI-1 sequences in *Iliamna* and *Malacothamnus* indicates that polyploidy and gene duplication have been contributing factors to the genetic diversity of the Tribe Malveae. The results indicate the need for further investigation. To provide substantiated hypotheses of evolution in *Iliamna* and *Malacothamnus* with GBSSI-1, sampling of species and paralogs would need to be increased in order to obtain representatives of each paralogous copy for each taxon. This problem was addressed, in part, by the development of Malvaceae-specific primers. However, it appears that additional primers to target each copy individually could be designed, which would increase the likelihood that all paralogs in a sampleís genome would be recovered. Increased sampling from several populations per species would also be valuable to further explore the utility of GBSSI-1 gene as a tool for phylogenetic analysis.

Literature Cited

Alice, L.A. and C.H. Campbell. 1999. Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. *American Journal of Botany* 86: 81-97.

Bates, D. M. 1993. *Malacothamnus*. In Hickman, J.C., ed. *The Jepson Manual: Higher plants of California*. Berkeley: University of California Press. Pp. 747-753.

Bates, D.M. and O.J. Blanchard, Jr. 1970. Chromosome numbers in the Malvales II: New or otherwise noteworthy counts relevant to classification in the Malvaceae tribe Malveae. *American Journal of Botany* 57: 927-934.

Benesh, D.L. and W.J. Elisens. 1999. Morphological variation in *Malacothamnus fasciculatus* (Torrey and A. Gray) E. Greene (Malvaceae) and related species. *Madroño* 46: 142-152.

Dower, W.J. 1989. Transformation of *E. coli* to extremely high efficiency by electroporation. *Molecular Biology Reports* 6: 3-4.

Evans, R.C., L.A. Alice, C.S. Campbell, E.A. Kellogg, T.A. Dickinson. 2000. The granule-bound starch synthase (GBSSI) gene in the Rosaceae: Multiple loci and phylogenetic utility. *Molecular Phylogenetics and Evolution* 17: 388-400.

Fryxell, P.A. 1988. Malvaceae of Mexico. *Systematic Botany Monographs*. 25.

Fryxell, P.A. 1997. The American genera of Malvaceae II. *Brittonia* 49: 204-269.

Hillis, D. M. and J. P. Huelsenbeck. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189-195.

LaDuke, J. C. and J. Doebley. 1995. A chloroplast DNA based phylogeny of the Malvaceae. *Systematic Botany* 20: 259-271.

Maddison, W.R. and D. R. Maddison. 1992. *MacClade: Analysis of Phylogeny and Character Evolution, ver. 3*. Sunderland, Mass: Sinauer Associates, Inc.

Mason-Gamer, R, C. Weil, and E. Kellogg. 1998. Granule-Bound Starch Synthase: Structure, function, and phylogenetic utility. *Molecular Biology and Evolution* 15: 1658-1673.

Miller, R. E., M. D. Rausher, and P. M. Manos. 1999. Phylogenetic systematics of *Ipomoea* (Convolvulaceae) based on ITS and *waxy* sequences. *Systematic Botany* 24: 209-227.

Peralta, I. E., and D. M. Spooner. 2001. Granule-bound starch synthase (GBSSI) gene phylogeny of wild tomatoes (*Solanum* L. section *Lycopersicon* [Mill.] Wettst. Subsection *Lycopersicon*). *American Journal of Botany* 88: 1888-1902.

Rydberg, P. A. 1904. Rocky Mountain Flora. *Bulletin of the Torrey Botanical Club* 31: 565-566.

Slotta, T. A. B. 2000. *Phylogenetic analysis of Iliamna (Malvaceae) using the internal transcribed spacer*. Master's Thesis, Virginia Polytechnic Institute and State University.

Small, R. 2002. The GBSSI gene family in Malvaceae. *Botanical Society of America Abstracts* S-126.

Small, R. 2004. Phylogeny of *Hibiscus* sect. *Muenchhusia* (Malvaceae) based on chloroplast *rpL*16 and ndhF, and Nuclear ITS and GBSSI sequences. *Systematic Botany* 29

Smedmark, J.E.E., T. Eriksson, R.C. Evans, and C.S. Campbell. 2003. Ancient allopolyploid speciation in Geinae (Rosaceae): Evidence from nuclear granule-bound starch synthase (GBSSI) gene sequences. *Systematic Biology* 52: 374-385.

Smith, M. J., J.T. Landers, and J. Jordan. 1990. High efficiency bacterial electroporation. *Focus* 12: 38-40.

Stewart, C.N. and D.M. Porter. 1995. RAPD profiling in biological conservation: An application to estimating clonal variation in rare and endangered *Iliamna* in Virginia. *Biological Conservation* 74: 135-142.

Swenson. S., G. Allan, M. Howe, W. Elisens, and L. Riesberg. 1995. Genetic analysis of *Malacothamnus fasciculatus* (Nutt.) Greene var. *nesioticus* (Rob.) Kearn. *Conservation Biology* 9: 404-415.

Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sunderland, Mass.: Sinauer Associates.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673-4680.

Walsh, B. M. and S. B. Hoot. 2001. Phylogenetic relationships of *Capsicum* (Solanaceae) using DNA sequences from two non-coding regions: The chloroplast atpB-rbcL spacer region and nuclear *waxy* introns. *International Journal of Plant Science* 162: 1409-1418.

Table 3.1. Taxa for which sequences of *Iliamna (I.), Malacothamnus (M.),* and additional genera were obtained, with voucher and number of sequences recovered. Vouchers as follows following: INH=Illinois Natural History Survey, JEPS=Jepson Herbarium, KK=Kevin Kane, MBG=Missouri Botanical Garden, living collection, TBS=T. Slotta, UC=University of California, Berkeley, TFW=T. Wieboldt. Populations sampled include *I. crandallii* Fish Creek Falls (FC) and Buffalo Pass (BP) near Steamboat Springs, Colorado and *I. remota* near Clifton Forge, Virginia (CC2) and Illinois (RO).

Taxon	Voucher	Number of GBSSI Sequences Recovered
I. corei	TFW 6761	4
I. crandallii	(FC) TBS 994	2
I. crandallii	(BP) TBS 993	4
I. grandiflora	TBS 995	1
I. longisepala	KK s.n. 6/1994	5
I. remota	(CC2) TBS 2001-4	5
I. remota	(RO) INH	3
I. rivularis	TFW 1704	3
M. aboriginum	TBS 2002-31	3
M. clementinus	TBS 2000-15	3
M. densiflorus	UC- Plant 99.0611	1
M. fasciculatus var. *fasciculatus*	TBS 2000-6	3
M. fasciculatus var. *nesioticus*	TBS 2000-11	1
M. fremontii	JEPS 570,829	3
M. jonesii	TBS 2002-19	3
M. palmeri var. *palmeri*	TBS 2000-10	2
Sidalcea candida	TBS 997	3
Sphaeralcea incana	TBS 996	3
Callirhöe digitata	MBG 1,113	1
Modiola caroliensis	JMG 121 (College of William and Mary)	3

Table 3.2. Primers used for amplification and sequencing of GBSSI.

Primer	Sequence (5' to 3')	Reference
1F	CTG GTG GAC TCG GTG ATG TTC TTG	Evans et al. 2000
TS1F	CTG CAT GGC TGT AGT ATG AG	this paper
3F	ACT GTY CGR TTC TTC CAC	Small 2004
4F		
6R	AGA GCA GTG TGC CAA TCA TTG	Small 2004
8R	TCA CCR GAW ACA AGC TCC TG	Small 2004
9R	CTC TTC TAG CCT GCC AAT GAA CC	Evans et al. 2000
TS9R	GGC TTA GCA TCC ATT ACC TGC	this paper

Table 3.3. Selection of transformed colonies**.** Abbreviations for populations as listed in Table 3.1.

Taxon	Number of White Colonies	Number Successful
Iliamna bakeri	9 (4F to 9R)	0
I. corei	7	4
I. crandallii BP	8	4
I. crandallii FC	2	2
I. grandiflora	9 (4F to 9R)	1
I. latibracteata	7 (4F to 9R)	0
I. latibracteata	6	0
I. longisepala	8	5
I. remota CC2	9	4
I. remota RO	6	3
I. rivularis UT	10	3
Malacothamnus abbottii	3	0
M. aboriginum	6	3
M. clementinus	7	3
M. densiflorus	5	1
M. fasciculatus var. *fasciculatus*	5	3
M. fasciculatus var. *nesioticus*	4	1
M. fremontii	5	3
M. jonesii	5	3
M. palmeri var. *palmeri*	4	2
M. palmeri var. lucianus	7	0
Callirhoe involucrata	4	1
Modiola caroliniana	4	3
Sidalcea candida (Big copy)	5	2
Sidalcea candida (Little)	4	1
Sphaeralcea incana	5	3

Table 3.4. Aligned length of coding and non-coding regions in GBSSI-1.

Identification	Sequence Length (bp)	Number of Amino Acids
Exon 2	90	30
Intron 2	105	
Exon 3	198	66
Intron 3	1403	
Exon 4	69	13
Intron 4	100	
Exon 5	111	37
Intron 5	94	
Exon 6	111	37
Intron 6	104	
Exon 7	252	84
Intron 7	77	
Exon 8	81	27 (incomplete)

Table 3.5. Classification of homologous sequences based on length mutations in Intron 3. Abbreviations for populations as listed in Table 3.1. Numbers (or letters) following taxa indicate sequence number. For *Sidalcea*, the large copy (Big) and the smaller copy (Little) are listed below as well.

Class A: Full Intron (1403 bp)

I. remota RO5
M. densiflorus 3
M. fasciculatus 2 and 4 and 5
Callirhoe involucrate 3
Sidalcea Big1 and BigA
Sphaeralcea 2 and 4

Class B: 400 bp

I. rivularis 1 and 5
I. remota 5A and 6A
I. crandallii BP2 and 3
I. corei 6
M. clementinus 4

Class C: ca. 140 bp

M. fremontii A
M. palmeri 8b
M. palmeri B

Class D: <100bp

I. corei 1, 2, 3
I. crandallii FC1, FC2, BPB, BP1
I. longisepala A, D, E, 2.3, 2
I. remota 6B, RO1, 6D, RO4
I. rivularis 2
Modiola
Sphaeralcea 5
M. aboriginum 4, 5, 6
M. clementinus 3, 3B
M. fasciculatus var. nesioticus 2.1
M. fremontii B, D
M. jonesii 1, 2, 4
Sidalcea Little

Figure 3.1. Generalized diagram of the GBSSI gene, arrows indicate approximate location of primers. Right pointing arrows are forward primers and left arrows are reverse primers in the labeled exons. Primer names are based on the exon in which it is located. A scale bar of 200 bp is provided for reference.

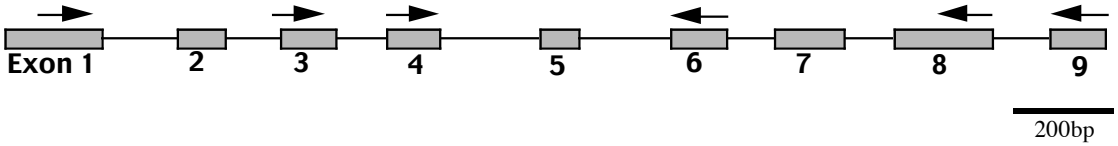


Figure 3.2. Phylogenetic tree of all included samples using exon sequences only. Numbers above branches are majority rule values, numbers below branches indicate bootstrap support indices. Five loci of GBSSI-1 identified in the included taxa are indicated by the vertical bar and label (A-D). Tree length was 861 steps for 598 equally parsimonious trees (CI= 0.5668 RI= 0.7149 g1= -0.5525). Of the 892 aligned nucleotides, 5.4% were parsimony informative and 57%were constant.

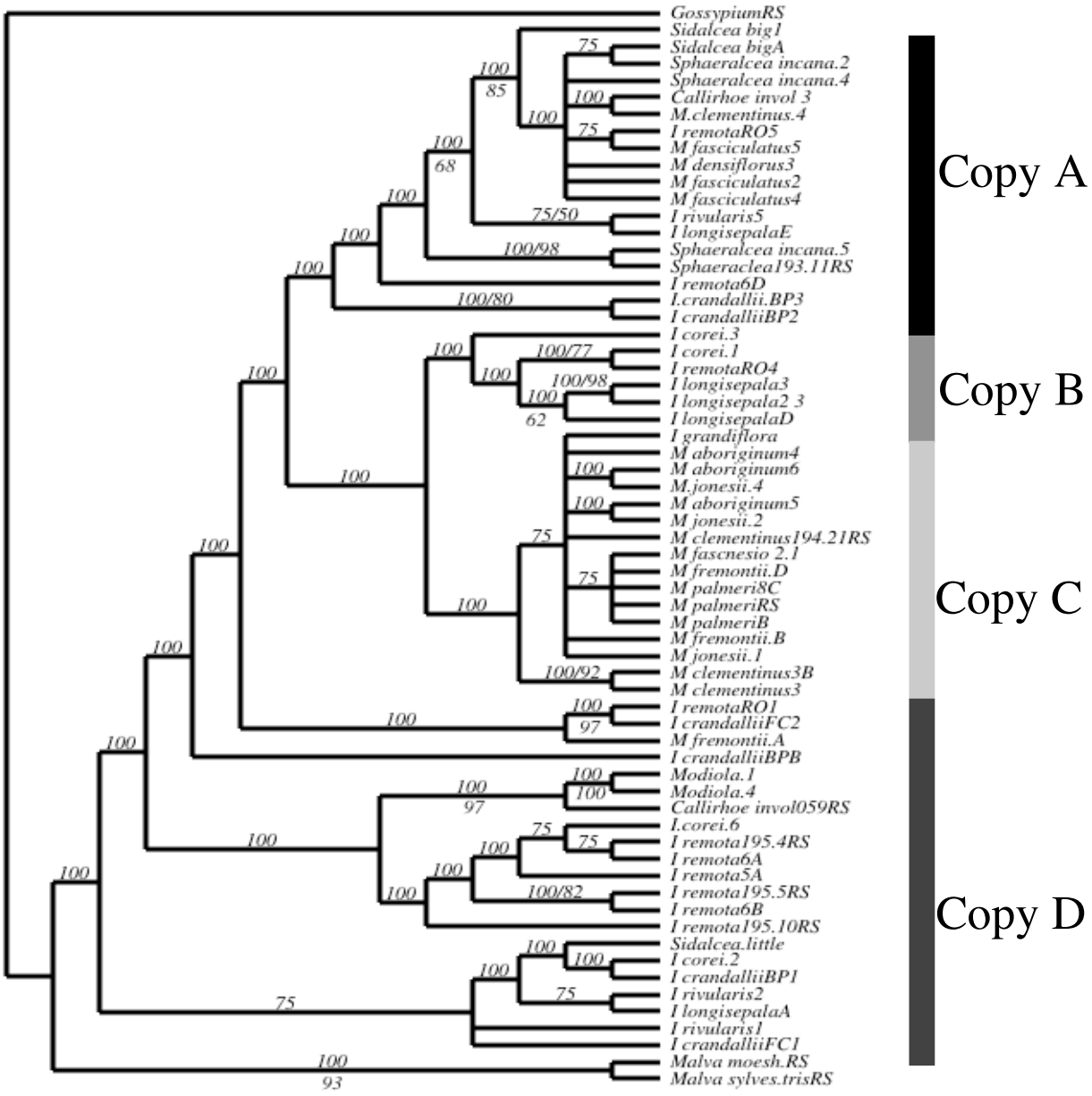
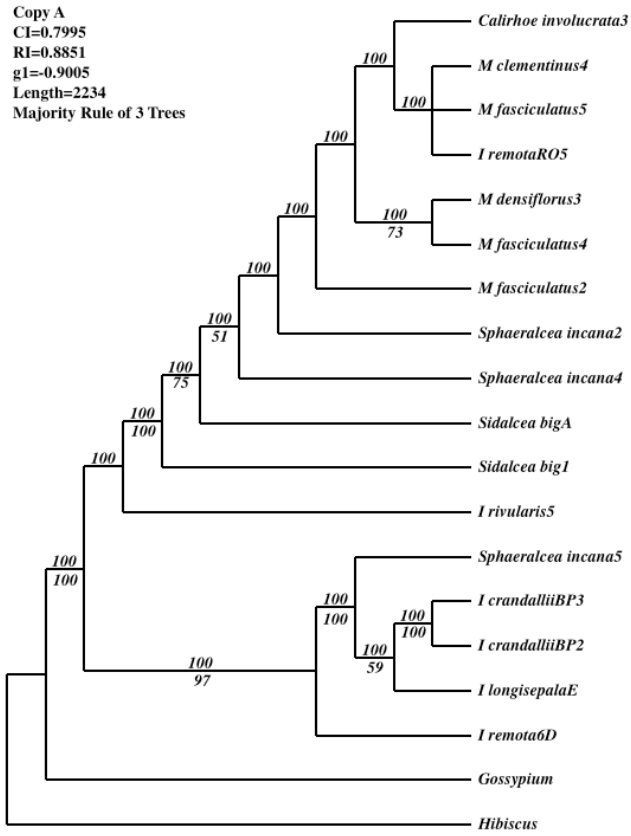


Figure 3.3. Majority rule tree for each paralogous copy of GBSSI-1 based on the alignment of exon and intron sequences. Copies A-D correspond to Groups A-D, respectively. Numbers above branches indicate majority rule support and those below are bootstrap support values.

A.



B.

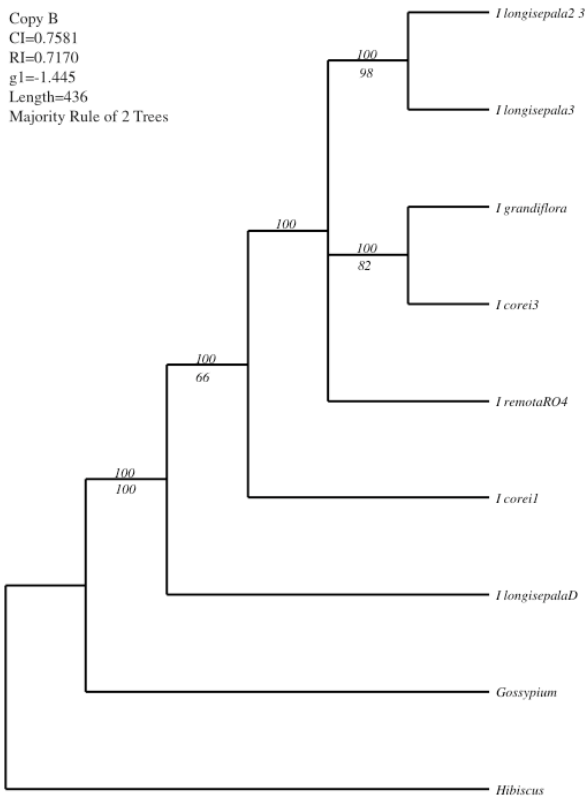
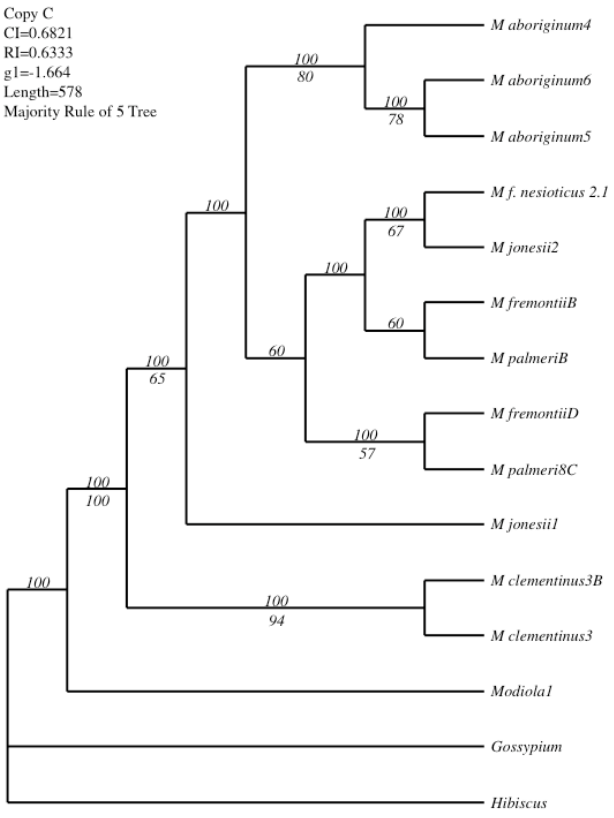
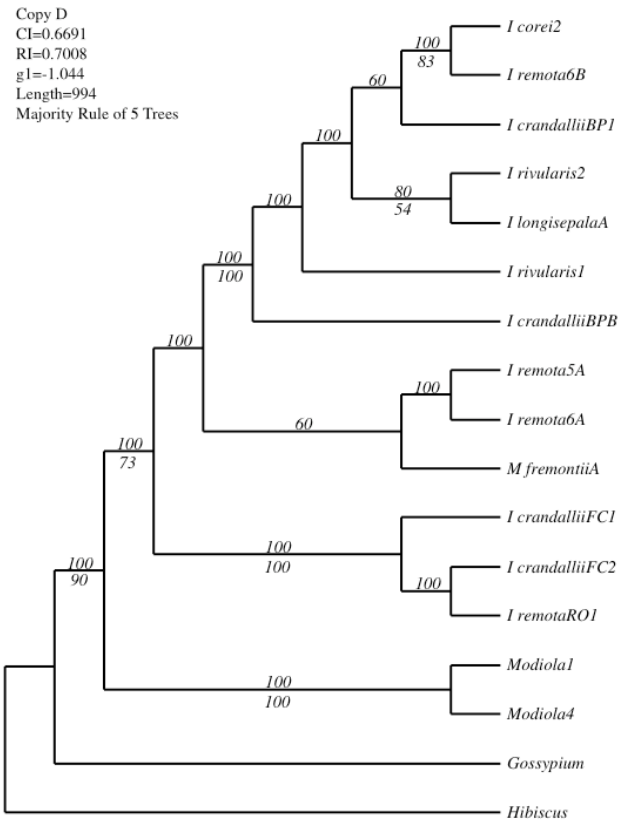


Figure 3.3, continued.

C.



D.



Chapter 4

Morphometric analysis of *Iliamna*: Implications for Taxonomic Revision

"We try to make sense of nonsense and put the world into some perspective which has order and harmony"
--Don Levin 1979

Abstract

Iliamna has a taxonomically complex history. Following its conception in 1906, the genus was not recognized for some time, and others placed species described by E.L. Greene in either *Sphaeralcea* or *Phymosia*. I. L. Wiggins revised *Iliamna* in 1936, to include seven species. The species in *Iliamna* are distributed in disjunct populations in temperate forests of North America. An analysis of morphological variation within *Iliamna* was conducted to determine appropriate specific and subspecific ranks of taxa. Morphometric analyses based on 26 characters from specimens representing the geographic range of the genus revealed significant differences between most of the species. Floral traits scored proved to be the most valuable in distinguishing between members of the genus in cluster and ordination analyses. However, distinction between two species in the southern Rocky Mountains was not obtained. A revised taxonomic key and descriptions for the seven distinguishable species in *Iliamna* is provided.

Introduction

Iliamna

The genus *Iliamna* Greene contains eight species distributed in disjunct populations throughout the contiguous United States and southern Canada (Wiggins 1936). *Iliamna* is a member of the *Malacothamnus* alliance in tribe Malveae of the Malvaceae along with *Malacothamnus* Greene and *Phymosia* Desv. ex Hamilton (Bates and Blanchard 1970). *Iliamna* is distinct from other genera in the *Malacothamnus* alliance in that members are perennial herbs emerging from a woody caudex and have carpels with single compartments that contain 2 to 3 seeds. Edward L. Greene (1906) described *Iliamna* briefly and included five species.

Ira L. Wiggins revised *Iliamna* in 1936 to include seven species: *I. bakeri*, *I. crandallii*, *I. grandiflora*, *I. longisepala*, *I. remota*, *I. rivularis*, and the newly-described *I. latibracteata*. Previous studies had included some of these species in *Malva*, *Phymosia*, or *Sphaeralcea* (Table 4.1). Wiggins (1936) based his separation of *Iliamna* from *Sphaeralcea* and *Phymosia* on several factors, including biogeography, morphology and anatomy of leaves, flowers, carpels, and seeds, and chromosome number. The North American species of *Sphaeralcea* St. Hilare range from northern Mexico to throughout the western United States, all in arid habitats. There are approximately 40 species of *Sphaeralcea* contained in 12 sections. *Iliamna*, in contrast, occurs in moist habitats along streams, in meadows, and on mountain hillsides. The eight species in *Phymosia* are small trees found in southern Mexico, the Bahaman Islands, Haiti, and Guatemala. Species in *Iliamna* often grow at elevations above 7000 feet in mountain habitats (see Figure 4.1). Furthermore, as Wiggins (1936) pointed out, the leaves of *Iliamna* are distinctly maple-like and are thinner and broader than those of *Sphaeralcea*, as well as less pubescent than those in *Phymosia*. Stipules in *Iliamna* are inconspicuous, as they are in *Sphaeralcea*, but they readily fall off, as they do in *Phymosia*. Wiggins further states that the

distinction of *Iliamna* from *Phymosia* is not well defined. Taxa in *Malacothamnus* are endemic to California and Baja California and Sonora, Mexico and have much smaller leaves and flowers than either *Iliamna* or *Phymosia* (Bates 1963).

Iliamna crandallii, *I. grandiflora*, *I. longisepala*, and *I. rivularis* are morphologically very similar and have overlapping geographical ranges (Weber 1987) (Fig. 4.1). Of these western species, *I. rivularis* has the widest distribution. *Iliamna rivularis* is described as ranging in height from 6-20 dm, having leaves with 5-7-lobes, the pedicel and calyx being covered with stellate trichomes, and petals being pink to white. This profile also describes *I. crandallii* and *I. grandiflora* (Harrington 1964, Weber 1987). A difference between *I. crandallii* and *I. rivularis* is found in the seeds; *I. crandallii* seeds are glabrous while *I. rivularis* seeds are sparsely puberulent with short trichomes (Wiggins 1936). *Iliamna grandiflora* has blunter leaves and larger flowers than does *I. rivularis*. In addition, differences have been noted within *I. grandiflora*; plants from Sierra Ancha, Arizona are more hirsute than plants from other populations (Rydberg 1932).

Iliamna corei and *I. remota* represent the easternmost distribution of the genus. *Iliamna remota* was first described in 1906 from specimens collected on the Kankakee River in eastern Illinois (Greene 1906). During the 1910s, *I. remota* was recognized as being in danger of extinction and seeds were collected by the Chicago Wildflower Society and reportedly dispersed along railways in Illinois, Indiana, and Virginia (Sherff 1949). The presence of *I. remota* in Virginia was not reported before this time. Earl Core first found *Iliamna corei* atop Peters Mountain in southwestern Virginia in 1932, although at that time, it was believed to represent a

disjunct population of *I. remota* (Sherff 1949). It was not until 1946 that Sherff named the Virginia plants as a different variety, *I. remota* var. *corei* (Sherff 1946). He later concluded that these two varieties were indeed separate species, citing floral scent, habitat, and leaf morphology among the differences (Sherff 1949).

The remaining species, *I. bakeri, I. latibracteata*, and *I. longisepala*, have not been questioned as to their status as distinct entities. *Iliamna latibracteata* is known historically from two disjunct populations, one in southern Oregon and another in northern California, and has rose-purple flowers in crowded racemes, with bracts being 10-14 mm in length, and seeds being minutely pubescent. *Iliamna bakeri* is found east of the *I. latibracteata* populations and has rose-purple flowers in axillary clusters, with bracts being 5-8 mm long, and with finely puberulent seeds. *Iliamna longisepala* is located in central Washington, overlapping with the northwestern distribution of *I. rivularis*. It has flowers in solitary or few-flowered axillary clusters, and has petals that are densely tomentose inside, a trait that has not been noted in other species. Bracts in *I. longisepala* are 5-10 mm long and sepals are 15-20 mm long, characters that distinguish this taxon from others in the genus.

Why is it so difficult to define plant species?

The biological species concept cannot often be applied to plant species. In this concept, a species is an entity that is geographically and reproductively isolated from other entities (Mayr 1942). In plants, however, complete reproductive isolation between closely- related species is rarely achieved (Stebbins 1982). Many plant species have poorly developed reproductive isolation mechanisms and pollination between species within a genus is frequent. Furthermore,

individuals within a species may be geographically isolated, yet could reproduce if populations were recombined.

The concept of a species among plants may be imaginary according to Levin (1979). We can consider a group of individuals that have an apparent morphological affinity and share a common gene pool that is independent from other similar entities to be unique units of evolution. Several factors promote speciation in plants. First, habitat shifts, the actions of seed carriers, plant migration, or colonization may lead to the introduction of individuals to novel habitats. In allopatric speciation, populations, or a group of populations, may diverge from a larger set of populations following isolation (Levin 2000). Isolation and subsequent speciation may be the result of physical separation from one or more larger populations (geographic speciation), divergence of smaller populations that were near the edge of a larger ancestral population (peripatric speciation), or divergence from long-distance disjunct populations. Adding to this, dramatic shifts in the size of populations can lead to the survival of individuals with unique characteristics (Levin 1979). Any specialized interactions with pollinators, substrate requirements, seed dispersal mechanisms, or other ecological variants could lead to speciation, with gradual shifts in phenotypes as well.

Conversely, populations may diverge without a geographical separation (sympatric speciation). A population may differentiate despite gene flow, or hybridization between two ancestral populations may lead to a new form. At the chromosomal level, polyploidy or autogamy can lead to novel forms that may become stable entities within a larger population. Applying a particular definition of a species may work well for one group but not another, even

within a genus (Levin 2000). In the present study, entities that are morphologically unique, as revealed by the analyses, will be considered as distinct species.

Caution must be exerted when using morphological characters in analyses assessing taxonomic delineation (Donoghue and Sanderson 1992). Traits may be subject to homoplasy and environmental variation. Numerous specimens from a number of habitats are often examined, and native populations are visited before deciding on appropriate characters to observe. Furthermore, statistical analyses such as discriminate functions, ANOVA (Analysis of Variance), principal coordinate analysis (PCoA), and multidimensional scaling (MDS) can be used to identify traits that express variation and not homoplasy.

The objectives of this study were to examine morphological variation in *Iliamna* in order to produce a revised taxonomic key and to identify morphologically unique entities in the genus. Numerous specimens representing the range of populations and habitats were examined to address speciation in *Iliamna*.

Methods

Analysis of morphological variation in *Iliamna* was based on measurements of 319 herbarium specimens. A total of 26 vegetative and floral characters were measured (Table 4.2). Eight characters were scored as qualitative and placed into designated categories as described in Table 4.2. Not all characters could be scored for all herbarium specimens. In examining specimens of *I. rivularis*, only specimens that had all floral and vegetative organs present were included in order to provide the greatest information per specimen. All available intact

specimens were included for the remaining taxa since they have not been collected as frequently as *I. rivularis*.

Quantitative morphological data were standardized by log transformation prior to multivariate analysis in order to remove character weighting (Timmerman-Erskine et al. 2002). Burnaby's (1966) method was used to reduce the effect of size in the data matrix. Burnaby's method eliminates factors related to age of the plants among samples and is a way to transform data that are not normally distributed prior to multivariate analyses by computing ratios of characters and computing the log of other characters. A one-way analysis of variance could not be performed on the data set since several assumptions were violated. First, random sampling of collections was not assured. Specimens replicating populations were included if the specimen was collected by different individuals or in different years or placed in different herbaria; since the species are perennials, the same individual may be included more than once without knowledge of the researcher. Secondly, the characters scored may not be independent. The six characters qualifying the density of pubescence may not be independent of the size of the structure (e.g., leaf or sepal). Third, the groups (e.g., species) did not have equal variance. This is exemplified in comparison of *I. rivularis* with any of the other species. Numerous specimens (154) of *I. rivularis* were scored versus 165 collections of the remaining nine taxa identified by the herbarium labels (Table 4.3). *Iliamna rivularis* has the largest distribution in the genus and is reported to exhibit the greatest variation (Wiggins 1936). Furthermore, values for characters within a species were not normally distributed since a variety of age classes and populations were sampled per species.

To resolve these problems while analyzing the significance of variance within characters, the Kruskal-Wallis test was implemented as the Wilcoxon npar-1way test (SAS Institute, Inc. 1999). The Kruskal-Wallis test is a non-parametric test that relies on the relative rank of the observations across all groups in assessing significance. Characters with significant variation ($P<0.05$) were then used in multivariate and cluster analyses. The %distance macro was implemented using the D-Gower algorithm in order to compute a distance matrix using qualitative, categorical, and ratio variables (Kuo 1997, SAS Institute, Inc. 1999). The algorithm calculates dissimilarity between two specimens by subtracting the sum of the similarity of all variables in the pairwise comparison from one. If either specimen does not have a value for a variable, the similarity for that variable is 0. If both specimens have a value for a variable, then the similarity is calculated as one minus the absolute value of difference between specimen 1 and specimen 2.

The dissimilarity matrix was implemented in PAUP 4.0b10 (Swofford 2002) to generate a phenogram by the unweighted pair-group method using arithmetic averages (UPGMA). The resulting phenogram is used to identify discrete taxonomic groups (Rohlf 1993). Multidimensional scaling results were generated for this study using the SAS software, Version 8 of the SAS System for PC (SAS Institute Inc. 1999). Multidimensional scaling (MDS) was chosen for ordination analysis since the algorithm does not exclude variables or specimens with missing data. All variables were used in the calculations of the MDS analysis.

Results

The Kruskal-Wallis test indicated that all but four characters had significant variation (Table 4.4). The maximum, minimum, mean, and standard deviation of variables within taxa was recorded (Table 4.5). The multidimensional scaling analysis retained all 319 specimens. The vectors produced in the MDS calculations were plotted in Excel (Figure 4.2). Most individuals were closely clustered in the plot of the first two dimensions. In the plot of all specimens (Fig. 4.2), points representing *I. rivularis* dominated and loosely clustered. When data from the *I. rivularis* specimen were omitted from the plot (Fig. 4.3), individual clusters were inferred for *I. bakeri*, *I. corei*, *I. latibracteata*, *I. longisepala*, and *I. remota*. Two taxa, *I. crandallii* and *I. grandiflora*, clustered as a single group and not as two separate clusters based on herbarium identifications.

When all vegetative and floral characters were included, except those found to have insignificant variation, the resulting UPGMA phylogram did not result in species-restricted clusters. Upon examination of the maximum, minimum, mean, and standard deviation of variables for each species, overlapping values for most vegetative characters were observed. Since vegetative characters are often a reflection of environmental features and are often found to be homoplasious (Donoghue and Sanderson 1992), these were removed from the cluster analysis. Distance values were calculated for 245 specimens using sepal pubescence, length, and width, pedicel length, bract length and width, and petal length. If the remaining floral features that were scored (staminal column length, petal margin and base width, and features of fruits and seeds) were included in the analysis, numerous specimens would have been omitted from the analysis because of missing values (see Table 4.4 for the number of taxa scored per variable).

The lower triangular distance matrix produced from the analysis of floral characters was implemented in PAUP4.0b10 for phenogram generation using UPGMA. The resulting phenogram (Fig. 4.4) placed the majority of *I. rivularis* specimens as a group (Cluster I). Distinct clusters also resulted for *I. latibracteata* (Cluster IV) and *I. longisepala* (Cluster V), including their respective type specimen. One cluster (Cluster III) consisted primarily of specimens of *I. bakeri* and *I. corei*. Only one representative of *I. crandallii*, three representatives of *I. grandiflora*, and ten of *I. rivularis* were present in Cluster III, which includes 56 specimens. Representatives of *I. crandallii*, *I. grandiflora*, *I. remota*, and *I. rivularis* dominated the final cluster (Cluster II). Specimens of *I. grandiflora* and *I. rivularis* from Utah (5 and 9, respectively) dominated Cluster IIb. Cluster IIa is comprised of *I. crandallii* (9 specimens), *I. grandiflora* (8), *I. remota* (9), and *I. rivularis* (18) and included the type for *I. grandiflora*.

Discussion and Conclusions

Populations in *Iliamna* differ more in floral morphology than in morphology of vegetative structures. Although pubescence of stems, leaves, and seeds has been used to distinguish species in this genus (Wiggins 1936), these characters did not prove valuable in distinguishing taxa in the cluster analysis here. The length of the terminal leaf lobe also has been indicated as a distinguishing feature in comparing *I. corei* to *I. remota* (Swinehart and Jacobs 1998). However, the ratio of the terminal leaf lobe length to overall leaf length did not have significant variation ($P=0.0254$, $\square=0.01$) among all specimens (Table 4.4) even though each character was significant individually.

The smallest-flowering member of the genus was found to be *I. rivularis*, which also has the smallest bracts when mean values are examined. This is consistent with prior reports of the species as being small-flowered (Harrington 1964, Weber 1987). The leaves of *I. rivularis* can become quite large (up to 18 cm wide and 17 cm long), a reflection of the forest understory habitat it occupies. In both the MDS and cluster analysis, the majority of *I. rivularis* specimens grouped together. In the cluster analysis, 79 specimens of *I. rivularis* are segregated from the remaining representatives of *Iliamna* (Cluster I, Fig. 4.4). The species can be quite variable morphologically (Slotta 2000), as is indicated by the positions of other specimens of *I. rivularis* integrated into other clusters in the UPGMA phylogram and in the MDS plot. Despite its wide geographic range, the morphology of *I. rivularis* is unique within the genus. Our findings show that *I. rivularis* is unlike *I. crandallii* and *I. grandiflora*, contrary to previous reports (Rydberg 1932, Harrington 1964). *Iliamna acerifolia* was described in synonymy with *I. rivularis* by Wiggins (1936). The variety of specimens examined that were labeled as *I. acerifolia* do not cluster together in either analysis and are often in groups with *I. bakeri* and *I. rivularis*. This study shows that the epithet does not warrant recognition.

Iliamna latibracteata is easily distinguished, with its large leaves and large broad bracts in the inflorescence. Currently, the distribution of *I. latibracteata* does not overlap with any other species in *Iliamna*. Its closest neighbor, *I. bakeri*, is found in the adjacent cluster in the UPGMA phylogram, but not in the MDS plot. The size range of characters measured in *I. latibracteata* is consistent with the description by Wiggins (1936). The recognition of *I. latibracteata* is upheld, as it is a unique entity that occurs in populations isolated from other taxa in *Iliamna*.

The remaining members of the Rocky Mountain region, *I. crandallii*, *I. grandiflora*, and *I. longisepala*, are distinct from *I. rivularis* to a limited extent. First, *I. longisepala*, with its long narrow sepals and long pedicels, is morphologically distinct. The species forms a loose group in the MDS analysis outside of all the other species. In the UPGMA phylogram, specimens, including the holotype, are in a cluster closest to *I. latibracteata*. The recognition of *I. longisepala* as a unique entity is upheld in both the multivariate and cluster analyses presented here.

Specimens of *I. crandallii*, *I. grandiflora*, and *I. remota* cluster together in the UPGMA analysis. In the MDS analysis, two close clusters indicate the morphological similarity of *I. crandallii* and *I. grandiflora*. However, in the lower portion of the mixed cluster (Fig. 4.4 cluster II. b.), the majority of specimens represent *I. grandiflora* and *I. rivularis* from Utah and Arizona; no specimens of *I. remota* are in this cluster. Plants in the Sierra Ancha region of Arizona have been noted to be different from more northern populations of *I. grandiflora* (Rydberg 1932). The type specimen for *I. grandiflora* is not found in this group (Fig. 4.4 cluster II. b.), however. The holotype for *I. grandiflora* is in the upper group (Fig. 4.4, cluster II. a.) of the larger mixed cluster along with the types for *I. crandallii* and *I. bakeri*. *Iliamna crandallii* and *I. grandiflora* can be distinguished from one another by flower size (larger in *I. grandiflora*) and pubescence (less in *I. crandallii*). The finding here that these two species are not easily delimited supports Harrington's (1964) view. It is proposed here that these two species are actually two geographic subspecies of a single species. Given their distribution and population ranges, the populations may be undergoing peripatric speciation and adapting to the habitats in which they occur.

The morphology of *I. remota* is highly variable with little distinction between it and other species in the genus. The morphological variation in *I. remota* is a reflection of the wide range of habitats in which it occurs. The type location is along the Kankakee River in northern Illinois in an open flood plain on Altorf Island (Swinehart and Jacobs 1998). In Indiana, it has been found in abandoned fields and along railways. In Virginia, *I. remota* is known from populations along roadsides, railways, and the James River (Stewart and Porter 1995). The Virginia populations have been shown to be genetically distinct from the Illinois/Indiana populations (see chapters 2 and 6). Morphologically, *I. remota* resembles *I. corei* and *I. grandiflora*, with large flowers and fruits, but is unlike them in having less pubescent stems, leaves and sepals. In summary, *I. remota* is an intermediate form that has diversified and is similar to other phenotypes found in *Iliamna*.

The species with the smallest leaves is *I. bakeri*, which occurs in dry lava ash soils in northern California (Slotta 2000). Individuals sampled here for *I. bakeri* also presented the densest pubescence of fruits, sepals, and leaves among the material examined. Such a modification is consistent with dry habitats as a means of reflecting light and retaining water vapor. In both the MDS and UPGMA plots, *I. bakeri* and *I. corei* were closely linked. In the UPGMA phenogram, specimens for the two species are in one large cluster. Given that these two species represent the westernmost and easternmost range limits in the distribution of the genus, their morphological similarity and their position in the MDS and UPGMA plots was unexpected. A molecular analysis of multiple regions did not show these two species to be closely related (see Chapter 2). The larger flower size and pubescence of surfaces expressed in

these two taxa are convergent adaptations to widely different habitats. *Iliamna corei* occurs as an understory plant on a sandstone outcrop in southwest Virginia, a habitat very unlike that of *I. bakeri*. However, *I. bakeri* and *I. corei* are distinct from the remaining species in the genus and from one another and, therefore, should be retained as distinct species.

The results of this survey of morphological variation in *Iliamna* support findings from molecular assessments that taxonomic revision in *Iliamna* is necessary (see Chapter 2 as well). The following revisions are proposed. First, *I. crandallii* and *I. grandiflora* are not separate species, but two subspecies that have adapted to ecological variation. Since Rydberg (1904) published both names at the same time, *I. grandiflora* will be retained as the species epithet as it has a larger distribution than *I. crandallii*. *Iliamna grandiflora* subsp. *crandallii* includes those populations in moist woods of New Mexico and Colorado with less pubescent stems and leaves and smaller flowers, as is illustrated by the type description of *I. crandallii* (Rydberg 1904). *Iliamna grandiflora* subsp. *grandiflora* includes those populations in more arid habitats, which have larger flowers and an overall increased density of pubescence.

Secondly, the epithets *I. acerifolia* and *I. angulata* do not represent unique morphological entities. As Wiggins (1936) proposed, these are in synonymy with *I. rivularis* and *I. grandiflora* (now *I. grandiflora* subsp. *grandiflora*). Lastly, the remaining species in *Iliamna* (*I. bakeri*, *I. corei*, *I. latibracteata*, *I. longisepala*, *I. remota*, and *I. rivularis*) are accepted as currently circumscribed.

Taxonomic Treatment

Iliamna Greene *Leaflets of Botanical Observation and Criticism* 1: 206. 1906

Type Species: *Malva rivularis* Douglas (*Flora boreali-americana* 2: 107. 1830.) [*Iliamna rivularis* (Douglas) Greene].

Lectotype designated by I.L. Wiggins (*Contributions from the Dudley Herbarium* 1: 228, 1936): Royal Botanical Gardens, Kew: D. Douglas, s.n. "On the banks in North-West America, from the ocean to the Rocky Mountains. Fl. June-August."

Stems erect, 0.6 m to 2 m long, emerging from woody rootstalk, pubescence stellate. Leaves palmately 3- to 7-lobed, sparsely to densely pubescent, cuneate, truncate, or cordate at base. Stipules lanceolate, not persistent. Inflorescence resembling an interrupted spike or corymbose raceme to solitary axilary flowers. Flowers 2 cm to 6 cm in diameter with white to lavendar-pink petals. Stamens numerous in compact ball. Fruit a schizocarp with 10 to 15 segments (mericarps), generally larger than calyx; segments with bristly and stellate pubescence along margin, side walls thin and smooth. Seeds 2 to 4 per mericarp, reniform in shape, glabrous to puberulent. Seven species in North America.

Taxonomic Key

1 Leaves shallowly 3-lobed; stipules persistent; petioles stout, 2-5 cm long. *I. bakeri*
1 Leaves 5- to 7-lobed; stipules deciduous (rarely persistent); petioles slender, >5 cm long. 2

2 Sepals ovate; seeds puberulent. 3

3 Sepals as wide as long, or nearly so; plants sparingly branched. 4

4 Bracts <1 cm long, half as long as sepals. *I. rivularis*
4 Bracts to 1 cm long, 2/3 as long or longer than sepals. 5

5 Bracts <1 cm wide; lobes of leaves broadest at base, leaf sinuses shallow to moderate, pubescence equal above and beneath. *I. grandiflora* subsp. *grandiflora*
5 Bracts nearly 1 cm wide or wider; lobes of leaves broadest at middle, leaf sinuses acute, pubescence greater beneath. *I. latibracteata*

3 Sepals longer than wide; plants profusely branched. 6

6 Leaf sinuses shallow; leaf teeth rounded to mucronate; sepals hirsute to tomentose. *I. remota*
6 Leaf sinuses acute; leaf teeth cuspidate; sepals pilose. *I. corei*

2 Sepals nearly lanceolate; seeds glabrous or nearly so. 7

7 Petals white to pale pink; petioles 1/2 length of leaf blades; sepals 1/3 to 1/2 length of petals; bracts shorter than sepals. *I. grandiflora* subsp. *crandallii*
7 Petals deep rose-purple; petioles nearly as long as leaf blades; sepals 1/2 length of petals; bracts longer than sepals. *I. longisepala*

***Iliamna bakeri* (Jepson) Wiggins**. *Contributions from the Dudley Herbarium* 1: 228, 1936.

Basionym: *Sphaeralcea bakeri* Jepson. *A Manual of the Flowering Plants of California* p. 635, 1925. Holotype: Fall River Valley, Shasta County, California. M.S. Baker 116, University of California.

Stems puberulent, 0.3-0.7 m long. Leaves shallowly 3- to 5-lobed, 1.5-4.5 cm long, 2-5 cm wide, puberulent. Leaf base tapered to truncate. Petioles 2-5 cm long. Leaf margin mucronate to crenate. Inflorescence of axillary clusters of flowers. Calyx 9-12 mm long, 1-2 mm wide, puberulent. Pedicels 5 mm long. Bracts 5-8 mm long. Flowers rose-purple, 3-6 cm in diameter. Seeds 3-4 per carpel, finely pubescent. Mountain slopes, juniper woodlands, lava beds. Southern Klamath Co., OR and Shasta Co., CA. 1000-2500 m.

***Iliamna corei* (Sherff) Sherff**. *American Journal of Botany* 36: 502, 1949.

Basionym: *Iliamna remota* var. *corei* Sherff. *Rhodora* 48: 96, 1946. Holotype: Giles County, Virginia. McNeill and Sherff, Oct. 5, 1945, Gray Herbarium.

Stems hirsute, 1 m long. Leaves deeply 5- to 7-lobed, 8-15 cm long, 10-15 cm wide, puberulent. Leaf base cordate-orbicular. Petioles 1-3 cm long. Leaf margin serrated. Inflorescence a raceme. Calyx 10-15 mm long, 5-10 mm wide, tomentose. Pedicels 5 mm long. Bracts <1 mm long. Unscented lavendar-pink flowers, 4-6 cm in diameter. Seeds 2-3 per carpel, glabrous. Restricted to Peters Mountain, Giles Co., VA along ridge in sandstone outcrops. 1500 m.

Iliamna grandiflora* (Rydberg) Wiggins subsp. *grandiflora

Basionym: *Sphaeralcea grandiflora* Rydberg. *Bulletin of the Torrey Botanical Club* 31: 564, 1904. Holotype: Mesa Verde, Colorado. Vreeland 882, New York Botanical Garden.

Synonyms: *Iliamna angulata* Greene. *Leaflets of Botanical Observations and Criticisms* 1: 206, 1906.

Phymosia grandiflora (Rydberg) Rydberg. *Bulletin of the Torrey Botanical Club* 40:60, 1913.

Sphaeralcea rydbergii Tidestrom, *Contributions from the United States National Herbarium* 25: 256, 1925.

Iliamna grandiflora (Rydberg) Wiggins. *Contributions from the Dudley Herbarium* 1: 223, 1936.

Stems sparingly stellate-pubescent, 1-2 m long. Leaves deeply 5- to 7-lobed, 3-5 cm long, 5-8 cm wide, pubescence sparse. Leaf base truncate to cordate. Petioles 5-10 cm long. Leaf margin with broadly triangular serration. Inflorescence a crowded raceme. Calyx 10 mm long, 5 mm wide, densely hirsute. Pedicels to 3-5 cm long. Bracts 2/3 length of calyx. Flowers pale pink, 4-8 cm in diameter. Seeds 2-4 per carpel, puberulent. Found along mountains of AZ, CO, NM, and UT, the Colorado River drainage basin. 2000 to 3000 m.

***Iliamna grandiflora* subsp. *crandallii* (Rydberg) Wiggins**, *comb. nov.*

Basionym: *Sphaeralcea crandallii* Rydberg. *Bulletin of the Torrey Botanical Club* 31: 564, 1904. Holotype: Steamboat Springs, Routte County, Colorado. C.S. Crandall 97 (July 14, 1894), New York Botanical Garden.

Synonyms: *Phymosia crandallii* (Rydberg) Rydberg. *Bulletin of the Torrey Botanical Club* 40:60, 1913.

Iliamna crandallii (Rydberg) Wiggins. *Contributions from the Dudley Herbarium* 1: 228, 1936.

Stems sparingly stellate-pubescent, 0.5 m long. Leaves deeply 5- to 7-lobed, 2-5 cm long, 5-10 cm wide, sparingly stellate. Leaf base cordate. Petioles 1-3 cm long. Leaf margin coarsely serrated. Inflorescence a raceme. Calyx 10 mm long, 5 mm wide, sparingly to finely puberulent. Pedicels to 1-3 cm long. Bracts as long as calyx. Flowers pale pink fading to white, 4-6 cm in diameter. Seeds 2-4 per carpel, glabrous. Restricted to the vicinity of Steamboat Springs, CO. 2000m

***Iliamna latibracteata* Wiggins.** *Contributions from the Dudley Herbarium* 1: 225, 1936.

Holotype: Prairie Creek, near Davidsonís, Humboldt County, California. Kildare 2,313 (July 5, 1926), Dudley Herbarium.

Stems sparingly stellate pubescent, 1-2 m long. Leaves deeply 5- to 7-lobed, 10-15 cm long, 10-15 cm wide, pubescence fine. Leaf base truncate to cordate. Petioles 5-14 cm long. Leaf margin broadly serrated. Inflorescence a crowded raceme. Calyx 8-10 mm long, 5 mm wide, hirsute. Pedicels <5 cm. Bracts 10-14 mm long. Flowers rose-purple, 4-6 cm in diameter. Seeds 2-4 per carpel, finely puberulent. Conifer forests, streamsides in Coos Co., OR and Humboldt Co., CA. 500-2000 m.

***Iliamna longisepala* (Torrey in Wilkes) Wiggins.** *Contributions from the Dudley Herbarium* 1: 227, 1936.

Basionym: *Sphaeralcea longisepala* Torrey in Wilkes. *United States Exploring Expedition* 17: 255, 1874. Holotype: Upper Columbia, Washington Territory. Upper Columbia Wilkes Expedition 883, New York Botanical Garden.

Synonym: *Phymosia longisepala* (Torrey in Wilkes) Rydberg. *Bulletin of the Torrey Botanical Club* 40: 61, 1913.

Stems sparingly stellate-pubescent, 1.2-2 m long. Leaves deeply 5- to 7-lobed, 5-8 cm long, 5-10 cm wide, pubescence sparse. Leaf base cordate-orbicular. Petioles half as long as leave blades. Leaf margin coarsely serrated. Inflorescence of few-flowered axilary clusters. Calyx 15-20 mm long, <5 mm wide, sparsely pubescent. Pedicels 2.5 cm long. Bracts 8-10 mm long. Flowers pink to lavendar, 2-4 cm in diameter. Seeds 3 per carpel, pubescence unknown. Stream banks and mountain slopes in Chelan, Douglas, and Kittitas Co., WA.

***Iliamna remota* Greene**. *Leaflets of Botanical Observation and Criticism* 1: 206. 1906.

Holotype: Altorf Island, Kankakee County, Illinois. Greenman 3,530, Gray Herbarium.

Synonyms: *Sphaeralcea remota* (Greene) Fernald. Rhodora 10: 52, 1908.
Phymosia remota (Greene) Britton and Brown. *Flora of Northern United States and Canada*, 2nd ed. 2: 522. 1913.

Stems densely stellate-pubescent, 2 m long. Leaves shallowly 5- to 7-lobed, 15-20 cm long and about as wide, pubescence dense. Leaf base cordate. Petioles 5-10 cm long. Leaf margin mucronate to rounded. Inflorescence of axilary clusters. Calyx 10-15 mm long, 5 mm wide, hirsute. Pedicels 1-3 cm long. Bracts half as long as calyx. Fragrant flowers rose-purple, 4-6 cm in diameter. Seeds 2-3 per carpel, sparsely puberulent. Kankakee River, Altoff, IL; Goshen Co., IN; and in the valley of the James River in Alleghany Co., Bedford Co. and Botetourt Co., VA.

***Iliamna rivularis* (Douglas ex Hooker) Wiggins.** *Contributions from the Dudley Herbarium* 1: 228, 1936.

Basionym: Malva *rivularis* Douglas ex Hooker. *Flora boreali-americana* 2: 107. 1830. Lectotype: Designated by I.L. Wiggins (*Contributions from the Dudley Herbarium* 1: 228, 1936): Royal Botanical Gardens, Kew: D. Douglas, s.n. “On the banks in North-West America, from the ocean to the Rocky Mountains. Fl. June-August.”

Synonyms: *Sphaeralcea acerifolia* Nuttall ex Torrey and Gray. *Flora of North America* 1: 228. 1838.

Sphaeralcea rivularis (Douglas ex Hooker) Torrey ex Gray. *Memoirs of the American Academy of Art and Science* 4:23. 1849.

Iliamna rivularis (Douglas ex Hooker) Greene. *Leaflets of Botanical Observation and Criticism* 1: 206. 1906.

Iliamna acerifolia (Nuttall ex Torrey and Gray) Greene. *Leaflets of Botanical Observation and Criticism* 1: 206. 1906.

Phymosia acerifolia (Nuttall ex Torrey and Gray) Rydberg. *Bulletin of the Torrey Botanical Club* 40: 61, 1913.

Phymosia rivularis (Douglas ex Hooker) Rydberg. *Bulletin of the Torrey Botanical Club* 40: 61, 1913.

Stems sparingly stellate-pubescent, 0.5-2 m long. Leaves shallowly to deeply 5- to 7-lobed, 5-15 cm long and about as wide, pubescence sparse. Leaf base cordate to reniform. Petioles 1-5 cm long. Leaf margin coarsely serrated. Inflorescence racemose to paniculate. Calyx 5-8 mm long, 3-5 mm wide, hirsute. Pedicels 1.5-4 cm long. Bracts half as long as calyx. Fragrant flowers rose-purple to light pink to white, 3-8 cm in diameter. Seeds 2-4 per carpel, sparsely puberulent. Found along mountain streams from British Columbia east to western South Dakota, west to eastern Washington, and south to Arizona, Colorado, and New Mexico.

Literature Cited

Bates, D.M. 1963. *The Genus Malacothamnus*. Ph.D. Dissertation, University of California, Los Angeles.

Bates, D.M. and O.J. Blanchard, Jr. 1970. Chromosome numbers in the Malvales II: New or otherwise noteworthy counts relevant to classification in the Malvaceae tribe Malveae. *American Journal of Botany* 57: 927-934.

Burnaby, T.P. 1966. Growth-invariant discriminant functions and generalized distances. *Biometrics* 22: 96-110.

Donoghue, M.J. and M.J. Sanderson. 1992. The suitability of molecular and morphological evidence in reconstructing plant phylogeny. In *Molecular Systematics in Plants*, P.S. Soltis, D.E. Soltis, and J.J. Doyle (eds.). New York: Chapman and Hall. Pp. 340-368.

Greene, E. L. 1906. Certain malvaceous types. *Leaflets of Botanical Observation and Criticism*. Pp. 205-209.

Harrington, H. D. 1964. *Manual of the plants of Colorado*. Chicago: Swallow Press. P. 373.

Kuo, A. 1997. The Distance Macro: Preliminary Documentation, 2nd ed. Cary, NC: SAS Institute Inc.

Levin, D. 1979. Nature of plant species. *Science* 204: 381-384.

Levin, D. 2000. *The Origin, Expansion, and Demise of Plant Species*. New York: Oxford University Press.

Mayr, E. 1942. *Systematics and the Origin of Species*. New York: Columbia University Press.

Rohlf, F.J. 1993. Multidimensional paleobiology. *Bulletin of Mathematical Biology* 55: 491-493.

Rydberg, P. A. 1904. Rocky Mountain Flora. *Bulletin of the Torrey Botanical Club* 31: 564-565.

Rydberg, P.A. 1932. *Flora of the Prairies and Plains of Central North America*. New York: New York Botanical Garden. P. 541.

SAS Institute, Inc. 1999. SAS software, Version 8 of the SAS System for PC. Copyright 1999 SAS Institute Inc. Cary, NC.

Sherff, E.E. 1946. Notes on certain plants in the Gray's Manual Range. *Rhodora* 48: 89-96.

Sherff, E.E. 1949. Further notes on *Iliamna*. *American Journal of Botany* 36: 502-503.

Slotta, T.A.B. 2000. Phylogenetic analysis of *Iliamna* (Malvaceae) using the internal transcribed spacer. M.S. Thesis. Virginia Polytechnic Institute and State University.

Stebbins, G. L. 1982. Plant Speciation. In *Mechanisms of Speciation: Proceedings from the International Meeting on Mechanisms of Speciation*, C. Barigozzi, ed. New York: Alan. R. Liss, Inc. Pp. 21-39.

Stewart, C.N. and D.M. Porter. 1995. RAPD Profiling in Biological Conservation: An Application to estimating Clonal Variation in Rare and Endangered *Iliamna* in Virginia. *Biological Conservation* 74: 135-142.

Swinehart, A. L. and M. E. Jacobs. 1998. Rediscovery, status and preservation of the endangered Kankakee Globe Mallow (*Iliamna remota*) in Indiana. *Rhodora* 100: 82-87.

Timmerman-Erskine, M., R.R. Dute, and R.S. Boyd. 2002. Morphometric analysis of the *Trillium pusillim* Michaux complex (Trilliaceae) of the Southeastern United States. *Castanea* 67: 109-119.

Weber, W.A. 1987. *Colorado Flora: Western Slope*. Boulder: Colorado Associated University Press. Pp. 330-331.

Wiggins, I.L. 1936. A resurrection and revision of the genus *Iliamna* Greene. *Contributions from the Dudley Herbarium, Stanford University* 1: 213-229.

Table 4.1. Species in *Iliamna* with authors and synonyms listed.

Taxa	Authority	Synonyms
Iliamna bakeri	(Jepson) Wiggins	*Sphaeralcea bakeri* Jepson
Iliamna crandallii	(Rydberg) Wiggins	*Phymosia crandallii* Rydb., *Sphaeralcea crandallii* Rydb.
Iliamna corei	(Sherff) Sherff	*Iliamna remota var. coreii Sherff*
Iliamna grandiflora	(Rydberg) Wiggins	*Phymosia grandiflora* Rydb., *Sphaeralcea grandiflora* Rydb., *Sphaeralcea rydbergii* Tidestrom, *Iliamna angulata* Greene
Iliamna latibracteata	Wiggins	none
Iliamna longisepala	(Torrey) Wiggins	*Phymosia longisepala* Rydb., *Sphaeralcea longisepala* Torr.
Iliamna remota	(Greene) Wiggins	*Phymosia remota* Britton, *Sphaeralcea remota* Fernald, *Iliamna remota* Greene
Iliamna rivularis	(Douglas ex Hooker) Wiggins	*Iliamna acerifolia* Greene, *Malva rivularis* Dougl. *ex* Hooker, *Phymosia rivularis* Rydb., *Phymosia acerifolia* Rydb., *Sphaeralcea acerifolia* Torr. & Gray, *Sphaeralcea rivularis* Torr. in Gray

Table 4.2. Characters scored in *Iliamna*.

	Character	Categories
Pubescence	1. Leaves (upper versus lower surface) 2. Stem Surfaces 3. Sepals 4. Dorsal Edge of Fruit 5. Seeds	1=Glabrous (no trichomes), 2=puberulent (arms do not touch), 3=moderate (arms touch), 4=hirsute (arms overlap some), 5=tomentose (leaf surface obstructed by trichomes), 6=pilose (long arms of trichomes overlap, stalks of trichomes abut)
Leaf Shape Interpretation	Leaf Serration	1=rounded, 2=mucronate, 3=cuspidate, 4=acute, 5=acuminate
	Shape of Leaf Base	1=cuneate (reflexed upward, wedge-shaped), 2=cordate (downward lobes, heart-shaped), 3=truncate (flat base, nearly straight), 4=auriculate (reflexed downward)
Other Qualitative	Stipules	1=present, 0=absent
Additional Vegetative Characters	Petiole Length, Leaf Length and Width, Length of Terminal Leaf Lobe	Quantitative Measurements
Additional Floral Characters	Pedicel Length, Sepal Length and Width, Bract Length and Width, Petal Length, Width of Petal Base and Margin, Length of Staminal Column, Length of Fruits, and Length of Seeds	Quantitative Measurements

Table 4.3. Specimens examined and included in the present morphological study. Citations in bold typeface are **type** specimens (lectotypes, isotypes, isolectotypes); underline and bold are **holotypes**.

***Iliamna acerifolia* (Synonym of *I. rivularis*)**
Colorado: A. Eastwood Aug. 1887 (COLO 28,105); G.E. Osterhout Aug. 23, 1899 (RM 16,955)
Idaho: L.F. Hendersen July 29, 1899 (DS 118,744); J.H. Sandberg 562 (CAS 52,332);
Oregon: **Isotype: T. Nuttall 1866(?) (GH 52,865);** *Other Specimen Examined*: L.F. Henderson June 12, 1886 (DS 117,454)
Utah: D.C. Eaton 59 (CAS 52,329)
Washington: I.C. Otis 588 (CAS 52,330; S.P. Sharples 147 (CAS 52,334)

***Iliamna angulata* (Synonym of *I. grandiflora* subsp. *grandiflora*)**
Colorado: **Holotype: A. Eastwood Aug. 1887 (US 12,502)**

Iliamna bakeri
California **Isotypes: M.S. Baker 116 (US 669,633); M.S. Baker Aug. 17, 1899 (MO 140,890); M.S. Baker Aug. 17, 1899 (COLO 280,147);** *Other Specimens Examined*: E.I. Applegate 3644 (CAS 508,053); M.S. Baker 554 (JEPS 67,375); E.K. Balls 20,958 (US 1,075,791); L. Constance 15,337 (DS 277,250); L. Constance 15,337 (COLO 28,106); W.B. Cooke 15,193 (JEPS 643,276); W.B.Cooke 16,264 (MO 1,212,550); W.B. Cooke 15,337 (US 643,277); R.K. Frenkel 667 (CAS 508,053); V. and A. Grant 8,019 (JEPS 932,081); A.A. Heller 15,253 (DS 256,626); A.A. Heller 15,253 (MO 1,162,033); A.A. Heller 15,253 (US 1,974,292); P.C. Hutchison 920 (JEPS 28,129); P.C. Hutchison 2,705 (JEPS 48,093); A.H. Kramer summer 1937 (CAS 247,380); W.C. McCalla 6365 (DS 290,971); A.L. and H.N. Moldenke 30,259 (CAS 619,693); M.A. Showers 1950 (CAS 662,390); M.A. Showers 1909a (CAS 662,391); T. Slotta 99-10A (VPI); T. Slotta 99-10B (VPI); T. Slotta 99-10C (VPI); T. Slotta 99-10D (VPI); D.W. Taylor 9,244 (JEPS 1,576,732); D.W. Taylor 10,044 (JEPS 1,561,017); D.W. Taylor 10,034 (CAS 781,539); D.W. Taylor 9244 (CAS 781,519); L.J. Uttall 11433 (VPI 62144); C.B. Wolf 6029 (CAS 298,433); C.B. Wolf 4330 (DS 343,793); C.B. Wolf 4330 (US 774,467); C.B. Wolf 6029 (US 1,630,028).

Iliamna corei
Virginia: **Holotype: E. Meade McNeill and E.E. Sherff Oct. 5, 1945 (GH 52,866); Isotypes: E. Meade McNeill and E.E. Sherff 1 Oct. 5, 1945 (DS 348,699); E. Meade McNeill and E.E. Sherff Oct. 5, 1945 (US 2,006,865); E. Meade McNeill and E.E. Sherff 1 Oct. 5, 1945 (DS 351,737);** *Other specimens examined:* Biol. Sta. Party Aug. 13, 1934, (VPI 20,869); E. Core Aug. 8, 1927 (MO 938,141); E. Core and E. M. Fling Roush Aug. 8, 1927 (MO 938,140); S.Q. Croy, D.M. Porter July 31, 1980, (VPI 68,100); J.M. Fogg, Jr. 15,045 (MO 1,182,015); G.P. Frank (VPI 443), A.B. Massey Aug. 12, 1934, (VPI 20,868); A.B. Massey July 7, 1935, (VPI 20,866); A.B. Massey Aug. 12, 1939, (VPI 20,870); P.A. Munz 13,505 (DS 239,900); D.P. Rogers Sept. 5, 1963 (COLO 191,498); A.J. Sharp June 29, 1940 (MO 1,258,374 (E.E. Sherff Topotype)); A.J. Sharp June 29, 1940 (MO 1,199,356 (E.E. Sherff Topotype)); T.F. Wieboldt 6761 (VPI 81,780).

Iliamna crandallii

Colorado: **Holotype: Crandall 97 (NY 222,081);** *Other specimens examined*: A. Eastwood July 1891 (COLO 28,104); A. Eastwood July 1891 (DS 31,905); L.N. Goodding July 21, 1903 (MO 140,911); L.N. Goodding 1,644 (RM 51,929); L.N. Goodding 1644 (US 581,541); L.N. Goodding July 21, 1903 (COLO 28,173); F. Ramaley 16,260 (COLO 43,393); T. Slotta 99-6 (VPI); T. Slotta 99-7 (VPI); T. Slotta 99-3 (VPI); T. Slotta 99-4 (VPI).

Iliamna grandiflora

Arizona: F.W. Gould and W.P. Martin 4,451 (JEPS 749,879); C.L. Hitchcock 25,597 (US 2,580,488); T. Slotta 99-11 (VPI).

Colorado: **Holotype: F.K. Vreeland 882 (NY 222,082);** *Other specimens examined:* Bessey 3933 (US 856,122); T.S. Brandegee 11,328 (MO 140,907); T.S. Brandegee Aug. 1877 (JEPS 109,388); T.J. Mathias 168 (US 1,073,393); G.E. Osterhout 5,376 (RM 166,619); E. Partone Hawyer July 13, 1932 (CAS 196,011); H.M. Schmoll 1,760 (RM 105,904); C.L. Shear 4122 (US 858,247); Spackman, Casey, and Fayette SS9629 (COLO 475,446).

New Mexico: T.S. Brandegee 13,271 (MO 140,908); C.C. Ellis 300 (MO 759,960); C.C. Ellis 300 (US 890,766).

Utah: M. Douglas 54-482 (COLO 134,601); C.C. Ellis 300 (US 662,707); W. Hodgson July 28, 1980 (COLO 352,828); O.A. Olsen 241 (RM 514,322); P.A. Rydberg and A.O. Garrett 9,834 (RM 80,276); P.A. Rydberg (9207) and A.O. Garrett (US 765,190); P.A. Rydberg (9834) and A.O. Garrett (US 765,338).

Iliamna latibracteata

California: **Holotype: D.K. Kildale 2,312 (DS 161,387);** Other *specimens examined:* W.R. Dudley 1899 (DS 16,199); V.P. Fox July 1919 (JEPS 67,375); D. Hagar L10 (CAS 407,018); B.R. Jackson Sept. 15, 1939 (CAS 269,830); D.K. Kildale 2,114 (DS 161,389); L.B. Kildale 10,535 (DS 199,734); H.E. Parks and S.T. Parks 5593 (MO 1,191,137); J.P. Tracy 18,535 (MO 1,660,098); J.P. Tracy 18,535 (DS 352,512); J.P. Tracy 16,104.5 (DS 337,106); J.P. Tracy 7,585 (JEPS 67,376); Y.W. Winbald June 22, 1941 (CAS 298,532); C.B. Wolf 2367 (DS 232,842); C.B. Wolf 2367 (US 1,630,021).

Oregon: E.I. Applegate 2694 (DS 214,632); E.*I.* Applegate 2694 (US 361,851); R.S. Ferris and R. Duthie 632 (DS 103,994); L.F. Henderson 8853 (CAS 148,666); K. Kane mid-June 1994 (VPI 89,297); K. Kane July 19, 1994 (VPI 89,298); W.E. Lawrence 427 (DS 100,818); M.E. Peck 10,142 (DS 14,751); M.E. Peck 20,337 (CAS 302,900); C.R. Quick 56-02 (DS 404,502); V. Stansell July 16, 1994 (VPI 89,295).

Iliamna longisepala

Washington: **Holotype: Wilke's Expedition (NY 222,090);** *Other specimens examined:* G. Garrison and D. Wooldridge 41-56 (RM 514,321); D. Hole H17 (RM 514,320); J.H. Sandberg and J.B. Leiberg July 1893 (DS 118,921); J.H. Sandberg and J.B. Leiberg July 1893 (Nasou) (JEPS 109,387); J.H. Sandberg and J.B. Leiberg 430 (JEPS 167,454); J.H. Sandberg and J.B. Leiberg July 1893 (Rock Island) (JEPS 189,037); J.H. Sandberg and J.B. Leiberg July 1893 (MO 134,430); J.H. Sandberg and J.B. Leiberg 430 (MO 189,297); J.H.Sandberg and J.B. Leiberg 430 (US 288,641); J.Wm. Thompson 10,783 (DS 228,883); J.Wm. Thompson 11,784 (DS 246,576); J.W. Thompson 11,784 (JEPS 871,593); J.Wm.

Thompson 10,783 (MO 1,081,363); J.Wm. Thompson 10,783 (US 1,633,266); J.Wm. Thompson 11,784 (US 1,697,536); K. Whited July 8, 1900 (US 371,638).

Iliamna remota

Illinois: **Holotype: J.M. Greenman 3530 (US 985,913); Isotype: J.M. Greenman 3530 (MO 740,802);** *Other specimens examined:* O.E. Lansing and E.E. Sherff 19 (US 820,149); O.E. Lansing and E.E. Sherff 8 (US 820,283);); R.A. Schneider 1,294 (JEPS 871,356); R.A. Schneider 1,294 (JEPS 871,357); E.E. Sherff July 25, 1945 (JEPS 771,086); E.E. Sherff 5,021 (JEPS 771,087); E.E. Sherff 5021 (MO 1,292,324); E.E. Sherff 5050 (MO 1,981,344); E.E. Sherff 5021 (US 2,006,866); J.A. Steyermark 68,636 (MO 1,739,932).

Indiana: R.C. Friesner 19,265 (CAS 340,322); R.C. Friesner 19,265 (JEPS 731,590); R.C. Friesner 19,265 (US 1,926,222); R.C. Friesner July 14, 1945 (VPI 20,871); E.E. Sherff 5033 (DS 348,704); E.E. Sherff 5,033B (JEPS 771,090).

Virginia: C.H. Leys (43,234), S. Leys, and N.L. Bottone (VPI 85,576); P.A. Munz 13,505 (JEPS 569,242); A.J. Sharp June 29, 1940 (JEPS 871,355); C. Stevens 15,733 (VPI 85,814); T.F. Wieboldt 4393 (VPI 72,353); T.F. Wieboldt 5,577 (VPI 77,936).

Iliamna rivularis

British Columbia, Canada: J.M. Macoun 63,694 (CAS 52,333); W.C. McCalla 2820 (DS 290,972); A. and R. Nelson 3,112 (JEPS 614,334);); R.L. Taylor and D.H. Ferguson 2,144 (JEPS 1,235,057); T.M.C. Taylor and A.F. Szczawinski 47 (JEPS 1,184,593J.Wm. Thompson 14,432 (US 1,828,144).

California: P.C. Hutchison 2705 (US 2,485,099); J.P. Tracy 16,104 (US 1,789,259); J.P. Tracy 18,151 (US 2,008,692).

Colorado: E.H. Bader 154 (COLO 28,175); J. Barrell July 18, 1955 (US 2,870,477); J. Barrell and S. Spongberg 131-66 (US 2,870,478); M. Douglas 54-482 (COLO 134,602); M. Douglas 54-231 (COLO 134,699); Johusau and Hedgrock 765 (RM 101,887); B.E. and M. Nelson 17,745 (RM 571,607); G.E. Osterhout 1,906 (RM 166,615); G.E. Osterhout 2,722 (RM 166,616); H. Schmoll 1509 (COLO 28,172); L.J. Uttal 4094 (VPI 49,045); L.J. Uttal 9196 (VPI 54,954).

Idaho: G.B. Aiton July 1892 (JEPS 189,026); Geo.B. Aiton July 1892 (MO 140,889); C.C. Epling 7077 (MO 966,048); B.W. Evermann 422 (US 242,539); A.A. and E.G. Heller July 14, 1896 (JEPS 109,390); N.H. Holmgren 5550 (CAS 559,339); M.E. Jones 6204 (MO no number); A. Kirn Aug. 3, 1994 (VPI 89,302); A.R. Kruckeberg 2,855 (JEPS 1,013,045); R.L. Lang 380 (JEPS 799,769); J.B. Leiberg 1160 (MO 140,897); W.H. Lewis 6,755 (JEPS 1,391,106); W.H. Lewis 6755 (MO 2,013,461); J.F. MacBride 582 (DS 31,662); J.F. MacBride 582 (MO 140,924); J.F. MacBride and E.B. Payson 3343 (MO 814,885); M. R. Mousseux June 27, 1994 (VPI 89,304); A. *I.* Mulford July 7, 1892 (MO 140,899); W. R. Owen July 27, 1994 (VPI 89,301); C.R. Quick 1,089 (JEPS 523,402); J.H. Sandberg July 1892 (JEPS 18,952); B.O. Schreiber 1,224 (JEPS 615,226); D. Soltis mid-July 1994 (VPI 89,303); J.Wm. Thompson 13,513 (CAS 265,218); J.Wm. Thompson 13,822 (US 1,731,253); L.J. Uttal 9161 (VPI 54,925).

Montana: H. and V. Bailey 402 (JEPS 1,323,208); J.E. Kirkwood 2,446 (JEPS 871,358); P.P. Lowry II 2453 (MO 2,944,428); F.J. Scully 1201 (US 1,971,226); P.C. Standley 16,599 (US 1,027,181); W.R. Sweadner 167 (JEPS 1,598,862); R.S. Williams (MO 14,093); R.S. Williams 871 (US 962,046); R.S. Wirt 51 (VPI 89,302).

Nevada: A.A. Heller 11,128 (DS 31,655); A.A. Heller 11,128 (MO 745,774); A.H. Holmgren 1561 (JEPS 676,185); A. Nelson 873 (MO 140,901); A. Pinzl 5625 (MO 3,687,770); A. Tiehm 5372 (CAS 715,745); A. Tiehm 5372 (MO 2,721,130); P. Train 405 (DS 276,561); P. Train 669 (JEPS 871,620).

Oregon: Wm.C. Cusick (MO 140,910); L.E. Detling 7,478 (DS 447,194); L.F. Henderson 1518 (MO 140,912); J.B. Leiberg 890 (MO 140,896); J.B. Leiberg 890 (US 288,640); M.E. Peck 18,419 (DS 236,294); E.P. Sheldon 1,897 (MO 140,902).

Utah: Mrs. J. Clemens Sept. 29, 1909 (JEPS 871,625); A. Eastwood 7735 (CAS 52,416); A.O. Garrett 1806 (DS 118,911); A.O. Garrett 8,218 (JEPS 871,221); E. H. Graham 9640 (MO 1,172,824); B.F. Harrison 8365 (DS 296,253); B.F. Harrison 8,365 (JEPS 647,568); C.L. Hitchcock 4,631 (JEPS 722,745); M.E. Jones July 27, 1895 (CAS 139,290); M.E. Jones Aug. 5, 1895 (DS 15,598); M.E. Jones July 27, 1895 (DS 679,489) and (DS 679,490); M.E. Jones July 27, 1895 (JEPS 303,416); M.E. Jones Aug. 5, 1895 (US 359,635); M.E. Jones 1085 (MO 140,913); M.E. Jones July 27, 1985 (MO 140,914); M.E. Jones Aug. 5, 1895 (MO 140,915); M.E. Jones Aug. 5, 1895 (MO 140,916); B. Maguire 3,548 (JEPS 532,841); H.W. Marion 241 (JEPS 156,616); E.C. and K.S. McCarty 31 (DS 683,186); A. *I.* Mulford 221 (MO 140,900); L.J. Uttal 9089 (VPI 54,794); L.J. Uttal 11,515 (VPI 62,370); T.F. Wieboldt (1704) and F. Smith (VPI 69,789); L. Williams 5802 (MO 1,040,173).

Washington: R. K. Beatie 11,746 (CAS 399,047); L. Boner and V. Weldert 206 (DS 301,172); L. Boner and V. Weldert 206 (JEPS 707,917); T.S. Brandegee 14,449 (MO 140, 909); A.D. Elmer 42 (MO 140,892); C.B. Filker 885 (MO 1,030,960); M.W. Gorman 4,607 (DS 103,623); J.M. Grant June 1930 (JEPS 438,826); J.T. Howell 28,501 (CAS 374,869); W.C. and M.W. Muenscher 11,185 (JEPS 727,601); L.S. Rose 37,438 (JEPS 871,219); W.N. Suksdorf June 16, 1883 (DS 10,892); W.N. Suksdorf June 16, 1883 (JEPS 189,579); J.Wm. Thompson 6685 (MO 1,012,408); J.Wm. Thompson 9061 (MO 1,085,608).

Wyoming: H. Bailey and V. Bailey 4199 (US 1,567,834); A.A. Beetle 5,057 (JEPS 753,916); Flora of Grand Teton National Park 4157 (MO 3,179,557); W. Haas June 22, 1994 (VPI 89,300); E.C. Johnston July 24, 1931 (CAS 191,997); G.N. Jones 23,798 (MO 2,626,706); P. Kamb 681 (JEPS 1,178,486); A. and R.A. Nelson 813 (MO 1,075,960); E.B. and L.B. Payson 2654 (MO 904,140); C.L Porter 3,712 (DS 320,706); C.L. Porter 5,560 (DS 354,304); C.L. Porter 3,712 (JEPS 742,386); G.B. Rossbach 5,584 (JEPS 1,351,416); Spindel 10 (MO 104,575,157); L.J. Uttal 10,771 (VPI 58,832); I.L. Wiggins 11,953 (DS 374,778); L. Williams 959 (MO 1,032,529).

***Iliamna rivularis* var. diversa (Synonym of *I. rivularis*)**

Idaho: **Holotype: J.F. Macbride 582 (NY 222,082).**

Table 4.4. **Summary of character variation.** Several characters were present on a limited number of specimens (N). The results of the Kruskal-Wallis test (P values), mean, standard deviation (StdDev), and maximum and minimum values for each character of the entire data set are listed. Floral characters used in the computation of the distance matrix for UPGMA analysis are indicated by an asterisk (*).

Variable	N	P=	Mean	StdDev	Minimum	Maximum
Leaf Serration	314	0.0042	1.758	1.054	0.000	5.000
Pubescence on Upper Surface of Leaves	311	<0.0001	2.473	1.056	0.000	5.000
Pubescence on Lower Surface of Leaves	314	<0.0001	2.838	1.028	0.000	6.000
Leaf Base Shape	309	<0.0001	2.573	0.878	0.000	5.000
Stipules	306	<0.0001	0.291	0.455	0.000	1.000
Pubescence of Sepals	317	<0.0001	4.050	1.375	1.000	6.000
Pubescence of Fruit Wall	172	<0.0001	4.802	1.478	1.000	6.000
Pubescence of Seed	130	<0.0001	2.546	1.336	0.000	6.000
Pubescence of Stem	317	<0.0001	3.243	1.388	1.000	6.000
Petiole Length/Leaf Length	311	<0.0001	0.700	0.349	0.091	4.700
Leaf Length/ Leaf Width	305	0.0233	0.940	0.239	0.133	7.050
Terminal Leaf Lobe/ Leaf Length	285	0.0254	0.564	0.239	0.000	4.100
Sepal Length/ Width	310	<0.0001	2.446	1.031	0.875	10.606
Pedicel Length/ Petal Length	278	<0.0001	0.343	0.375	0.053	2.880
Bract Length/ Width	307	<0.0001	8.661	6.430	0.625	76.667
Bract Length/ Petal Length*	278	<0.0001	0.309	0.225	0.000	3.286
Staminal Column Length/ Petal Length	255	<0.0001	0.557	0.165	0.025	1.200
Petiole Length	311	<0.0001	5.320	2.407	1.100	16.000
Leaf Width	305	<0.0001	8.650	3.166	2.400	18.100
Leaf Length	311	<0.0001	7.908	2.917	1.000	17.200
Sepal Length*	310	<0.0001	1.060	0.429	0.300	3.500
Sepal Width*	311	<0.0001	0.452	0.152	0.150	1.100
Pedicel Length*	314	<0.0001	0.766	0.826	0.030	7.200
Bract Length*	309	<0.0001	0.686	0.478	0.000	6.900
Bract Width*	309	<0.0001	0.112	0.122	0.000	0.970
Petal Length*	279	<0.0001	2.224	0.475	1.000	3.700
Width Petal Base	153	0.0042	0.282	0.346	0.100	4.000
Width Petal Margin	202	<0.0001	1.787	0.495	0.200	3.700
Staminal Column Length*	257	<0.0001	1.237	0.402	0.070	3.000
Fruit Length	144	0.0983	0.804	0.319	0.200	3.000
Terminal Leaf Lobe Length	285	<0.0001	4.374	1.909	0.000	11.500
Seed Length	85	0.6414	0.166	0.038	0.050	0.310

Table 4.5. **Summary of morphological variation in *Iliamna*.** The number of specimens (N) per taxon, and the mean, minimum, maximum and standard deviation for each variable examined in the study are provided.

		Petiole Length	Leaf Width	Leaf Length	Leaf Serration	Upper Pubscence	Lower Pubescence	Leaf Base	Stipules	Sepal Length	Sepal Width	Pedicel Length	Bract Length	Bract Width	Petal Length	Sepal Pubescence	Petal Base	Petal Margin	Staminal Column	Pubscence Fruit	Pubscence Seed	Pubsence Stem	Fruit Length	Terminal Lobe Length	Seed Length
I. acerifolia	mean	3.74	7.34	6.95	1.63	2.50	2.88	2.38	0.25	0.72	0.37	0.54	0.50	0.07	2.07	3.75	0.20	1.57	1.34	3.83	1.50	3.38	0.55	3.64	0.13
N=8	stdev	1.33	2.48	1.67	0.74	0.76	0.64	1.06	0.46	0.14	0.08	0.24	0.19	0.04	0.47	1.28	.	0.58	0.33	1.60	0.71	1.19	0.20	1.06	0.05
	max	5.30	11.00	9.00	3.00	3.00	4.00	3.00	1.00	1.00	0.50	1.00	0.80	0.14	2.90	5.00	0.20	2.10	1.80	5.00	2.00	5.00	0.82	5.50	0.16
	min	1.20	3.00	3.40	1.00	1.00	2.00	0.00	0.00	0.57	0.27	0.30	0.28	0.02	1.31	2.00	0.20	0.62	0.88	1.00	1.00	2.00	0.23	1.90	0.09
I. angulata	N=1	7.00	13.00	12.00	3.00	1.00	3.00	3.00	0.00	1.90	0.35	0.50	1.00	0.04	2.50	3.00	0.20	2.10	1.30	5.00	.	3.00	0.60	5.00	.
I. bakeri	mean	3.36	5.13	4.44	2.37	3.31	4.00	2.03	0.97	1.35	0.55	0.67	0.76	0.09	2.49	5.58	0.22	1.82	1.50	5.95	2.07	3.81	0.85	2.32	0.16
N=36	std	1.72	2.17	1.54	1.14	1.16	1.10	1.16	0.17	0.31	0.15	0.41	0.27	0.04	0.44	0.60	0.05	0.50	0.50	0.22	1.22	1.24	0.38	1.21	0.03
	max	6.50	9.90	8.70	4.00	5.00	6.00	4.00	1.00	2.00	1.10	2.50	1.50	0.20	3.50	6.00	0.30	3.00	3.00	6.00	5.00	6.00	1.70	5.70	0.21
	min	1.10	2.40	2.20	0.00	1.00	2.00	1.00	0.00	0.80	0.40	0.20	0.30	0.04	1.60	4.00	0.14	1.20	0.70	5.00	1.00	2.00	0.30	0.00	0.11
I. corei	mean	6.28	8.65	7.85	1.64	2.91	3.41	2.50	0.36	1.33	0.58	0.87	0.78	0.08	2.54	5.52	0.29	1.93	1.04	5.17	4.06	4.95	0.79	4.36	0.18
N=22	stdev	2.13	1.48	1.36	1.22	0.87	0.80	0.86	0.49	0.29	0.10	0.41	0.18	0.04	0.26	0.60	0.08	0.19	0.21	0.86	1.24	1.02	0.22	0.88	0.02
	max	12.00	11.00	11.00	5.00	4.00	4.00	4.00	1.00	1.86	0.78	1.90	1.20	0.20	3.00	6.00	0.39	2.30	1.30	6.00	6.00	6.00	1.10	6.10	0.20
	min	3.00	6.10	4.30	1.00	2.00	2.00	1.00	0.00	0.90	0.50	0.09	0.60	0.03	2.00	4.00	0.20	1.60	0.55	4.00	2.00	1.00	0.38	3.00	0.14
I. crandallii	mean	3.72	6.27	6.06	1.72	1.94	2.29	2.50	0.18	1.12	0.47	0.64	0.77	0.11	2.31	4.06	0.23	1.81	1.10	4.80	1.00	2.11	0.65	3.42	0.17
N=18	stdev	2.01	2.62	2.08	1.07	1.09	0.47	0.92	0.39	0.27	0.13	0.31	0.28	0.03	0.39	0.80	0.06	0.54	0.36	0.45	1.00	0.76	0.30	1.09	0.03
	max	9.00	13.50	12.00	5.00	3.00	3.00	4.00	1.00	1.60	0.80	1.30	1.30	0.15	3.00	5.00	0.30	3.00	1.60	5.00	2.00	3.00	1.10	6.00	0.20
	min	1.20	3.00	3.00	0.00	0.00	2.00	1.00	0.00	0.70	0.30	0.20	0.10	0.06	1.70	2.00	0.15	1.00	0.50	4.00	0.00	1.00	0.30	2.10	0.15
I. grandiflora	mean	4.76	8.30	7.88	1.42	2.32	2.53	2.42	0.53	1.18	0.52	0.69	0.86	0.13	2.49	3.68	0.25	2.24	1.29	4.86	2.60	3.16	0.82	4.46	0.19
N=19	stdev	2.67	2.79	2.50	1.12	1.11	0.96	0.61	0.51	0.23	0.13	0.34	0.19	0.06	0.50	1.29	0.08	0.46	0.34	0.90	0.89	1.02	0.29	1.49	0.03
	max	12.60	12.60	12.00	4.00	4.00	4.00	3.00	1.00	1.50	0.70	1.40	1.18	0.32	3.70	6.00	0.40	3.60	1.90	6.00	4.00	6.00	1.25	7.40	0.23
	min	1.70	3.00	3.20	0.00	0.00	0.00	1.00	0.00	0.75	0.30	0.20	0.60	0.08	1.80	2.00	0.10	1.30	0.70	4.00	2.00	1.00	0.50	2.20	0.17
I. latibracteata	mean	6.68	10.91	9.88	1.80	2.76	3.52	3.04	0.38	1.12	0.57	0.65	1.04	0.40	2.31	4.40	0.25	1.84	1.39	4.80	2.42	3.76	0.87	6.09	0.17
N=25	stdev	2.03	2.42	2.32	1.29	0.83	0.92	0.88	0.50	0.27	0.17	0.23	0.36	0.22	0.56	1.16	0.05	0.35	0.37	0.78	1.24	1.27	0.40	1.78	0.03
	max	10.30	16.00	13.60	5.00	4.00	5.00	4.00	1.00	1.60	0.95	1.30	1.60	0.97	3.20	6.00	0.30	2.50	1.90	6.00	5.00	6.00	1.89	9.50	0.21
	min	3.70	6.70	3.50	0.00	1.00	2.00	1.00	0.00	0.62	0.30	0.25	0.32	0.05	1.30	2.00	0.18	1.20	0.75	4.00	1.00	2.00	0.20	3.50	0.13
I. longisepala	mean	4.99	7.46	5.88	2.10	2.38	2.71	2.90	0.58	1.63	0.45	3.03	1.02	0.15	2.28	2.57	0.26	1.60	1.49	4.88	2.00	4.43	0.88	3.06	0.16
N=21	stdev	1.55	2.19	1.62	1.51	0.92	0.90	0.91	0.51	0.39	0.13	1.83	0.36	0.07	0.36	0.87	0.06	0.37	0.33	1.36	0.71	0.93	0.10	0.97	0.05
	max	8.20	12.50	9.50	5.00	4.00	4.00	4.00	1.00	2.80	0.70	7.20	2.00	0.30	3.00	4.00	0.30	2.20	2.40	6.00	3.00	6.00	1.00	5.50	0.23
	min	1.50	4.20	3.30	1.00	1.00	2.00	1.00	0.00	1.10	0.15	0.48	0.10	0.03	1.50	1.00	0.17	0.90	1.10	3.00	1.00	3.00	0.76	1.60	0.12
I. remota	mean	6.86	8.87	8.38	1.53	2.66	2.73	2.94	0.18	1.29	0.54	0.59	0.92	0.09	2.30	4.97	0.30	2.21	0.91	4.92	3.25	4.27	0.85	4.24	0.17
N=24	stdev	1.98	2.08	2.50	0.86	1.07	1.07	1.00	0.39	0.32	0.13	0.29	1.12	0.08	0.50	0.92	0.07	0.47	0.38	1.22	1.26	1.44	0.17	1.01	0.03
	max	10.20	12.90	15.00	4.00	5.00	5.00	4.00	1.00	1.90	0.80	1.20	6.90	0.50	3.50	6.00	0.50	3.70	1.60	6.00	6.00	6.00	1.20	6.50	0.23
	min	2.80	4.00	1.00	0.00	1.00	1.00	0.00	0.00	0.42	0.30	0.03	0.30	0.02	1.50	4.00	0.12	1.30	0.48	2.00	2.00	1.00	0.45	1.20	0.11
I. rivularis	mean	5.49	9.69	8.96	1.65	2.19	2.46	2.53	0.06	0.77	0.36	0.53	0.45	0.07	2.05	3.41	0.31	1.61	1.20	4.36	2.06	2.44	0.79	4.98	0.17
N=134	stdev	2.44	3.25	2.90	0.84	0.96	0.81	0.69	0.24	0.35	0.11	0.29	0.22	0.08	0.44	1.17	0.52	0.44	0.35	1.16	1.04	0.95	0.39	1.98	0.05
	max	16.00	18.10	17.20	5.00	4.00	5.00	5.00	1.00	3.50	0.90	1.60	1.40	0.82	3.00	6.00	4.00	2.80	2.50	6.00	6.00	5.00	3.00	11.50	0.31
	min	1.30	2.80	3.50	0.00	0.00	1.00	1.00	0.00	0.30	0.20	0.10	0.00	0.00	1.00	1.00	0.10	0.20	0.07	2.00	1.00	1.00	0.35	1.60	0.05

Figure 4.1. Distribution of taxa in *Iliamna*. Shapes provide approximate distributions of taxa. In dark purple is *I. bakeri,* red *I. corei*, bright green *I. crandallii,* light blue *I. grandiflora,* lime green *I. latibracteata,* lavendar *I. longisepala,* dark blue *I. remota,* yellow *I. rivularis*.

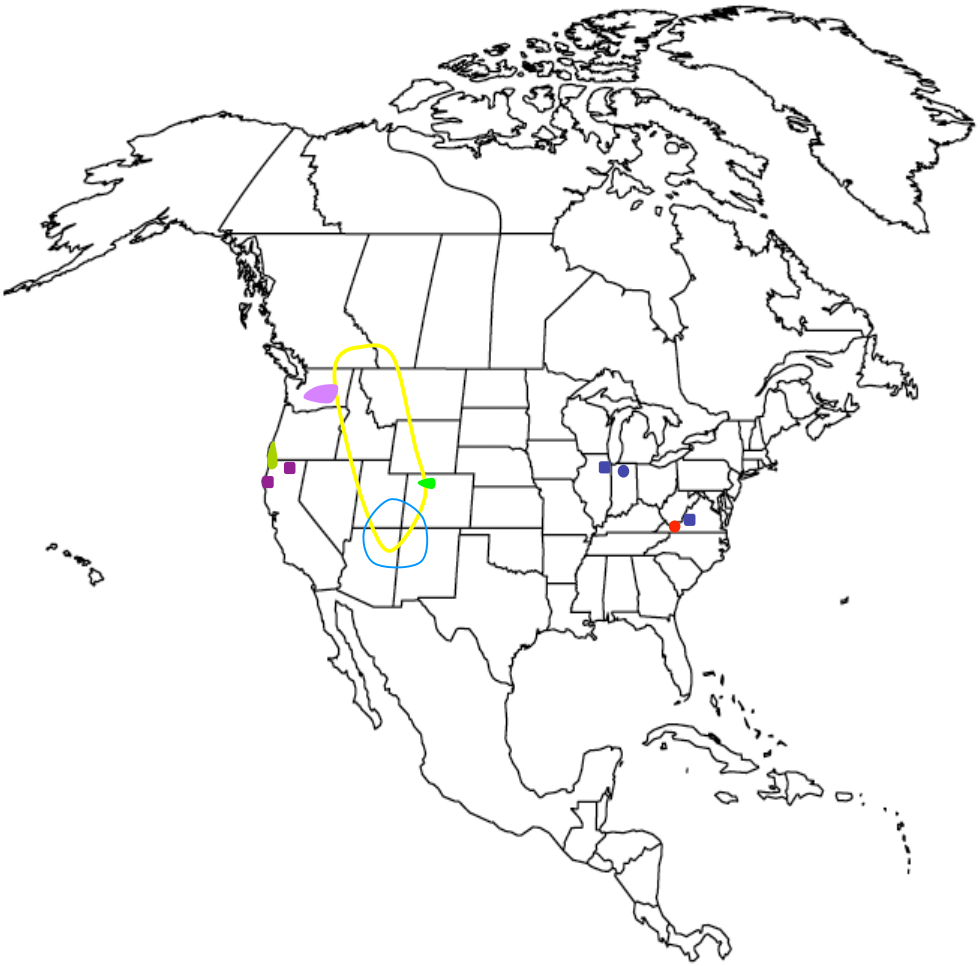


Figure 4.2. Multidimensional scaling plot of all specimens. Symbols for taxa as indicated in the legend.

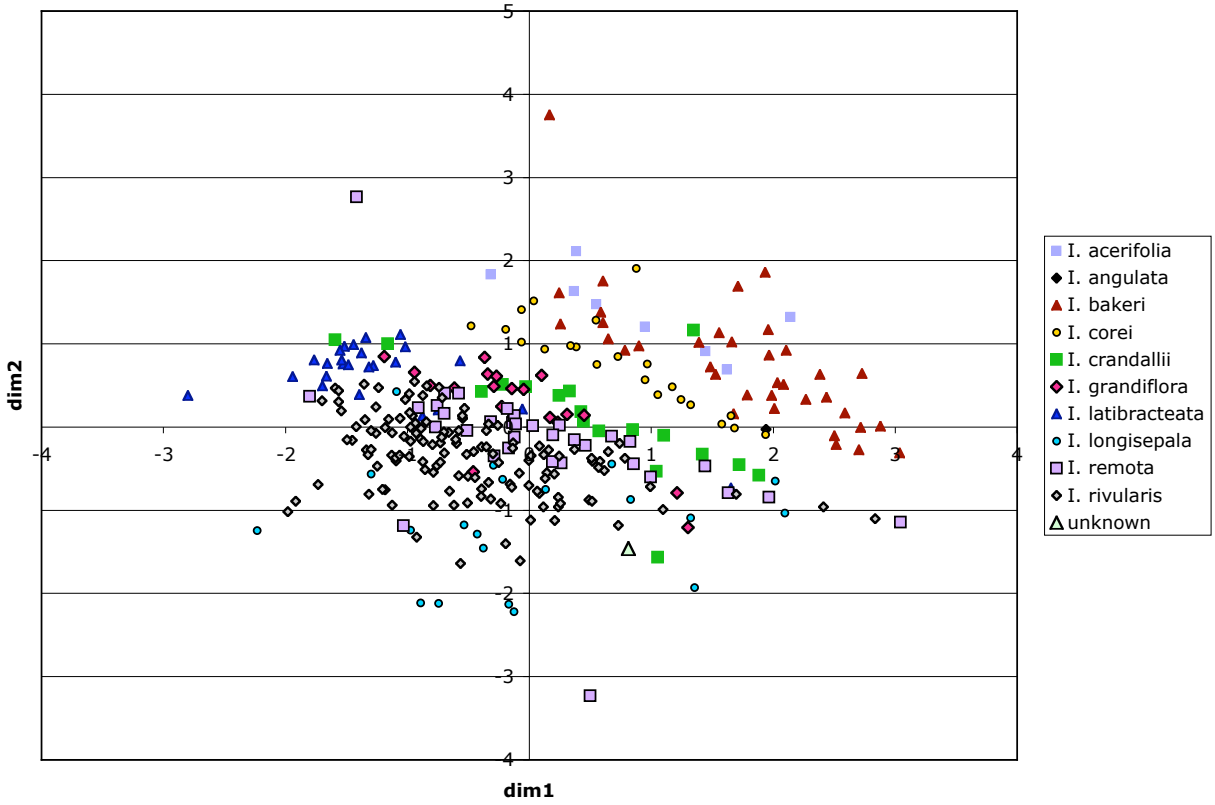


Figure 4.3. Multidimensional scaling plot of all specimens excluding those identified as *Iliamna rivularis*. Lines drawn surrounding points indicate clusters corresponding to taxa according to the author. Symbols for taxa as indicated in the legend.

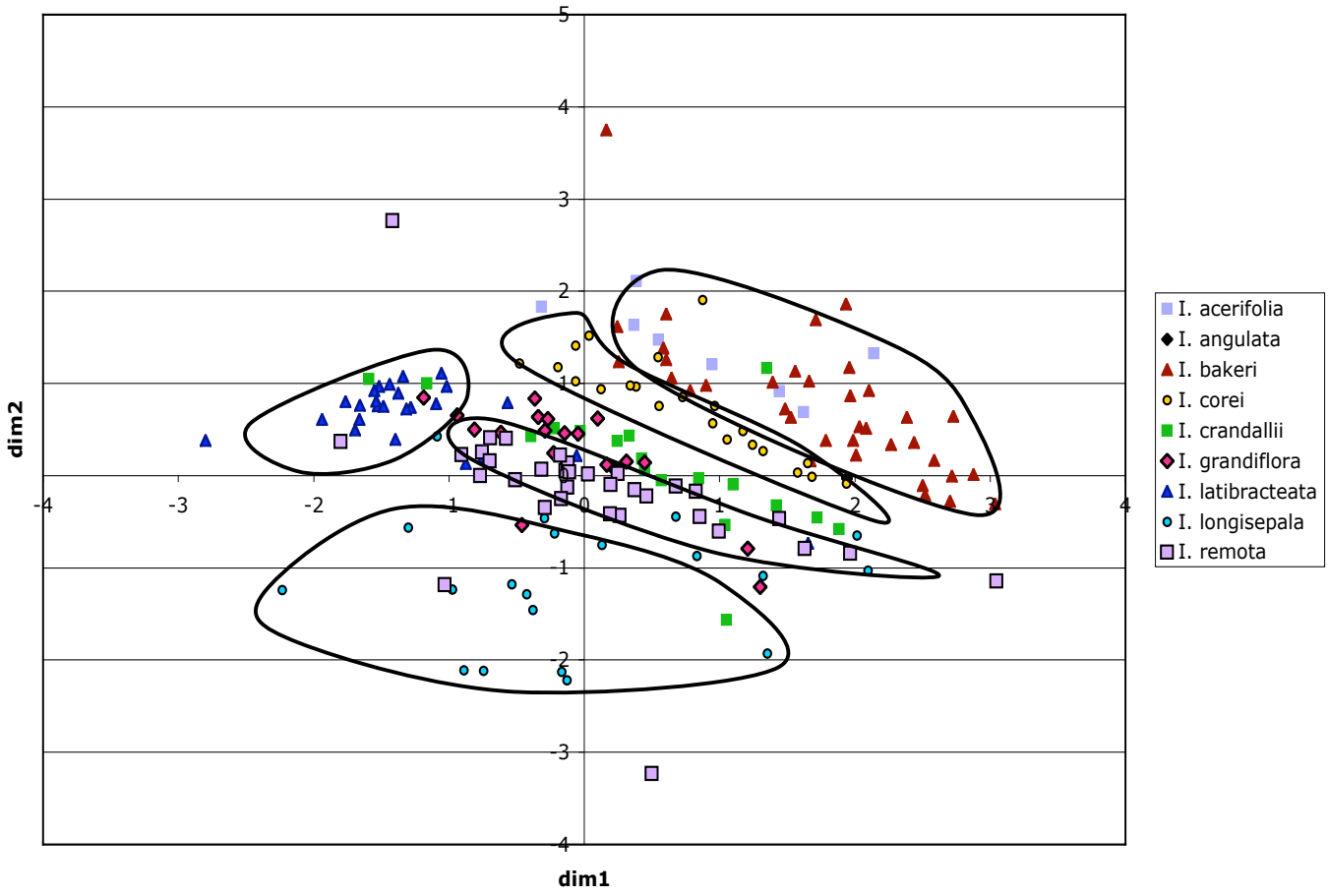
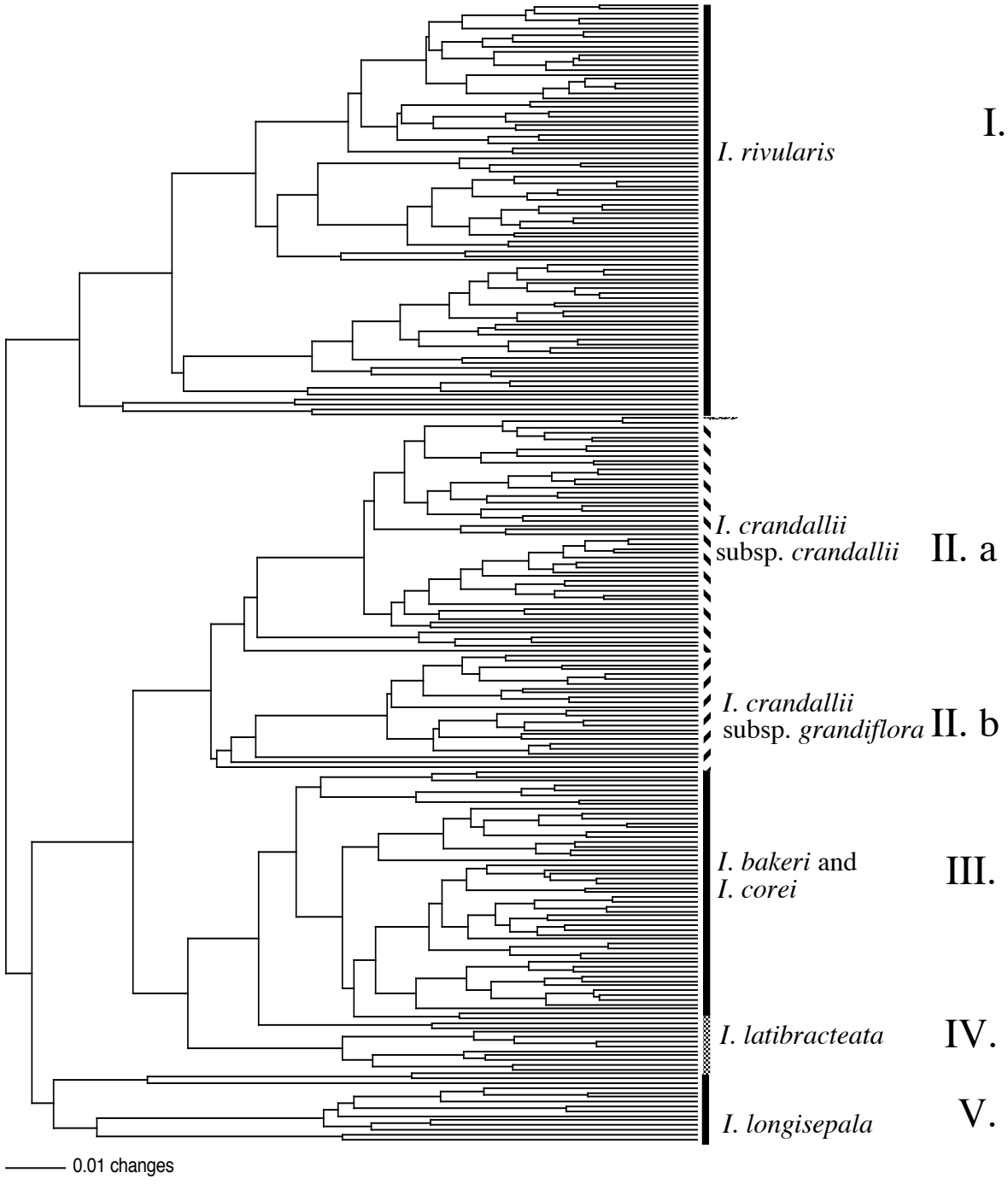


Figure 4.4. UPGMA phylogram of *Iliamna* based on floral morphology. Specimens labeled as *I. remota* are located throughout clusters II. (a and b) and III.



Chapter 5

Morphological and Molecular Variation in *Malacothamnus*: Implications for Species Delineation

Abstract

Malacothamnus has undergone several taxonomic revisions with two to 27 species recognized. Taxonomic boundaries among the species are unclear since morphology within species can be highly variable. To evaluate species delineation within the genus, 25 traits were examined for 419 herbarium specimens representing all described species, recognized or not. The morphological characters were analyzed using cluster and statistical methods. To complement the examination of morphological features, fresh material was collected throughout the distribution of *Malacothamnus* and sequenced for the ITS region. Maximum likelihood analysis was used to assess relationships between 42 exemplar sequences. Although significant variation was detected among all vegetative and floral traits examined, use of floral traits alone enabled species discrimination for several entities in both multidimensional scaling and cluster analyses. Specimens identified as *M. arcuatus* clustered with either *M. clementinus* or *M. davidsonii*. Within the widely designated species *M. fasciculatus*, three of the four accepted varieties were found to be distinct in the MDS analysis but not in the cluster analysis. Two previously recognized species, *M. hallii* and *M. orbiculatus*, were found to be statistically different than their synonymous species, *M. fasciculatus* and *M. fremontii*, respectively. Analysis of sequence data indicates gene flow between taxa has occurred. Continued integration and/or

recent divergence prevent taxa in *Malacothamnus* from forming well-defined taxonomic boundaries with little overlap in morphology.

Introduction

Malacothamnus Greene (Malvoideae: Malvaceae: Malvales) is a genus of eleven taxa endemic to California and Baja California and Sonora, Mexico (Bates 1993) (Fig. 5.1). Greene (1906) first described the genus and included eight species, six of which he transferred from *Malvastrum*. Greene did not indicate a type specimen for his genus. Taxa in *Malacothamnus* differ from *Malvastrum* in having numerous rays on the stellate trichomes, and the trichomes may or may not be stipitate in *Malacothamnus* (Kearney 1951). Greene's taxonomic classification was not recognized by some later botanists (Estes 1925, Eastwood 1936, 1939).

Within *Malacothamnus* there is a great deal of morphological variation, which has led to the description of numerous species and varieties that may or may not be valid (Abrams 1907, Estes 1925, Eastwood 1936, Kearney 1951, Bates 1993). With the numerous revisions of *Malacothamnus*, uncertainty of species delimitations resulted, with 75% or more of *Malacothamnus* taxa being referred to as taxonomically uncertain (Skinner et al. 1995). Furthermore, hybridization is reported to freely occur among the species where the taxa overlap in distribution (Bates 1993). Two taxa are listed as endangered in California (*M. clementinus* and *M. fasciculatus* var. *nesioticus*), ten others as rare (Thomas 2000), and two are believed extinct (Bates 1993).

Treatments by Eastwood (1936) and Kearney (1951) recognized two groups within *Malacothamnus* with morphological affinities to either *M. fasciculatus* or *M. fremontii*. Eastwood divided the shrubby *Malvastrums* into 27 species, and Kearney into 21 with six varieties (Table 5.1). Bates (1963) reexamined relationships in *Malacothamnus* using the distribution, ecology, population structure, breeding, and hybrid potential of the genus in order to provide a "different orientation for their classification" (Bates 1963, p. 4). The classification provided included two species, *M. chilensis* (in South America) and *M. fasciculatus* (in North America). Floral characters clearly delineated the two species. Flowers that had the petal bases covered completely with trichomes and the pubescence continuing up the staminal column characterized *M. chilensis*. In *M. fasciculatus* the petal bases were not considered to be pubescent and the staminal column was glabrous or had a fine pubescence limited to its base. Recently, *Malacothamnus chilensis* has been transferred to a new genus, *Andeimalva*, which includes four species in Chile (Tate 2003). This revision was based, in part, on chromosome number; *M. chilensis* was observed to have x=6, whereas the North American *Malacothamnus* species are x=17 (Bates and Blanchard 1970). Morphologically, *M. chilensis* can be grouped with *Tarasa geranioides* and *T. meyeri*, since the three have smooth-walled and completely dehiscent mericarps. The species designated in the genus are *Andeimalva chilensis* (Gay) J.A. Tate, *A. machupicchenis* (Krapov.) J.A. Tate, *A. mandonii* (Baker f.) J.A. Tate, and *A. spiciformis* (Krapov.) J.A. Tate.

Many of the taxa previously described by Eastwood (1936) and Kearney (1951) were treated as synonyms of Bates' new combinations for the eleven subspecies of *M. fasciculatus*. For example, *M. fasciculatus* subsp. *jonesii* was based on *Malvastrum jonesii* Munz (Munz and

Johnston 1925), *Malvastrum dudleyi* Eastwood (1936), *Malvastrum fragrans* Eastwood (Eastwood 1936), *Malvastrum gracile* Eastwood (Eastwood 1936), and *Malacothamnus fragrans* (Eastwood) Kearney (Kearney 1951). The sub-species of *M. fasciculatus* described by Bates included subsp. *abbottii*, subsp. *aboriginum*, subsp. *clementinus*, subsp. *davidsonii*, subsp. *densiflorus*, subsp. *exfibulosus*, subsp. *fasciculatus*, subsp. *foliosus*, subsp. *jonesii*, subsp. *marrubioides*, and subsp. *palmeri*. The subspecies could be defined by geographical location, suggesting an ecological (e.g., climate, elevation, habitat) influence on the morphological variation seen.

In a recent study examining morphological variation between five taxa from Southern California (*M. clementinus*, *M. davidsonii*, *M. densiflorus*, *M. fasciculatus*, and *M. fremontii*), little distinction between taxa was found (Benesh and Elisens 1999). This reflected a limited number of characters with taxonomic unity used in the phenetic analysis. The data were analyzed by cluster analyses, principle component analyses (PCA), and discriminant analyses. The PCA revealed loosely clustered groups of species with much overlap. This finding reflects the frequency at which taxa in *Malacothamnus* hybridize, given that the populations operate as a mixed mating system dominated by out-crossing. The 26 morphological characters chosen (11 quantitative, 15 qualitative) indicated floral traits to be the most consistent. However, many characters used to distinguish species in *Malacothamnus* are qualitative (e.g., degree of leaf pubescence) and could benefit from quantitative analysis (e.g., number of trichomes per cm2) and the use of phylogenetic analyses.

It was a goal of this study to further elucidate relationships and taxonomic boundaries in this taxonomically difficult genus. To meet this objective two data sets were examined, DNA sequence data from the ITS region was used to examine gene flow between populations, and morphology of herbarium specimens was examined to determine physical attributes characterizing taxa in *Malacothamnus*.

Methods

Morphological Analyses

Analysis of morphological variation in *Malacothamnus* utilized 419 herbarium specimens encompassing the taxonomic and geographic range of the genus. A total of 27 vegetative and floral characters was measured (Table 5.2). Specimens were examined prior to scoring and only those with all characters present were selected, excluding type specimens, in order to limit the amount of missing data in the final matrix. Efforts were taken to ensure duplicate specimens were not scored as well.

Quantitative morphological data were standardized by log transformation prior to multivariate analysis in order to remove character weighting (Timmerman-Erksine et al. 2002). The Kruskal-Wallis test was implemented as the Wilcoxon npar-1way test (SAS Institute, Inc. 1999) to assess significance of variation within variables. Characters with significant variation ($P<0.001$) were then used in multivariate and cluster analyses.

The %distance macro was implemented using the D-Gower algorithm in order to compute a distance matrix using qualitative, categorical, and ratio variables (Kuo 1997, SAS Institute, Inc. 1999). The distance matrix was implemented in PAUP 4.0b* (Swofford 2002) to generate a phenogram by unweighted pair-group method using arithmetic averages (UPGMA). The resulting phenogram was used to identify discrete taxonomic groups (Rohlf 1993). Multidimensional scaling (MDS) was chosen for ordination analysis since the algorithm does not exclude variables or specimens with missing data, therefore retaining maximum representation of material measured (SAS Institute Inc. 1999).

Molecular Analyses

Analysis of genetic variation in *Malacothamnus* utilized sequences of the nuclear ribosomal internal transcribed spacer region (ITS). Sequences were generated directly from products amplified with the ITS4 and ITS5 primers of White et al. (1990). Methods of DNA extraction and reaction conditions for DNA amplification were as those of Slotta (2000). Prior to sequencing, products were cleaned with the Qiaquick PCR Purification kit (Qiagen Corp., Madison, WI) and quantified by gel electrophoresis. Sequencing reactions were prepared with PE Biosystems Big Dye Terminator Kit (Foster City, CA) and electrophoresis conducted at Virginia Bioinformatics Institute (Blacksburg, VA). Sequences were manually edited and contiguous sequences generated using the SeqMan program of DNA Star/Lasergene (Madison, WI) and aligned in MegAlign using the CLUSTAL W algorithm. Boundaries for the ITS-1, ITS-2, and 5.8s rDNA regions were determined by comparing sequences available in GenBank (*Gossypium darwinii* U12716). Maximum parsimony was performed in PAUP*4b10 (Swofford 2002) with 1000 heuristic searches using tree bisection-reconnection (TBR).

Prior to maximum likelihood analysis, the ModelTest 3 program was used to determine the appropriate model of evolution to use (Posoda and Crandall 1998). The program chooses the appropriate model that best fits the data by testing 56 models of evolution and estimating base change frequencies and shape of the gamma distribution. Maximum likelihood was conducted in PAUP*4b10 (Swofford 2002) with heuristic searches using the TrNeF model (Tamura and Nei 1993) with an estimated gamma shape parameter (gamma). This model was chosen as a result of the examination of the ModelTest results.

Results

Multivariate Analyses of Morphological Data

Variables included in the generation of the MDS matrix and those for the UPGMA distance matrix are indicated in Table 5.2. Characters which had the most significant correlation ($P<0.001$) to the first two dimensions in the MDS analysis were the amount of pubescence on stems and leaves, shape of the sepals, ratio of sepal length to width, number of stellate rays, and length of the rays. Figures 5.2 to 5.5 illustrate the results of the MDS, in Fig. 5.3 to 5.5 some taxa are excluded in order to better visualize relationships between specimens within taxa.

Specimens labeled by collectors as *M. arcuatus* were either grouped with *M. aboriginum*, *M. clementinus*, or *M. davidsonii*. Those labeled as *M. niveus* clustered with *M. jonesii*. The most diverse cluster includes those specimens identified as *M. fasciculatus*. In Fig. 5.2, the varieties of *M. fasciculatus* (var. *catalinense*, var. *fasciculatus*, var. *nesioticus*, and var. *nuttallii*)

are assigned a single symbol. In Fig. 5.3 each variety is assigned a different symbol and the relationships between these varieties can be examined. The second most diverse cluster included specimens identified as *M. fremontii*, *M. hallii*, *M. helleri*, and *M. howellii*. The majority of points for these taxa cluster closely together. These also overlap with the cluster of *M. fasciculatus* var. *fasciculatus* and *M. fasciculatus* var. *nesioticus*.

In the generation of the distance matrix, the data set was subdivided into one set with stipitate trichomes and one without. Figure 5.6 includes those specimens without stipitate trichomes and includes *M. abbottii*, *M. fasciculatus* var. *fasciculatus*, var. *nesioticus*, var. *nuttallii*, *M. fragrans*, *M. gracile*, *M. hallii M. jonesii*, *M. marrubioides*, *M. mendocinense*, *M. niveus*, *M. thurberi* and the specimens classified as unknown. Figure 5.7 includes those specimens with stipitate trichomes and includes *M. aboriginum*, *M. catalinense*, *M. clementinus*, *M. davidsonii*, *M. densiflorus*, *M. fasciculatus* var. *laxiflorum*, *M. fremontii*, *M. helleri*, *M. howellii*, *M. orbiculatus*, *M. palmeri* (var. *involucratus*, var. *lucianus*, and var. *palmeri*), and *M. paniculatus*.

Maximum Likelihood Estimates of Molecular Data

The ITS region in *Malacothamnus* consists of 719 aligned nucleotides with 539 invariable sites and 59 parsimony informative sites. The phylogenetic tree resulting from the maximum parsimony analysis (not shown) indicated all representatives of *Malacothamnus* as an unresolved, well-supported (96% bootstrap support) clade (244 steps long with 845 trees being equally parsimonious). One clade with representatives of *M. fasciculatus*, *M. densiflorus* and *M.*

marrubioides and another with *M. davidsonii* and *M. palmeri* formed, each with weak bootstrap support (54% and 63%, respectively).

Maximum likelihood was calculated using TrNef + G with the following parameters. The model included base transformation rates of R(a)=1.0000, R(b)=1.4124, R(c)=1.0000, R(d)=1.0000, R(e)=2.7374, and R(f)=1.0000. The gamma distribution shape parameter was estimated to be 0.368. This model was chosen since the results of ModelTest revealed TrNef-G to best fit the data in comparison to all other models ($P<0.000001$). The ML tree (Fig. 5.8) indicates *Malacothamnus* to be monophyletic. The relationships suggested in the analysis are *M. davidsonii* and *M. palmeri* and *M. fasciculatus* with *M. densiflorus* and *M. marrubioides* as another clade. An additional, and unexpected, clade of *M. aboriginum* and *M. fasciculatus* var. *nesioticus* resulted in the ML analyses as well.

Discussion

Character Variation

This study has attempted to encompass the geographical distribution of taxa in *Malacothamnus* in a survey of the complexity of morphological variation within the genus. Variation of morphological traits in the genus accounts for the numerous taxa described in the available treatments (Estes 1925, Eastwood 1936, Kearney 1951, Bates 1963, 1993). Adaptations to local stresses (e.g., water availability, herbivores, or competition) have led to minor variations within some taxa, which have then been classified as unique species or varieties (Bates 1963).

Our results from examination of 419 specimens concur with those of Benesh and Elisens (1999) that vegetative characters used to delimit species in *Malacothamnus* are too variable and are difficult to quantify in describing species in the genus. For example, Kearney (1951) noted *M. orbiculatus* as the species most difficult to satisfactorily define. He distinguished *M. orbiculatus* from *M. fremontii* by the thinner and less pubescent leaves and calyx of *M. orbiculatus*. In distinguishing *M. orbiculatus* from *M. marrubioides*, Kearney stated that leaves in *M. orbiculatus* were larger than in *M. marrubioides* (<4cm) and that leaves of *M. orbiculatus* were cordate, versus truncate and unlobed in *M. marrubioides*. The distribution of *M. orbiculatus* is also anomalous, with records from the eastern slopes of the Sierra Nevada and at elevations higher (1200m to 2800m) than any other species in the genus. Although differences in elevation may be informative in delimiting taxa in *Malacothamnus*, elevation was not included in this analysis since this datum is often omitted from herbarium collections and, when provided, it may not be accurate for older collections.

All characters measured, except the shape of the leaf base, exhibited significant variation between taxa in the Kruskal-Wallis test. Characters quantifying pubescence, calyces, and the involucre proved to be the most valuable in distinguishing taxa in the MDS and UPGMA analyses. The characters most informative in the MDS analysis were pubescence of the upper and lower leaf surfaces, the number of rays per stellate trichome, type of pubescence, and the length of the trichome rays. Five qualitative and five quantitative characters were retained for the UPGMA analysis characterized the inflorescence. The remaining characters were not used in this analysis since they did not contribute to the formation of the dendrogram. Prior to UPGMA

tests, the data set was subdivided into two groups, those with stipitate trichomes and those without. This characterization of trichomes was part of the basis for subdividing the genus into two groups by Eastwood (1936) and Kearney (1951).

Genetic variation within *Malacothamnus* was also characterized by sequences of the ITS region. The maximum likelihood analysis revealed two sister clades within the genus. The lower clade with *M. densiflorus*, *M. fasciculatus* var. *fasciculatus*, *M. fasciculatus* var. *nuttallii*, and *M. marrubioides* represents the southernmost collections included in the study, with taxa from populations in Los Angeles, Santa Barbara, and Ventura Counties. The morphology within this clade includes stipitate and non-stipitate trichomes, an open panicle inflorescence, a thryse-like inflorescence, and various numbers of stellate rays. Although the taxa can be distinguished morphologically, gene flow has recently occurred between them according to the segregation of these samples from the second clade.

The second, and larger, clade unites all of the remaining samples sequenced for ITS. However, little variation in the ITS region was detected between the samples as indicated by the zero branch lengths in the ML tree. One noteworthy group within this large clade is that of *M. aboriginum* and *M. fasciculatus* var. *nesioticus*. The accessions of *M. aboriginum* included in this clade are from San Benito County east of Pinnacles National Monument in the Diablo Range. Accessions of *M. fasciculatus* var. *nesioticus* represent two populations of a taxa that is endemic to Santa Cruz Island, south of Santa Barbara. Morphologically the taxa are quite distinct. *Malacothamnus fasciculatus* var. *nesioticus* has an open panicle inflorescence, non-stipitate trichomes, sparse pubescence on the upper leaf surface, dense pubescence on the lower

leaf surface, slender pedicels (0.5 to 1.4 cm long), and an overall white pubescence. In *M. aboriginum*, the inflorescence is a thyrse with more than 5 flowers per node, with dense pubescence on both the upper and lower leaf surfaces, reduced pedicels (<0.5 cm long), and the pubescence is tawny. It is unlikely that recent gene flow has occurred between the species given their isolated populations. Most likely, both taxa are recently derived from a common ancestor, possibly *M. fasciculatus* var. *fasciculatus*, which overlaps the distribution of the other two taxa.

Taxonomic Implications

Bates (1993) surmised that species in *Malacothamnus* could potentially hybridize and do so when they occur in close proximity. He further noted that characters within *Malacothamnus* were highly polymorphic, especially the pubescence and characters of the inflorescence. Both of these characteristics in *Malacothamnus* have led to complex patterns of variation within the species as well. Kearney (1951) stated that *Malacothamnus* is a recently derived genus, with some taxa being widespread and exhibiting broad ranges of morphological traits, and other taxa occurring in small disjunct populations. A recent origin or recurrent hybridization events within the genus are equally plausible explanations for the lack of distinct species in *Malacothamnus*. In this study, the multiple methods used were able to distinguish between species in the genus; some taxa are more clearly distinct than others.

As Eastwood (1936) and Kearney (1951) had suggested, *Malacothamnus* can be divided into two sections. Section one is comprised of the *M. fasciculatus* group, which can be characterized as having an appressed (non-stipitate) pubescence of short-rayed trichomes (Kearney 1951). My findings include the following taxa in the *M. fasciculatus* group: *M.*

abbottii, *M. catalinensis*, *M. fasciculatus* var. *fasciculatus*, *M. fasciculatus* var. *nesioticus*, *M. fasciculatus* var. *nuttallii*, *M. jonesii*, and *M. marrubioides*. Among these taxa, an open panicle dominates, although the inflorescence in *M. marrubioides* approaches a thyrse with an average of six flowers per node.

The second section, the *M. fremontii* group, as Eastwood (1936) and Kearney (1951) defined it, is characterized as having a stipitate pubescence, which may appear woolly, and the calyx is densely lanate. The following species should be considered members of the group: *M. aboriginum*, *M. clementinus*, *M. davidsonii*, *M. densiflorus*, *M. fremontii*, *M. orbiculatus*, *M. palmeri*, and *M. paniculatus*. All members of the group have a shaggy pubescence with the stellate rays being up to 0.65 mm in length, and all, except *M. clementinus* and *M. fremontii*, have a tawny pubescence. Within this group, three types of inflorescences occur, capitulum (*M. palmeri*), panicle-like (*M. davidsonii* and *M. paniculatus*), and thyrse-like (*M. aboriginum*, *M. clementinus*, *M. densiflorus*, and *M. fremontii*). Two species in this group, *M. densiflorus* and *M. palmeri*, have populations with glandular trichomes and bristle trichomes in addition to the stellate pubescence.

Malacothamnus davidsonii approaches being an intermediate between the two sections in *Malacothamnus*. The trichomes in *M. davidsonii* may or may not be stalked, and the plants' habit is most similar to *M. fasciculatus*. The species, however, does occur in two disjunct locations, north in Monterey and San Luis Obispo (SLO) Counties and to the south in Los Angeles (LA) and San Bernardino (SB) Counties. Whether both locations are truly *M. davidsonii* has not been determined. The two accessions included in the ML phylogeny are not

located in the same clade. Material from a LA population is in a clade with two individuals fitting the description of *M. hallii*, both from Contra Costa County. The SLO material is nested in a clade with Monterey County accessions of *M. abbottii* and *M. palmeri*, which is sister to an accession of *M. clementinus* from San Clemente Island. Material from Contra Costa County identified as *M. hallii* and an unidentified specimen (from a population referred to as *M. fasciculatus* in herbaria) are located in a clade with the *M. davidsonii* material. The two sequences (non-*M. davidsonii*) were retrieved from material collected at the northern edge of the natural range of *Malacothamnus*. The clade has the longest length of any clade in the ML tree with the fewest taxa included. Specimens surveyed for *M. hallii* in the morphological analyses did not fit within the range of all characters for *M. fasciculatus*, to which it has been attributed in synonomy. In the UPGMA analysis, several specimens of *M. hallii* clustered together on a branch. In analyzing the MDS results, *M. hallii* is not allied with *M. fasciculatus*, but has closer affinities with *M. fremontii*. The species, *M. hallii*, may be the progeny of a past hybridization event between *M. fasciculatus* and *M. fremontii* since it shares morphological characters with both, however, it does resolve as a unique entity in this study.

Numerous specimens were identified by collectors as *M. arcuatus* or *M. orbiculatus*. As the MDS plot (Fig. 5.2) indicates, specimens identified as *M. arcuatus* may be correctly classified as *M. aboriginum*, *M. clementinus*, or *M. davidsonii*. Typically, these specimens had a tawny pubescence, moderately pubescent leaves, a few-flowered thyrse or a panicle, deltoid sepals, and a wide range as to the number of rays per trichome. In neither analysis, MDS nor UPGMA, do specimens of *M. arcuatus* form a cohesive group.

Regarding *M. orbiculatus*, again a wide range of morphology was found in this set of specimens, many of which were incorrectly assigned. The species is described as having round, unlobed leaves, a more or less shaggy pubescence, and widely varying shapes for calyx lobes (Kearney 1951). In my analyses, many specimens identified as *M. orbiculatus* are *M. fremontii* and others are *M. marrubioides*. Kearney (1951) did note that these are difficult to separate. However, *M. orbiculatus* can be distinguished from *M. fremontii* and *M. marrubioides* by several characters (Kearney 1951). The taxa were found in separate clusters in the UPGMA analysis (Fig. 5.6). Populations in the Sierra Nevada at high elevations are unique. As with *M. marrubioides*, these have a tawny pubescence and a thyrse-like inflorescence, but *M. orbiculatus* has fewer flowers and bracts per inflorescence. As Kearney noted, leaves in *M. orbiculatus* are larger than in *M. marrubioides*, this was found in the examination of specimens in this study as well. The species is different from *M. fremontii* in that the pubescence in *M. fremontii* is white, has a greater number of rays per trichome, is evenly distributed on both upper and lower leaf surfaces, and is denser on the calyces. The sepals in *M. fremontii* are also more deltoid than lanceolate as in *M. orbiculatus*.

Taxonomic Treatment

Malacothamnus Greene

Plants perennial shrubs that may have numerous spreading or nearly erect branches. Stems, leaves, bracts, and calyces more or less densely pubescent; pubescence typically stellate (stalked or un-stalked) with some taxa occasionally having glandular or bristle trichomes as well. Leaves nearly round and more or less shallowly to somewhat deeply lobed (3-5 lobes); base varies from cuneate to cordate to truncate to rounded to auriculate; texture smooth to rugose, rarely corrugated with prominent veins beneath. Inflorescence panicle-like, thyrse-like, or a terminal capitulum; if a panicle, peduncles long and slender and with few flowers in an open

inflorescence; if a thyse, pedicels approaching sessile and nodes may have numerous flowers; if a capitulum, bracts may obscure pedicels and calyces. An involucre of 3 subulate or filiform bractlets (either black or concolorous with the calyx) may be longer than calyx. Calyx 5-lobed, smaller than petals and sparsely to densely pubescent. Petals white to lavender and connate at the base. Staminal column as long as or shorter than petals. Stigmas capitate and usually equaling number of carpels. Fruits schizocarps with smooth thin walls and each section containing one seed. Seeds reniform and often with a fine pubescence.

Type species: *M. fasciculatus* (Nuttall) Greene.

Taxonomic Key to the Species of Malacothamnus

1. Flowers in dense, terminal clusters, subtended by a conspicuous involucre. 2
1. Flowers not in terminal clusters, either in open panicles or clusters along the stems; involucre of narrow bracts. 3

2. Involucre comprised of bracts 2cm wide, often longer than petals; leaves glabrous or nearly so above. *M. palmeri* var. *involucratus*
2. Involucre comprised of bracts 2.5cm long and half as wide, nearly as long as petals; leaves equally pubescent above and beneath. *M. palmeri* var. *palmeri*

3. Flowers in open panicle-like inflorescence; pedicels >2 mm long. 4
3. Flowers in dense clusters along stems; pedicels greatly reduced, approaching sessile. 5

4. Pubescence tawny, branches stout; <25 rays per trichome. 6
4. Pubescence white, branches slender; numerous (>25) short rays per trichome. 7

6. Leaves thick, >7cm long and wide; trichomes with few (<10) rays; calyx lobes lanceolate. *M. davidsonii*
6. Leaves thin, to 5cm long, wider than long; trichomes with >10 rays; calyx lobes cordate. *M. paniculatus*

7. Leaves <4cm wide and long, pubescence close velutinous; involucral bracts black; stem and leaf surfaces ash green. *M. jonesii*
7. Leaves >4cm wide and long, pubescence from lanate to velutinous; involucral bracts not black; stem and leaf surfaces appearing white with pubescence or green. 8

8. Branches erect or spreading; leaves velutinous, rugose to corrugated; pedicels <1cm long. 9
8. Branches spreading; leaves lanulose to villous, not rugose.; pedicels >1 cm long. 10

9. Branches erect; leaves thick, <5cm wide, round, veins very prominent beneath, upper surface with grooves, pubescence equal above and beneath. *M. abbottii*
9. Branches spreading; leaves thin, approaching 7cm wide, round, slightly rugose, pubescence above (lanulose) less than beneath (lanate). *M. hallii*

10. Pubescence woolly with long-rayed trichomes; pedicles stout (<1.5 cm long); calyx lobes cordate. *M. fasciculatus* var. *catalinensis*
10. Pubescence not woolly, rays short; pedicels slender, approaching 1.5cm long; calyx lobes subulate to lanceolate. 11

11. Pubescence of leaves equal above and beneath, leaves shallowly lobed to unlobed; staminal column half as long as petals. *M. fasciculatus* var. *fasciculatus*
11. Pubescence on upper leaf surface less than beneath, leaves distinctly lobed; staminal column greater than half as long as petals. 12

12. Leaves thin, <8cm wide, nearly round, acutely lobed, upper leaf surfaces lanulose, villous below. *M. fasciculatus* var. *nuttallii*
12. Leaves thick, wider (<8cm) than long (<7cm), shallowly lobed, upper leaf surfaces nearly glabous, villous beneath. *M. fasciculatus* var. *nesioticus*.

5. Pubescence white, woolly; branches erect. 13
5. Pubescence tawny, not woolly; branches erect or spreading. 14

13. Branches stout; leaves shallowly to moderately lobed with pubescence equal above and beneath; inflorescence with less than 5 flowers per node, bracts shorter than calyx, occasionally turning black upon maturity. *M. fremontii*
13. Branches slender; leaves sharply lobed with pubescence sparse above and villous beneath; inflorescence with up to 10 flowers per node, bracts longer than calyx, green. *M. clementinus*

14. Branches slender; leaves thin; bracts as long as or longer than calyx. 15
14. Branches stout; leaves thick; length of bracts approaching calyx length. 16

15. Pubescence of leaves equal above and beneath; leaves obscurely lobed; 3-6 flowers per node; pedicles <4mm long. *M. marrubioides*
15. Pubescence of leaves sparse above and villous beneath; leaves sharply 3-5 lobed; usually 10 flowers or more per node; pedicels 1-1.5c*M. M. densiflorus*

16. Leaves sharply lobed, pubescence equal above and beneath; up to 10 flowers per node and numerous leafy bracts per inflorescence. *M. aboriginum*

16. Leaves rounded, usually unlobed, rugose beneath, pubescence more dense on lower leaf surfaces than above; 3-6 flowers per node and few leafy bracts per inflorescence. *M. orbiculatus*

***Malacothamnus abbottii* (Eastwood) Kearney** *Leaflets of Western Botany* 6(6): 129. 1951

Basionym: *Malvastrum abbottii* Eastwood. *Leaflets of Western Botany* 1(18): 215. 1936. Holotype: E.K. Abbott in 1889 (California Academy of Sciences, not seen).

Plants <1.5m high; pubescence dense, unstalked, white; branches slender, erect, velutinous; leaf blades <5cm long, round, shallowly 3-lobed, rugose, pubescence on upper and lower surfaces equal, stellate trichomes with numerous (>30) short rays (<0.01mm); inflorescence panicle-like, 3-5 flowers per peduncle, few bracts within inflorescence; flowers pedicels <1cm, long calyx 1cm long by 0.4cm wide, velutinous, buds acuminate, petals 1-2cm long, staminal column shorter than petals. Type locality: Salinas River, Monterey County. Very rare, known only from a few locations in Monterey County.

***Malacothamnus aboriginum* (B.L. Robinson) Greene** *Leaflets of botanical observation and criticism* 1: 208. 1906.

Basionym: *Malvastrum aboriginum* B.L. Robinson in Gray, *Synoptic Flora of North America* 1(1): 311. 1897. Holotype: M.K. Curran in 1885 (California Academy of Sciences not seen).

Synonym: *Sphaeralcea aboriginum* (B.L. Robinson) Jepson *Flora of California* 2: 498. 1936.

Plants 2-3m high; pubescence coarse, dense, yellow; branches stout, erect, velutinous; leaf blades <7cm long, round to ovate, sharply 3-5-lobed, pubescence on upper and lower surfaces equal, stellate trichomes with numerous short rays (<0.05mm); inflorescence thyrse-like, to 10 flowers per node, numerous leafy bracts within inflorescence; flowers with pedicels <0.5cm long, bracts nearly as long as calyx, calyx +/- 1cm long and half as wide, lanate, buds acuminate, petals 1-2cm long, staminal column shorter than petals; carpels 2.5-3mm long, almost as wide, shallowly to deeply incised. Type locality: Indian Valley, Monterey County; Fresno, Monterey, and San Benito Counties.

***Malacothamnus clementinus* (Munz & I.*M.* Johnston) Kearney** *Leaflets of Western Botany* 6(6): 127. 1951.

Basionym: *Malvastrum clementinum* Munz and I.M. Johnston, *Bulletin of the Torrey Botanical Club* 51(7): 296. 1924. Holotype: P.A. Munz 6,684 (Pomona College Herbarium, now integrated with Rancho Santa Ana Botanical Garden) not included in the morphological study, but seen. Isotypes at NY and US seen.

Synonym: *Sphaeralcea orbiculata* var. *clementina* (Munz and I.M. Johnston) Jepson, *Flora of California* 2: 499. 1936.

Plants <1m high; pubescence shaggy, dense, white; branches slender, erect, woolly; leaf blades <6cm long, round to ovate, sharply 3-5-lobed, pubescence on upper surface sparse (6 trichomes per 0.5cm) and villous below (25+ trichomes per 0.5cm), stellate trichomes with 10-20 slender rays (<0.15mm long); inflorescence thyrse-like, to 10 flowers per node, few bracts within inflorescence; flowers with pedicels <0.5cm long, bracts longer than calyx, calyx 1cm long and half as wide, lanate, petals 1-2cm long, staminal column shorter than petals; carpels 2.5-3mm long. Type locality: San Clemente Island; Los Angeles County. Endemic to San Clemente Island.

***Malacothamnus davidsonii* (B.L. Robinson) Greene** *Leaflets of Botanical Observation and Criticism* 1: 208. 1906.

Basionym: *Malvastrum davidsonii* B.L. Robinson in Gray, *Synoptical Flora of North America* 1(1): 312. 1897. Lectotype: A. Davidson in 1895 (Gray Herbarium seen); (chosen by Kearney, *Leaflets of Western Botany* 6: 127. 1951). Isolectotypes, US and DS seen.

Synonym: *Sphaeralcea davidsonii* (B.L. Robinson) Jepson, *A Manual of the Flowering Plants of California*. P.634. 1925.

Plants 3-5m high; pubescence coarse, dense, yellow; branches stout, erect, velutinous; leaf blades <7cm long, round to ovate, sharply 3-5-lobed, pubescence on upper and lower surfaces equal, stellate trichomes with few short rays (<0.08mm long); inflorescence panicle-like, 3-7 flowers per node, few leafy bracts within inflorescence; flowers with pedicels 1cm long, bracts half as long as calyx, calyx to 1cm long and half as wide, villous, buds acuminate, petals 1-2cm long, staminal column half length of petals; carpels 2.5-3.5mm long, ovate, moderately incised. Type locality: San Fernando Valley, Los Angeles County; Los Angeles, Monterey, San Luis Obispo, and Ventura Counties.

***Malacothamnus densiflorus* (S. Watson) Greene** *Leaflets of Botanical Observation and Criticism* 1: 208. 1906.

Basionym: *Malvastrum densiflorum* S. Watson, *Proceedings of the American Academy of Arts and Sciences* 17: 368. 1882. Lectotype: Parish Brothers 738 (Gray Herbarium seen); chosen by Kearney (*Leaflets of Western Botany* 6: 113. 1951). Isolectotypes at DS and MO seen.

Synonyms: *Malvastrum viscidum* Abrams, *Bulletin of the Torrey Botanical Club* 34: 264. 1907. Isotypes seen (MO and US).

Sphaeralcea densiflora (S. Watson) Jepson, *Manual of the Flowering Plants of California* 633. 1925.

Sphaeralcea densiflora var. *viscida* (Abrams) Jepson, *Flora of California* 2: 498. 1936.

Malacothamnus densiflorus var. *viscidus* (Abrams) Kearney, *Leaflets of Western Botany* 6: 134. 1951.

Plants 2m high; pubescence dense, yellow, occasionally glandualar trichomes present; branches slender, erect, woolly; leaf blades thin, <6cm long, round to ovate, sharply 3-5-lobed, pubescence on upper surface sparse (6 trichomes per 0.5cm) and villous below (25+ trichomes per 0.5cm), stellate trichomes with 15-20 rays (<0.3mm long); inflorescence thyrse-like, to 10 flowers per node, <3 bracts within inflorescence; flowers with pedicels 1-1.5cm long, bracts as long or longer than calyx, calyx 1cm long and 0.4cm wide, villous, petals 1-2cm long, staminal column half as long as petals; carpels 2.2-3.8mm long, oval, shallowly incised. Type locality: San Jacinto Mountains, Riverside County; Orange, Riverside, San Diego Counties, California and Baja California Norte, Mexico.

Malacothamnus fasciculatus* (Nuttall) Greene var. *fasciculatus *Leaflets of Botanical Observation and Criticism* 1: 208. 1906.

Basionym: *Malva fasciculata* Nuttall, *A Flora of North America*: 1(2): 225. 1838. Lectotype: Nuttall s.n., Santa Barbara County (Gray Herbarium, seen), not included in the morphological study (designated by Kearney, *Leaflets of Western Botany* 6(6): 134. 1951).

Synonyms: *Malvastrum thurberi* A. Gray, *Memoirs of the American Academy* ser. 2, 5: 307. 1855. Holotype: *Malvastrum thurberi* (G. Thurber 709, NY) seen.

Malvastrum splendidum Kellogg, *Proceedings of the California Academy of Sciences* 1: 65. 1855. Holotype: W.A. Wallace, Los Angeles, not seen.

Malvastrum thurberi var. *laxiflorum* A. Gray, *Proceedings of the American Academy* 22: 291. 1887.

Malvastrum fasciculatum (Nuttall) Greene, *Flora of San Franscico* 108. 1891.

Malveopsis arcuata Greene, *Manual of the Region of the San Franscico Bay* 66. 1894. Lectotype: Greene in 1886 (UC) not seen; (designated by Kearney, *Leaflets of Western Botany* 6(6): 113. 1951).

Malvastrum arcuatus (Greene) Greene, *Leaflets of Botanical Observation and Criticism* 1: 208. 1906.

Malacothamnus fasciculatus var. *splendidus* (Kellogg) Abrams, *Bulletin of the New York Botanical Garden* 6: 417. 1910.

Sphaeralcea fasciculata (Nuttall) Arthur, *Torreya* 21: 11. 1921.

Malvastrum laxiflorum Davidson and Moxley, *Flora of Southern California* 233. 1923.

Malvastrum fasciculatum var. *laxiflorum* (A. Gray) Munz and I.M. Johnston, *Bulletin of the Torrey Botanical Club* 51: 296. 1924.

Sphaeralcea fasciculata var. *laxiflora* (A. Gray) Jepson, *Manual of the Flowering Plants of California* 634. 1925.

Malvastrum fasciculatum var. *laxiflorum* (Davidson and Moxley) Eastwood, *Leaflets of Western Botany* 1: 219. 1936.

Malvastrum parishii Eastwood, *Leaflets of Western Botany* 1: 219. 1936. Holotype: Parish 3,804 (CAS) seen.

Malacothamnus parishii (Eastwood) Kearney, *Leaflets of Western Botany* 6(6): 113. 1951.

Plants 1-5m high; pubescence unstalked, sparse to lanulose, white; branches slender, spreading; Leaves: blades 2-6cm long, round, shallow 3-5-lobed to unlobed, pubescence on upper and lower surfaces generally equal, stellate trichomes with numerous (>35) short rays (<0.01mm long); inflorescence panicle-like, 3-7 flowers per node with long slender peduncles, <3 leafy bracts within inflorescence; flowers with pedicels 1cm +/- 0.3cm long, bracts half as long as calyx, calyx to 1cm long and half as wide, pubescence sparse to lanulose, petals 1-1.5cm long, staminal column half the length of petals; carpels 2.5-3.2mm long, deeply incised. Type locality: Santa Barbara County; Los Angeles, Orange, Riverside, San Bernardino, Santa Barbara, and Ventura Counties, California and Baja California Norte and Sonora, Mexico.

***Malacothamnus fasciculatus* var. *catalinensis* (Eastwood) Kearney** *Leaflets of Western Botany* 6(6): 138. 1951.

Basionym: *Malvastrum catalinense* Eastwood, *Leaflets of Western Botany* 1(18): 215. 1936. Holotype: Eastwood 6,442 (California Academy of Sciences) not seen.

Synonym: *Malvastrum* var. *catalinense* (Eastwood) McMinn. *Manual of California Shrubs* 348. 1939.

Plants 1-3m high; pubescence stipitate, woolly, white; branches slender, spreading upright; leaves thin, blades <7cm long and <8cm wide, acutely 3-5-lobed, pubescence on upper surface glabrous to sparse and lower surface villous to lanate with long-rayed trichomes, stellate trichomes with <20 arms rays <0.05mm long; inflorescence panicle-like, 3-7 flowers per node with long slender peduncles, <3 leafy bracts within inflorescence; flowers with pedicels <1cm

long, bracts half as long as calyx, calyx <0.7cm long, lobes deltoid to cordate, villous to lanate, petals 1.3-2.6cm long, staminal column half length of petals; carpel 3.2-3.8mm long, deeply incised. Type localiy: Santa Catalina Island, Los Angeles County. Endemic to Santa Catalina Island.

***Malacothamnus fasciculatus* var. *nesioticus* (B.L. Robinson) Kearney** *Leaflets of Western Botany* 6(6): 134. 1951.

Basionym: *Malvastrum nesioticum* B.L. Robinson in Gray, *Synoptic Flora of North America* 1(2): 312. 1897. Holotype: E.L. Greene in 1886 (Gray Herbarium) seen.

Synonyms: *Malacothamnus nesioticus* (B.L. Robinson) Abrams, *Bulletin of the New York Botanical Garden* 6: 419. 1910.

Sphaeralcea nesiotica (B.L. Robinson) Jepson, *A Manual of the Flowering Plants of California*. 634. 1925.

Sphaeralcea fasculata var. *nesiotica* (B.L. Robinson) Jepson, *Flora of California* 2: 501. 1936.

Malvastrum fasciculatum var *nesioticum* (B.L. Robinson) McMinn, *Manual of California Shrubs* 348. 1939.

Plants 1-3m high; pubescence unstalked, sparse to lanulose, white; branches slender, spreading upright; leaves thick, blades <7cm long and <8cm wide, shallowly 3-5-lobed, pubescence on upper surface glabrous to sparse and lower surface villous, stellate trichomes with <30 arms, rays <0.03mm long; inflorescence panicle-like, <3 flowers per node with long slender branches spreading, <3 leafy bracts within inflorescence; flowers with pedicels <1.5cm long, bracts less than half as long as calyx, calyx 0.6-0.8cm long and half as wide, lobes lanceolate, lanulose to villous, petals <2cm long, staminal column half length of petals; carpels approximately 4mm long, deeply incised. Type locality: Santa Cruz Island, Santa Barbara County. An endangered variety of *M. fasciculatus* and endemic to Santa Cruz Island.

***Malacothamnus fasciculatus* var. *nuttallii* (Abrams) Kearney** *Leaflets of Western Botany* 6(6): 113. 1951.

Basionym: *Malacothamnus nuttallii* Abrams, *Bulletin of the New York Botanical Garden* 6: 419. 1910. Holotype: Abrams in 1908 (Dudley Herbarium) seen.

Synonyms: *Malvastrum nuttallii* (Abrams) Davidson and Moxley, *Flora of Southern California* 233. 1923.

Sphaeralcea fasciculata var. *nuttallii* (Abrams) Jepson, *Flora of California* 2: 501. 1936.

Malvastrum fasciculatum var. *nuttallii* (Abrams) McMinn, *Manual of California Shrubs* 348. 1939.

Plants 2-3m high; pubescence unstalked, sparse to lanulose, white; branches slender, spreading or erect; leaves thin, blades <8cm long, approximately equally wide, acutely 5-lobed, pubescence on upper (lanulose) less than lower (villous) surfaces, stellate trichomes with <20 arms, rays <0.02mm; inflorescence panicle-like, 3-5 flowers per node with long slender peduncles spreading, <3 leafy bracts within inflorescence; flowers with pedicels <1.5cm long, bracts less than half as long as calyx, calyx to 1cm long and less than half as wide, lobes subulate to lanceolate, lanulose to villous, petals to 2.5cm long, staminal column to 1.5cm long; carpels 3-5mm long, deeply incised. Type locality: Casitas Pass, Ventura County; Santa Barbara and Ventura Counties.

***Malacothamnus fremontii* (Torrey ex A. Gray) Greene** *Leaflets of Botanical Observation and Criticism* 1: 206. 1906.

Basionym: *Malvastrum fremontii* Torrey ex A. Gray, *Memoirs of the American Academy* ser. 2, 4: 21. 1849. Holotype; Fremont in 1846 (New York Botanical Garden) seen.

Synonyms: *Malvastrum foliosus* S. Wats., *Proceedings of the American Academy* 20: 356. 1885. Holotype: Orcutt, in 1884 (Gray Herbarium) not seen.

Malvastrum fremontii var. *cercophorus* B.L. Robinson, *Synoptical Flora of North America* 1(1): 311. 1897.

Sphaeralcea fremontii (Torrey ex A. Gray) Jepson, *Manual of Flowering Plants of California* 633. 1925.

Malvastrum helleri Eastwood, *Leaflets of Western Botany* 1(18): 216. 1936. Holotype: Heller 13,242 (CAS) not seen, isotypes (US and MO) seen.

Malvastrum howellii Eastwood, *Leaflets of Western Botany* 1(18): 216. 1936. Holotype: J.T. Howell May 12, 1931 (CAS) not seen, isotypes (US and MO) seen.

Malvastrum howellii var. *cordatum* Eastwood, *Leaflets of Western Botany* 1(18): 216. 1936. Holotype: C. Dudley in 1935 (CAS) not seen.

Malacothamnus foliosus (S. Watson) Kearney, *Leaflets of Western Botany* 6(6): 113. 1951. Lectotype: C.R. Orcutt in 1884 (MO) seen. Isolectotype (US) seen.

Malacothamnus helleri (Eastwood) Kearney. *Leaflets of Western Botany* 6(6): 113. 1951.

Malacothamnus howellii (Eastwood) Kearney, *Leaflets of Western Botany* 6(6): 113. 1951.

Malacothamnus fremontii subsp. *cercophorus* (B.L. Robinson) Munz., *Aliso* 4: 94. 1958.

Plants <3m high; pubescence stipitate, lanate to velutnious, white; branches stout, erect; leaves thick, blades to 9cm long and 9cm wide, shallowly 5-lobed to unlobed, pubescence on upper and lower surfaces velutinous, obscuring leaf tissue, stellate trichomes with to 30 arms, rays <0.10mm long; inflorescence thyrse-like, 3-5 flowers per node, <3 leafy bracts within inflorescence; flowers with pedicels <5cm, long bracts half as long as calyx turning black, calyx to 1.2cm long and 1/3 as wide, lobes subulate to lanceolate, velutinous, petals to 2cm long, staminal column to 1cm long; carpels 2-4mm long, obovate, shallowly incised. Type locality: "interior of California;" Alameda, Contra Costa, Merced, Monterey, Santa Clara, and Stanislaus Counties.

***Malacothamnus hallii* (Eastwood) Kearney** *Leaflets of Western Botany* 6(6): 113. 1951.

Basionym: *Malvastrum hallii* Eastwood, *Leaflets of Western Botany* 1: 216. 1936. Holotype: Hall and Essig 10,131 (University of California, Berkeley) seen.

Synonyms: *Sphaeralcea fasciculata* var. *elmeri* Jepson, *A Flora of California* 1: 501. 1936. Holotype: Elmer 4,395 (JEPS) seen.

Malvastrum mendocinense Eastwood, *Leaflets of Western Botany* 2: 188. 1939. Lectotype: Eastwood and Howell 4,582, CAS) not seen, isolectotype (NY) seen (designated by Kearney, 1951).

Malacothamnus mendocinensis (Eastwood) Kearney, *Leaflets of Western Botany* 6(6): 134. 1951.

Plants 3m high; pubescence unstalked, white; branches stout, spreading, villous to velutinous; leaf blades <7cm long, wider than long, cordate, rugose, shallowly 3-5 rounded lobes, pubescence on upper surface lanulose (<20 trichomes per 0.5cm) and lanate below (30+ trichomes per 0.5cm), stellate trichomes with 10-20 rays (<0.07mm long); inflorescence panicle-like, 3 to 7 flowers per node, 3-4 leafy bracts within inflorescence; flowers with pedicels <0.5cm long, bracts half as long as calyx, calyx <1cm long and 0.4cm wide, subulate to lanceolate, lanate, petals <1.7cm, staminal column half as long as petals; carpels: 2-3mm long, almost as wide, very shallowly incised. Type locality: Mt. Diablo, Contra Costa County; Contra Costa, Lake, Mendocino, Merced, San Joaquin, and Santa Clara Counties.

***Malacothamnus jonesii* (Munz) Kearney**, *Leaflets of Western Botany* 6(6): 135. 1951.

Basionym: *Malvastrum jonesii* Munz, *Bulletin of the Southern California Academy of Sciences* 24: 88. 1925. Holotype: M.E. Jones 223 (Pomona College Herbarium, now integrated with Rancho Santa Ana Botanical Garden) observed, not included in the morphological study.

Synonyms: *Sphaeralcea fasciculata* var *jonesii* (Munz) Jepson, *Flora of California* 2: 501. 1936.

Malvastrum dudleyi Eastwood, *Leaflets of Western Botany* 1(18): 218. 1936. Holotype: C. Dudley in 1929, (California Academy of Sciences) seen.

Malvastrum fragrans Eastwood, *Leaflets of Western Botany* 1(18): 218. 1936.

Malvastrum gracilis Eastwood, *Leaflets of Western Botany* 1(18): 219. 1936. Holotype: Eastwood 14,996 (CAS) not seen, but isotype (US) seen.

Malvastrum niveum Eastwood, *Leaflets of Western Botany* 1(18): 232. 1936. Holotype: M.E. Wall in 1933 (CAS), seen.

Malvastrum fremontii var. *niveum* (Eastwood) McMinn, Manual of California Shrubs 343. 1939.

Malacothamnus gracilis (Eastwood) Kearney, *Leaflets of Western Botany* 6(6): 130. 1951.

Malacothamnus niveus (Eastw.) Kearney, *Leaflets of Western Botany* 6(6): 123. 1951.

Plants <3m high; pubescence unstalked, closely lanate to velutnious, white; branches slender, erect, occasionally turning black with age; leaves thick, blades <4cm long and <4cm wide, shallowly 3-lobed to unlobed, approaching a diamond shape, pubescence on upper and lower surfaces close velutinous, giving leaves an ash-green appearance, surface rugose with prominent veins beneath, stellate trichomes with to 30 arms, rays <0.06mm long; inflorescence panicle-like, 3-5 flowers per node, <3 leafy bracts within inflorescence; flowers with pedicels slender, <7cm long, bracts more than half as long as calyx turning black, calyx to 1cm long and half as wide, lobes subulate to lanceolate, velutinous, petals to 2cm long, pale pink, staminal column to 1cm long, buds and flowers sweet smelling; carpels 3mm long, round, deeply incised. Type locality: Paso Robles, San Luis Obispo County; Monterey and San Luis Obispo Counties.

***Malacothamnus marrubioides* (Durand and Hilgard) Greene**, *Leaflets of Botanical Observation and Criticism* 1: 208. 1906.

Basionym: *Malvastrum marrubioides* Durand and Hilgard, *Journal of the Academy of Natural Science of Philadelphia* ser. 2, 3: 38. 1855. Holotype: Heermann in 1853, (Gray Herbarium) seen.

Synonyms: *Malvastrum gabrielense* Munz and I.M. Johnston, *Bulletin of the Torrey Botanical Club* 52: 223. 1925. Holotype: F.W. Peirson 774 (Pomona College, now integrated with Rancho Santa Ana Botanical Garden) seen, not included in the morphological study.

Sphaeralcea densiflorus var. *gabrielensis* (Munz and I.M. Johnston) Jepson. *Flora of California* 2: 501. 1936.

Plants 2m high; pubescence more or less stipitate, closely lanate to velutnious, tawny; branches slender, erect; leaves thin, green, blades <6cm long and <6cm wide, obscurely 3-5-lobed to

unlobed, cordate, reticulate beneath, pubescence on upper and lower surfaces <25 trichomes per 0.5cm, surface rugulose, stellate trichomes with <20 arms, rays <0.06mm long; inflorescence thyrse-like, 3-6 flowers per node, 3-6 leafy bracts within inflorescence nearly as large as leaves; flowers with pedicels stout, <0.4cm long, bracts nearly as long as or slightly longer than calyx, calyx to 1.2cm long and 1/3 as wide, lobes lanceolate to deltoid, lanulose to villous, petals to 2cm long, pale to deep pink, staminal column to 1cm long; carpels 2.5-3.5mm long, round, shallowly incised. Type locality: Fort Miller, Fresno County; Fresno, Kern, Los Angeles, San Luis Obispo, and Ventura Counties.

***Malacothamnus orbiculatus* (Greene) Greene** *Leaflets of Botanical Observation and Criticism* 1: 208. 1906.

Basionym: *Malvastrum orbiculatum* Greene, *Flora of San Francisco* 109. 1891. Holotype: Greene in 1889 (University of Notre Dame) not seen.

Synonyms: *Malvastrum fremontii* var. *orbiculatum* (Greene) I.M. Johnston, *Plant World* 22: 109. 1919.

Sphaeralcea orbiculata (Greene) Jepson, *Flora of California* 2: 499. 1936.

Plants 2m high; pubescence more or less stipitate, lanate to velutnious, tawny, shaggy; branches stout, erect; leaves thick, blades to 7cm long and 8cm wide, obscurely 3-lobed (uncommon) to unlobed, nearly entire, rugose beneath, pubescence on upper surface half that of lower surfaces (<20 trichomes per 0.5cm), stellate trichomes with <15 arms, rays <0.1mm long; inflorescence thyrse-like, 3-6 flowers per node, 3-5 leafy bracts nearly as long as leaves at base of inflorescence; flowers with pedicels stout, <0.6cm long, bracts 3/4 as long as calyx, calyx to 1.2cm long and 1/3 as wide, lobes subulate to lanceolate, lanate to villous, petals to 2cm long, staminal column more than half length of petals; carpels: 2.2-3.2mm long, ovate, slightly incised. Type locality: “mountains of Tehachapi,” Kern County; Inyo, Kern, Los Angeles, and San Bernardino Counties. Reaches elevations above the pine belt, 1300-2800M.

Malacothamnus palmeri* (S. Watson) Greene var. *palmeri *Leaflets of Botanical Observation and Criticism* 1: 208.

Basionym: *Malvastrum palmeri* S. Watson, *Proceedings of the American Academy* 12: 250. 1877. Holotype: Palmer 50, in 1876 (Gray Herbarium) seen. Isotypes (UC and US) seen.

Synonyms: *Sphaeraclea palmeri* (S. Watson) Jepson, *Manual of the Flowering Plants of California* 633. 1925.

Malacothamnus palmeri var. *lucianus* Kearney. *Leaflets of Western Botany* 7: 289. 1955. Holotype: Howell 30,642 (New York Botanical Garden) seen. Isotypes (CAS and US) seen.

Plants up to 2.5m high; pubescence stipitate, tawny, occasionally glandular and bristle along with stellate trichomes; branches stout, erect, very leafy, petioles >6cm long; leaves thin, blades up to 7cm long and 8cm wide, green, sharply 5-lobed, pubescence on upper and lower surfaces nearly equal (20-30 trichomes per 0.5cm), stellate trichomes with 7-14 arms, rays >0.1mm long, pubescence may appear granular; inflorescence a capitulum, >10 flowers per node, >10 leafy bracts per inflorescence; flowers with pedicels stout to sessile, bracts to 2.5cm long and half as wide, calyx to 1.5cm long and half as wide, lobes subulate to lanceolate, lanate, petals 2-3.5cm long, staminal column to 2cm long; carpels to 4mm long, ovate, deeply and narrowly incised, stalked. Type locality: Cambria, San Luis Obispo County; Monterey and San Luis Obispo Counties.

***Malacothamnus palmeri* var. *involucratus* (B.L. Robinson) Kearney** *Leaflets of Western Botany* 6(6): 121. 1951.

Basionym: *Malvastrum involuctratum* B.L. Robinson, *Synoptic Flora of North America* 1: 310. 1897. Holotype: T.S. Brandegee (Gray Herbarium) seen. Paratype, Eastwood in 1893 (California Academy of Science) seen.

Synonym: *Malvastrum palmeri* var *involuctratum* (B.L. Robinson) McMinn, *California Shrubs* 339. 1939.

Plants up to 2.5m high; pubescence stipitate, tawny, occasionally glandular and bristle along with stellate trichomes; branches stout, erect, very leafy, petioles 4-6cm; leaves: thin, cordate, blades up to 5cm long and 7cm wide, green, sharply 5-lobed, pubescence on upper surface sparse (6 trichomes per 0.5cm) and lower surfaces lanate (25-30 trichomes per 0.5cm), stellate trichomes with 10 arms, rays >0.1mm long, pubescence may appear granular; inflorescence a capitulum, >8 flowers per node, >8 leafy bracts per inflorescence; flowers with pedicels stout to sessile, bracts to 2cm long and nearly as wide, calyx to 1.5cm long and 1/3 as wide, lobes lanceolate, villous to lanate, petals 1-3cm long, staminal column up to 1.5cm long; carpels: to 4mm long, ovate, deeply and narrowly incised, unstalked. Type locality: Jolon, Monterey County; Monterey and San Luis Obispo Counties.

***Malacothamnus paniculatus* (A. Gray) Kearney** *Leaflets of Western Botany* 6(6): 123. 1951.

Basionym: *Malvastrum marrubioides* var. *paniculatum* A. Gray, *Proceedings of the American Academy* 22: 290. 1897. Holotype: Orcutt in 1886 (Gray Herbarium) seen. Isotype (US) seen.

Synonym: *Malvastrum* paniculatum (A. Gray) Wiggins, Madroño 10: 184. 1950.

Plants up to 1-2.5m high; pubescence stipitate, tawny; branches stout, erect, petioles <2cm; leaves thin, deltoid, blades up to 5cm long and 6cm wide, moderately 3-5-lobed, pubescence on upper and lower surfaces lanate (25-30 trichomes per 0.5cm), stellate trichomes with 10-16 arms, rays 0.1mm long; inflorescence panicle-like with long slender peduncles (to 30cm long), 3-7

flowers per node, 3-8 leafy bracts per inflorescence; flowers with pedicels slender, to 2cm, bracts half as long as calyx and filiform, calyx to 1.3cm long and 1/3 as wide, lobes cordate, 0.5-0.8cm wide at base, villous to lanate, petals to 2cm long, staminal column to 1cm; carpels to 2.5mm long, suborbicular, moderately incised, unstalked. Type locality: Ensenada de Todos Santos, Baja California Norte, Mexico.

Literature Cited

Abrams, L. 1907. *Malvastrum viscidum* sp. nov. *Bulletin of the Torrey Botanical Club* 34: 264.

Bates, D.*M*. 1963. *The Genus Malacothamnus*. Ph.D. Dissertation, University of California, Los Angeles.

Bates, D. *M*. 1993. *Malacothamnus*. In Hickman, J.C., ed. *The Jepson Manual: Higher plants of California*. Berkeley and Los Angeles: University of California Press. Pp. 747-753.

Bates, D.*M*. and O.J. Blanchard, Jr. 1970. Chromosome numbers in the Malvales II: New or otherwise noteworthy counts relevant to classification in the Malvaceae tribe Malveae. *American Journal of Botany* 57: 927-934.

Benesh, D.L. and W.J. Elisens. 1999. Morphological variation in *Malacothamnus fasciculatus* (Torrey and A. Gray) E. Greene (Malvaceae) and related species. *Madroño* 46: 142-152.

Eastwood, A. 1936. The shrubby *Malvastrum*s of California with descriptions of new species and a key to the known species. *Leaflets of Western Botany* 1: 213-220.

Eastwood, A. 1939. New California Plants. *Leaflets of Western Botany* 2: 186-188.

Estes, F. 1925. The Shrubby *Malvastrums* of Southern California. *Bulletin of the Southern California Academy of Science* 24: 81-87.

Greene, E. L. 1906. Certain malvaceous types. *Leaflets of Botanical Observation and CriticisM*. Pp. 205-209.

Harris, J.G. and *M*. Woolf Harris. 2001. *Plant Identification Terminology*: *An Illustrated Glossary*, 2nd ed. Spring Lake, UT: Spring Lake Publishing.

Kearney, T.H. 1951. The American genera of Malvaceae. *American Midland Naturalist* 46: 93-131.

Kuo, A. 1997. The Distance Macro: Preliminary Documentation, 2nd ed. Cary, NC: SAS Institute Inc.

Munz, P.A. and I. Johnston. 1925. California Plants. *Bulletin of the Torrey Club* 52: 223-224.

Posada, D. and K.A. Crandall. 1998. ModelTest: testing the model of DNA substitution. *Bioinformatics* 14: 817-817.

Rohlf, F.J. 1993. Multidimensional paleobiology. *Bulletin of Mathematical Biology* 55: 491-493.

SAS Institute, Inc. 1999. SAS software, Version 8 of the SAS System for PC. Copyright 1999 Cary, NC: SAS Institute Inc.

Slotta, T.A.B. 2000. *Phylogenetic analysis of* Iliamna *(Malvaceae) using the internal transcribed spacer*. *M*.S. Thesis. Virginia Polytechnic Institute and State University.

Skinner, *M*., D. Tibor, R. Bittman, B. Ertter, T. Ross, S. Boyd, A. Sanders, J. Shevock, and D. Taylor. 1995. Research needs for conserving California's rare plants. *Madroño* 42: 211-241.

Swofford, D. 2002. *PAUP*4.10: phylogenetic analysis using parsimony*. Sunderland, Mass.: Sinauer and Assoc.

Tamura, K. and *M*. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512-526.

Tate, J. 2003. *Andeimalva*, A new genus of Malvaceae from Andean South America. *Lundella* 6: 10-18.

Thomas, T. 2000. *Thirteen Plant Taxa from the Northen Channel Islands Recovery Plan* Report to Region 1, U.S. Fish and Wildlife Service, Portland, Oregon. Pp. 43-46.

Timmerman-Erskine, *M*., R.R. Dute, and R.S. Boyd. 2002. Morphometric analysis of the *Trillium pusillim* Michaux complex (Trilliaceae) of the Southeastern United States. *Castanea* 67: 109-119.

White, TJ, T Bruns, S Lee and J Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protoocols: A guide to Methods and Applications,* D.G.*M*. Innis, J. Sninsky and T. White, eds. San Diego, CA: Academic Press. Pp. 315-322.

Table 5.1. Subdivision of *Malacothamnus* by Eastwood (1936) and Kearney (1951) based on characters of the inflorescence and pubescence features.

	Eastwood	**Kearney**
M. fasciculatus	*Malvastrum fasciculatum*	*Malacothamnus fasciculatus*
Group	*M. abbottii, M. aboriginum,*	*M. arcuatus, M. hallii*
	M. arcuatum, M. catalinense,	*M. mendocinensis, M. parishii*
	M. davidsonii, M. densiflorum,	*M. fasciculatus* var. *catalinensis,*
	M. gabrielense, M. hallii,	var. *fasciculatus,* var. *laxiflorus,*
	M. involucratum, M. laxiflorum,	*var. nesioticus,var. nuttallii*
	M. marrubioides, M. nesioticum,	
	M. nuttallii, M. orbiculatum,	
	M. palmeri, M. parishii	
	M. spendidum,	
	M. mendocinense (1939)	
M. fremontii	*M. fremontii*	*M. fremontii*
Group	*M. clementinum, M. dudleyi,*	*M. helleri, M. howellii,*
	M. fragrans, M. gracile, M. helleri,	*M. marrubioides, M. niveus,*
	M. howellii var. *howellii,*	*M. orbiculatus*
	M. howellii var. *cordatum,*	
	M. jonesii	
Unplaced		*M. abbottii, M. aboriginum,*
		M. clementinus, M. davidsonii,
		M. densiflorus, M. foliosus,
		M. jonesii, M. paniculatus,
		M. palmeri var. *palmeri,*
		M. palmeri var. *involucratus*

Table 5.2. Morphological characters examined in assessing taxonomic boundaries in *Malacothamnus*. Significant characters are indicated by an asterisk (*) and those used in the cluster analysis are indicated by a plus symbol (+). Terminology for characters as defined in Harris and Woolf Harris (2001).

1. Trichomes: appressed (0), stipitate (1)
2. Type of Pubescence in Inflorescence: stellate (1), bristle (2), glandular (3)
3. Leaf Texture: smooth (1), rugulose (2), rugose (3), corrugated (4)
4. Leaf Base Shape: cuneate (1), cordate (2), truncate (3), rounded (4), auriculate (5)
5. Petiole Pubescence: glabrous(1), sparse(2), lanulose(3), villous(4), lanate(5), velutinous(6)
6. *+Stem Pubescence: glabrous(1), sparse(2), lanulose(3), villous(4), lanate(5), velutinous(6)
7. Stem Pubescence Type: stellate (1), bristle (2), glandular (3)
8. Pubescence Color: white (1), yellow or tawny (2)
9. *+Inflorescence Type: panicle (1), thyrse (2), capitulum (3)
10. *+Number of Flowers per Node: <3 (1), 4-9 (2), 10+ (3)
11. *+Number of Bracts per Inflorescence: <3 (1), 4-9 (2), 10+ (3)
12. *+Sepal Shape: subulate (1), lanceolate (2), deltoid (3), cordate (4)
13. *Bract Color: green or concolorous with sepals (1), brown or black (2)
14. *+Calyx Pubescence: glabrous(1), sparse(2), lanulose(3), villous(4), lanate(5), velutinous(6)
15. Petiole length
16. Leaf Width/Leaf Length
17. *Pubescence of Upper Leaf Surface: number of trichomes per 0.5cm transect
18. *Pubescence of Lower Leaf Surface: number of trichomes per 0.5cm transect
19. *+Pedicel Length
20. *+Sepal Length/Sepal Width
21. *+Bract Length
22. *+Number of Rays per Stellate Trichome
23. *+Petal Length
24. Staminal Column Length
25. *Length of Stellate Rays

Table 5.3. Specimens examined. **Holotypes** are indicated in bold underline, other **types** (isotypes, lectotypes, etc.) in bold.

M. abbottii

Monterey County: V. Yaden June 7, 1990 (US3,189,029); V. Yaden June 25, 1990 (JEPS 85,583)

M. aboriginum

Fresno County: C. Dudley 5 (CAS140,378); R.S. Ferris and R. Bacigalupi May 28, 1941 (DS290,430); L.R. Short 129 (UC661,093); L.R. Short and I.H. Johnston 20,978 (UC1,137,184); E.C. Twisselmann 2,457 (CAS608,465)

Monterey County: B.F. Howitt 1,462 (CAS519,982)

San Bernardino County: A. Eastwood and J.T. Howell 2,487 (CAS235,093)

San Benito County: R. Bacigalupi July 1, 1923 (DS129,386); A. Eastwood and J.T. Howell 2,491 (US1,678,213); *M*. and C. Epling 8,395 (MO964,614); C.B. Hardham 12,614 (CAS508,050); R.F. Hoover 4,715 (UC762,995); R.F. Hoover 4,715 (US2,002,564); R.F. Hoover 11,399 (UC1,392,975); P. Raven 8,809 (MO1,733,923); L.S. Rose 36,294 (MO1,230,530); E.C. Twisselmann 2,450 (CAS608,464); C.B. Wolf and P.C. Everett 4,372 (UC774,863)

Santa Clara County: J.L. McMurphy 39 DS104;

M. arcuatus

Baja California Norte, Mexico: W. R. Dudley May 18, 1895 (DS31,513);

Monterey County: H.L. Mason 5,893 (UC1,191,163)

San Mateo County: A. Eastwood 346 (MO836,861); A. Eastwood 346 (UC871,285); A. Eastwood May 1896 (UC18,767); H.*M*. Hall and F.*M*. Essig 10,131 (MO931,072); E.L. Greene 3,438 (MO 1,777,614); E.L. Greene 3,438 (US444,326); J.D. Randall 249 (DS63,046); L.S. Rose 35,213 (UC575,701); J.H. Thomas 5,309 (MO2,998,152); C.B. Wolf 3,746 (DS281,700)

Santa Clara County: W.R. Dudley May 21, 1898 (DS63,048); J.D. Randall 250 (DS606,132); H.K. Sharsmith 3,963 (UC1,001,162); I. Wiggins 8,544 (UC665,339); C.B. Wolf 3,746 (UC729,624)

M. clementinus

San Clemente Island, Los Angeles County: **Isotypes: P.A. Munz 6,684 (NY221,891);** P.A. Munz 6,684 (US1,368,336); *Other specimen examined*: S. Junak SCI862 (US2,886,797); S. Junak SCI865 (UC1,738,524); R. Moran 22,697 (MO no number); R. Moran 22,697 (UC1,430,061); N. Murbarger 224 (UC557,734); F.W. Pierson 3,458 (DS139,690); F.W. Pierson 3,485 (JEPS26,403)

M. davidsonii

Los Angeles County: **Holotype: A. Davidson June 24, 1895 (GH52,893); Isotypes: A. Davidson June 24, 1895 (US236,702); A. Davidson 1,907 (DS31,523);** *Other specimen examined:* J. A. Ewan 11,047 (MO1,129,923); T. Ross and S. Boyd 7,387 (CAS904,764); R.A. Schlising 3,115 (MO3,206,881); L.C. Wheeler 2,053 (UC519,947); L.C. Wheeler 2,053 (DS694,338); C.B. Wolf 2,264 (UC527,634); C.B. Wolf 4,076 (UC1,303,155); C.B. Wolf 2,264 (CAS227,828)

Monterey County: H.A. Jansen July 20, 1930 (UC1,121,998); A. Simontacchi Oct. 30, 1938 (UC1,122,032)

San Luis Obispo County: C.B. Hardham 2,484 (CAS519,924)

M. densiflorus

Baja California Norte, Mexico: C.R. Orcutt 1,311 (UC138,333)

Inyo County: E.K. Balls 22,642 (UC1,080,660)

Riverside County: J.T. Howell 6,596 (CAS204,342); J.T. Howell 1,056 (UC484,191); W.L. Jepson 1,387 (JEPS26,394); A.*M.* White 74-671 (CAS782,172)

San Diego County: **Lectotype: S.B. and W.F. Parish 738 (GH52,894); Isotypes: S.B. and W.F. Parish 738 (DS118,315); S.B. and W.F. Parish 738 (MO no number);** *Other specimen examined:* R.D. Alderson 418 (UC109,095); T.S. Brandegee May 28, 1894 (UC109,099); F.R. Fosberg 8,368 (MO no number); N. French 208 (UC1,122,002); H.*M.*

Hall May 1899 (UC63,860); C.C. Parry March 1881 (MO1,777,612); S.B. Parish 4,427 (JEPS26,395); S.B. Parish 4,427 (MO1,767,185); S.B. Parish 4,427 (US313,382); I.L. Wiggins 2,617 (UC650,646);

M. densiflorus* var. *viscidus

Baja California North, Mexico: F.R. Fosberg 8,368 (DS217,906); F.R. Fosberg 8,368 (UC483,760)

San Diego County: **Isotypes: L. Abrams 3,528 (MO no number); L. Abrams 3,528 (US614,032);** *Other specimen examined:* L.R. Abrams 4,898 (DS31,560); R.D. Alderson 1893 (UC 18,755); E.R. Blakley 5,422 (CAS565,719); F.E. and E.S. Clements Ap. 25, 1914 (MO79,792); F.E. and E.S. Clements Ap. 25, 1914 (canceled MO79,793); W.H. Evans July 23,1891 (MO812,056); F.E. Gardner June 26,1935 (UC797,617); C.B. Hardman 4,858 CAS427,599; J.T. Howell 30,952 CAS467,824; P.A. Munz 9,470 DS155,647; E. Palmer 25 (MO 3,937,049); I.L. Wiggins 1,883 DS153,341; I.L. Wiggins 1,925 DS153,358; C.B. Wolf 2,247 CAS173,926; C.B. Wolf 2,131 (US1,630.009)

M. fasciculatus* var. *catalinensis

Santa Catalina Island, Los Angelos County: K. Brandegee no date (UC198,130); J.I. Carlson June 13, 1915 (US1,200,397); P.H. Raven 17,778 (DS513,955); J. Ricketson 4,801 (MO3,209,650); T. Ross 6,924 (CAS986,212); R.F. Thorne and D. Probst 37,646 (MO2,004,275); R. Thorne and D. Probst 37,646 (UC1,369,831); B. Trask May 1898 (MO1,767,196)

M. fasciculatus* var. *elmeri

Contra Costa County: **Holotype: A.D.E. Elmer 4,395 (GH58,121)**

***M. fasciculatus* var. exfibulosus**

Colusa County: E. Brauton 1,144 (US469,867);C.A. Purpus 5,664 (MO1,767,181)

Kern County: B. Ertter 6,126 (UC1,561,298)

Los Angeles County: R.F. Thorne 37,348 (UC1,409,009)

M. fasciculatus* var. *fasciculatus

Baja California Norte, Mexico: (San Antonio del Mar) S. Boyd 3,299 (MO 4,000,558); I. Wiggins 11,899 (UC1,093,283)

Contra Costa County: M.L. Bowerman 2,223 (DS336,469); F.R. Fosberg S4770 (UC625, 105)

Los Angeles County: L. Abrams 2,656 (DS31,542); L. Abrams 1,382 (DS31,543); T.S. Brandegee 1,626 (MO1,767,198); R. Buck 1,217 (JEPS85,114); H.G. Chandler 2004 (UC 56,439); I.M. Clokey 4,553 (UC 871,576); A. Kellogg and W.G.W. Harford 107 (MO 1,767,186); unknown (MO1,767,202); W. Wheeler July 5, 1904 (MO1,767,202)

Orange: A. Lewis 398 (UC1,122,014)

Riverside County: H.M. Hall 528 (UC63,855); J.T. Howell 28,867 (CAS380,353); L.C. Wheeler March 20, 1976 (US3,373,575); L.C. Wheeler 218 (DS694,340); T. Winfield 2,379 (CAS782,173)

San Bernardino County: I.J. Condit June 24, 1910 (UC454,926); P.H. Raven 16,759 (JEPS30,354); P.H. Raven 16,025 (JEPS30,355); P.H. Raven 16,759 (UC1,234,730)

San Diego County: L. Abrams 3,429 (MO3,937,045); L. Abrams 2,656 (MO no number); A.*M.* Carter 3,195 (UC1,745,787); W.R. Dudley January 1909 (DS31,574); C.V. Meyer 219 (UC488,980); S.B. and W.F. Parrish Aug. 1890 (MO767,228); W. Palmer Stockwell 1,514 (DS274,488); I. Wiggins June 17, 1928 (UC3,274); C.B. Wolf 2,074 (DS230,365); H.S. Yates 6,785 (UC1,122,013)

Santa Barbara County: G.W. Dunn July 1888 (UC188,103); A. Eastwood Sept. 12, 1908 (MO no number); A. Eastwood Sept. 12, 1908 (collection 198 ?) (US610,077); A. Eastwood 198 (UC130,845); A.D.E. Elmer 3,730 (UC184,296); A.L. Reina 97-909 (MO no number)

Santa Catalina Island, Los Angeles County: *M.*E. Jones 5M1926 (CAS154,803); P.H. Raven 17,852 (CAS519,923)

Santa Clara County: C.H. Anderson (MO793,159); R.L. Pendleton 1,494 (JEPS26,400)

Ventura County: J.T. Howell 5,175 (UC 473,452);

M. fasciculatus* var. *laxiflorus

Los Angeles County: A.D. Gifford 406 (UC1,122,023); P.J. Hohannsen 203 (UC1,122,018); T. Ross 6,237 (UC1,595,304)

Santa Catalina Island: A. Eastwood 15,400 (UC410,546); F.R. Fosberg S4,770 (MO no number); F.R. Fosberg S4,440 (US1,766,303); L.P. Janeway 4,601 (MO5,088,302)

Ventura County: L.C. Wheeler 736 (UC1,280,816); C.B. Wolf 2,024 (US1,630,006) C.B. Wolf 654 (US1,845,007)

M. fasciculatus* var. *nesioticus

Santa Cruz Island, Santa Barbara County: **Holotype: E.L. Greene July-Aug. 1886 (GH52,907);** *Other specimen examined:* T.S. Brandegee April 1888 (DS31,552); T.S. Brandegee 1888 (UC168,556); T.F. Niehaus 477 (UC1,442,848); C.F. Smith 8,671 (UC1,586,825); D. Wilken 15,837 (JEPS 98,059)

M. fasciculatus var. *nuttallii*

Santa Barbara County: L.R. Abrams 5,030 (DS13,622); I.W. Clokey and B. Templeton 4,553 (MO1,086,308); A.D.E. Elmer 3,730 (CAS141,387); A.D.E. Elmer 3,730 (DS147,519); N. French 678 (UC1,122,028); L.R. Heckard 6,734 (JEPS86,616); H.*M.* Pollard Nov. 14, 1954 (CAS412,385); H.*M.* Pollard Nov. 5, 1952 (CAS383,060); H.*M.* Pollard July 15,1956 (US2,262,924); C.B. Wolf 10,963 (CAS373,965)

Ventura County: **Holotype: L. R. Abrams July 25, 1908 (DS60,885)**

M. foliosum

Baja California Norte, Mexico: **Lectotype: C.R. Orcutt Sept. 1884 (MO52,896); Isotype: C.R. Orcut 1884 (US47,888);** *Other specimen examined*: C.R. Orcutt 1,237 (UC109,091);

M. fragrans

San Luis Obispo County: **Holotype: M.E. Wall May 28, 1933 (US1,678,297)**

M. fremontii

Baja California, Norte, Mexico: I.L. Wiggins 11,899 (DS346,869);

California: **Holotype: Fremont's Expedition to California Apr. 28, 1846 (NY221,823);** *Other specimen examined:* J.C. Fremont 428 (MO no number)

Alameda County: W.H. Brewer 1,223 (MO1,0767,183)

Amador County: D.M. Hutt 1,083 (JEPS35,240); H.E. McMinn 5,037 (UC1,280,814)

Calaveras County: D.E. Breedlove 3,730 (DS494,551); W.R. Howden 235 (UC567,042); V. Rattan June 15, 1885 (DS10,895); V. Rattan June 1885 (US2,310,680)

Contra Costa County: M.L. Bowerman 353 (UC692,612); M.L. Bowerman 1,590 (UC692,693); M.L. Bowerman 2,158 (UC692,620); A.D.E. Elmer 4,395 (MO no number); K.L. Fenley May 3, 1931 (JEPS26,391); E.L. Greene June 20, 1892 (UC18,798)

Inyo County: G.F. Pratt July 23, 1995 (CAS934,833)

Kern County: E.C. Twisselmann 8,369 (CAS608,415)

Lake County: M.S. Baker 8,303 (DS252,350)

Los Angeles County: L. Abrams and E.A. McGregor 631 (DS31,547); A.M. Carter 993 (UC1,745,884)

Madera County: D.E Schlobohm 184 (UC570,133)

San Benito County: S.B. and W.F. Parrish 15,114 (MO1,767,184)

San Bernardino County: A.C. Sanders 19,460 (CAS934,813)

San Mateo County: B.O. Schreiber May 29, 1932 (UC614,867)

Santa Barbara County: R. Nichols 664 (JEPS85,890)

Santa Clara County: H.E. McMinn 227 (UC1,280,815)

Stanislaus County: R.F. Hoover 4,879 (UC762, 998); L.S. Rose 35,213 (MO 1,100,577); H.K. Sharsmith 3,130 (DS341,220)

Sutter County: L. Ahart 5,019 (CAS776,956); L. Ahart 5,019 (MO3,322,104); E. Braunton 1,144 (UC66,963); J.A. Ewan 9,597 (JEPS68,661)

Tulare County: F.V. Coville and F. Funston 1,310 (US11,968); J.T. Howell 33,159 (CAS427,597); *M*.S. Jussel June 21, 1927 (CAS229,218); P.H. Raven and H. Lewis 9,353A (CAS402,340); P.H. Raven and H. Lewis 9,359 (CAS402,339); P.H. Raven and H. Lewis 9,359 (US2,326,178); J. Sherock 315 (CAS713,332)

M. gracilis

San Luis Obispo County: **Isotype: A. Eastwood 14,996 (US1,678,331);** *Other specimen examined:* R.F. Hoover 7,905 (CAS399,065)

Santa Barbara County: R.F. Hoover 6,534 (UC1,285,300)

M. hallii

Contra Costa County: **Holotype: H.M. Hall and F.M. Essig 10,131 (UC198,957); Isotypes: H.M. Hall and F.M. Essig 10,131 (US1,328,168);** *Other specimen examined:* M.L. Bowerman 2,223 (UC692,676); A.A. Heller 15,719 DS311,812; J.T. Howell and T.H. Kearney 26,634 (CAS358,447); J.T. Howell and T.H. Kearney 26,634 (MO1,649,219); V. Rattan June 14, 1886 (DS10,891); L.S. Rose 54,082 CAS391,607; L.S. Rose 41,228 (MO no number)

Merced County: D.W. Taylor 14,296 (JEPS89,282)

San Benito County: I. Wiggins 8,519 (UC871,308)

Santa Clara County: J.T. Howell 28,968 CAS377,735

M. helleri

Colusa County: **Holotype: A.A. Heller 13,242 (US1,086,619); Isotype: A.A. Heller 13,242 (MO no number);** *Other specimen examined:* A.A. Heller 15,719 (MO1,199,104); A.A. Heller 15,719 (UC725,449); A.A. Heller 15,719 (US1,974,669)

Lake County: F. Bowcutt 596 (UC1,552,839); F. Bowcutt 596 (UC1,552,840); J. T. Howell 13,112 (CAS248,087)

Napa County: P. Raven 20,205 (MO2,596,576)

M. howellii

Contra Costa County: **Isotypes: J.T. Howell 6,470 (MO1,047,578); J.T. Howell 6,470;** *Other specimen examined:* (US1,434,220); J.T. Howell and T.H. Kearney 26,635 (DS337,827); J.T. Howell and T.H. Kearney 26,635 (MO1,649,220); J.T. Howell 26,635 (UC961,415); E. McClintock 184 (CAS473,471)

Madera County: R. Bacigalupi 6,394 (JEPS22,742); R.S. Ferris and R.C. Bacigalupi 13,374 (DS404,026)

San Joaquin County: J.T. Howell 48,110 (CAS554,872); J.T. Howell 28,932 (CAS2,326,418); J.T. Howell 28,932 (UC1,177,002)

Stanislaus County: J.T. Howell 32,355 CAS406,728

M. jonesii

Monterey County: B.F. Howitt 1,074 (CAS508,040); H.A. Jensen 19 (UC1,121,999)

San Luis Obispo County: J.H. Barber 1,901 (MO2,596,576); B. Bolt 721 (UC1,122,045); W.H. Brewer 554 (MO1,767,97); C.B. Hardham 633 (CAS508,042); C.B. Hardham 938 (CAS402,123); C.B. Hardham 4,414 (CAS427,595); C. Hardham 973 (UC1,177,007); R.F. Hoover 8,500 (CAS456,006); G.T. Nordstrom 19,938 (UC1,122,015); E. Painter and E. Neese June 16, 1998 CAS998,804; L.S. Rose 61,043 (CAS72,902); L.S. Rose 61,043 (DS562,997)

M. marrubioides

Baja California Norte, Mexico: C.R. Orcutt July 14, 1888 (UC109,093)

Fresno County: **Isotype: A.L. Heermann July 1853 (US49,307)**

Los Angeles County: K. Brandegee 1889 (UC198,500); T.S. Brandegee June 1885 (UC109,101); H.E. McMinn 2,579 (UC1,280,807); F.W. Peirson 774 (JEPS26,402); T. Ross 2,889 (UC1,584,242)

Ventura: C.B. Wolf 686 (DS290,338)

M. marrubioides* var. *paniculatum

Baja California Norte, Mexico: **Holotype: C.R. Orcutt July 14, 1885 (GH52,906); Isotype: C.R. Orcutt July 14, 1885 (US1,381,940)**

M. mendocinensis

Mendocino County: **Holotype: A. Eastwood and J.T. Howell 6,092 (NY221,831);** *Other specimen examined*: L.S. Rose 39,194 (CAS269,452); L.S. Rose May 30, 1939 (UC871,312);

M. niveus

Monterey County: W.H. Brewer 554 (UC18,787); C.B. Hardham 10,404 (CAS519,922); E.C. Twisselmann 2,161 (CAS396,245); V. Yadon June 5, 1982 (CAS742,124)

San Luis Obispo County: **Holotype: A. Eastwood 14,996 NY(221,824);** *Other specimen examined:* R. Bacigalupi 7,426 (JEPS27,047); J.H. Barber (UC55,293); A. Eastwood and J.T. Howell 5,910 (UC871,313); A. Eastwood and J.T. Howell 5,910 (CAS264,201); T.M. Hendrix 250 (US1,602,142); R.F. Hoover 793 (CAS358,677); R.F. Hoover 6,177 (DS562,691); R.F. Hoover 7,396 (UC1,285,302); J.T. Howell 24,379 (UC904,946)

Santa Barbara County: H.C. Lee 297 (UC1,122,029)

M. orbiculatus

Inyo County: A.M. Alexander and L. Kellogg 3,138 (DS331,987); A.M. Alexander 3,138 (UC694,461); R.S. Ferris 9,020 (DS230,556); R.S. Ferris 9,020 (UC562,886); J.T. Howell and T.H. Kearney 27,434 (DS337,822); T.H. Kearney and J.T. Howell 27,434 (MO1,649,172); T.H. Kearney and J.T. Howell 27,434 (UC177,004); J.C. Roos Aug. 9, 1965 (CAS905,598)

Kern County: J.T. Howell 43,500 (CAS833,393); J.T. Howell and G.H. True 42,766 (CAS832,779); J.T. Howell and G.H. True 42,364 (CAS847,463); R. Olmsted 816 (UC1,728,665); R.A. Schlising 3,057 (MO3,206,880); E.C. Twisselmann 1,199 (CAS608,414); E.C. Twisselmann 2,230 (CAS399,743); E.C. Twisselmann 2,324 (CAS399,527); E.C. Twisselmann 8,369 (CAS482,550)

Los Angeles County: P.C. Everett 23,806; (UC1,293,713); J.A. Ewan 8,448 (UC586,923); W.O. Griesel Aug. 9,1961 (DS495,382); J.T. Howell 23,413 (CAS354,246); I.M. Johnston 1,673 (UC205,366); T.S. Ross 3,702 (CAS933,271); T.S.. Ross 5,072 (CAS393,609); T.S. Ross 7,927 (CAS986,093); T. Ross 5,638 (UC1,584,926); T. Ross 7,263 (UC1,605,451); R.F. Thorne 40,710 (UC1,375,262); C.B. Wolf 1,629 (UC871,314); C.B. Wolf 2,496 (US1,630.025)

San Bernardino County: W.O. Griesel Aug. 15, 1961 (DS495,388); M.E. Jones 24,968 (CAS172,766); M.E. Jones 84,968 (MO no number); P.A. Munz 12,705 (MO1,072,123); P.A. Munz 12,705 (UC494,822) L.C. Wheeler 1,284 (1,280,812)

Tulare County: P. Raven and H. Lewis 9,351 (US2,326,177); L.S. Rose 58,047 (US2,326,247)

Ventura County: W.R. Dudley 4,557 (DS31,568); A.D. Elmer 3,895 (DS247,520); A.D.E. Elmer 3,895 (MO1,767,179); A.D. Elmer 3,895 (US466.098); H.E. McMinn 3,909 (UC1,280,811)

M. palmeri* var. *palmeri

San Luis Obispo County: **Holotype: E. Palmer 50 (GH 52,909);** Isotypes**: E. Palmer 50 (UC109,096); E. Palmer 50 (US15,215);** *Other specimen examined*: W. Davis Ap. 13, 1931 (JEPS26,416); A. Eastwood 13,606 (CAS399,964); A. Eastwood 15,027 (CAS147,742); A. Eastwood Aug. 1927 (MO1,016,007); A. Eastwood 15,027 (UC410,082); A. Eastwood 15,013 (UC no number); R.F. Hoover 8,356 (CAS139,964); R.F. Hooker 6,200 (UC1,285,301); R.F. Hoover 8,356 (CAS399,068); J.T. Howell 27,491 (MO no number); H.E. McMinn 3,845 (UC763,042); B.E. and H.J. Miossi 266 (UC 639,007); L. Roush Apr. 19, 1929 (JEPS26,413);L.S. Rose 41,196 (MO no number); L.S. Rose 44,307 (MO 1,283,546)

Monterey County: L.R. Abrams 5,642 (US1,326,096); L.R. Abrams 5,678 (US1,326,098) H.M. Hall 10,084 (JEPS26,412);H.E. McMinn 5,168 (UC1,280,813)

M. palmeri* var. *involucratus

Monterey County: **Holotype: T.S. Brandegee (GH52,904);** *Other specimen examined*: W.R. Dudley May 14, 1895 (DS31,550); A. Eastwood 4,049 (CAS52,723); A. Eastwood 4,049 (UC871,311); A. Eastwood May 1-12, 1897 (UC18,799); A. Eastwood 4,049 (US1,074,263); W.I. and E. Follett May 21, 1959 (CAS420,719); J.R. Griffin 3,867 (JEPS73,832); H.M. Hall 10,084 (MO no number); H.M. Hall 10,084 (US769,370); C. Hardham 4,512 (UC1,294,049); R.F. Hoover 4,117 (UC763,042); J.T. Howell 11,599 (US1,582,995); T.H. Kearney 27,491 (UC961,421); T.H. Kearney and J.T. Howell 27,492 (UC1,177,005); D.D. Keck and J. Clausen 3,057 (DS694,347); L.S. Rose 41,196

(UC871,310); L.S. Rose 54,086 (UC1,042,344); L.S. Rose 44,307 (US1,896,210); D.W. Taylor 14,180B (JEPS91,053)

M. palmeri* var. *lucianus

Monterey County: **Holotype: J.T. Howell July 8, 1955 (NY221,798); Isotypes: J.T. Howell 30,642 (CAS 396,251); J.T. Howell 30,642 (US2,326,451);** *Other specimen examined*: D.E. Breedlove 36,263 (CAS574,314); J. Carpenter 335 (CAS983,204); J.T. Howell 30,128 (US2,326,429); V. Yaden July 6, 1974 (CAS576,000)

M. paniculatus

Baja California, Norte, Mexico: R. Moran 22,923 (CAS612,892); R. Moran 22,923 (MO no number); R. Moran 16,515 (UC1,363,903); R. Moran 16,515 (US2,571,608); I.L. Wiggins 11,869 (DS346,868); I. Wiggins 4,013 (MO no number); I. Wiggins 11,869 (UC1,093,282)

M. parishii

San Bernardino County: **Holotype: S.B. Parish 3,804 (UC18,789)**

***M.* splendidum**

Santa Barbara County: A.D.E. Elmer 3,370 (MO no number)

M. thurberi

Monterey County: W.H. Brewer 554 (US351,541)

San Diego County: F.E. Clements Ap. 25, 1914 (UC197,060)

Sonora, Mexico: **Holotype: G. Thurber 709 (NY221,841); Isotype: G. Thurber 709 (GH52,911);** *Other specimen examined:* S.G. Stokes June 15, 1895 DS31,538;

Unknown

Shasta County: E. Dean 28 (1,553,464)

Table 5.4. Summary of morphological data observed in herbarium specimens of *Malacothamnus*. Definitions for categorical variables are provided in Table 5.2. Quantitative variables were measured in centimeters, except ray length (mm).

Species			petiole length	Leaf width	leaf length	leaf texture	leaf base	petiol pubes.	pubes. Upper	pubes. Lower	stem pubes.	stem pubes. Type	pubes. Color	inflores. Type	#FL/ Node	#Bract/INFL	Pedicel	sepal length	sepal width	bract length	sepal shape	bract color	pubes dens INFL	# Rays	IFL pubes. Type	petal length	stamen length	stipitate	ray length
M. abbottii	N=2	max	3.7	5	5	4	1	6	25	30	6	1	1	1	2	2	0.5	1.1	0.4	0.6	3	1	6	35	1	1.7	1	0	0.01
		min	1.2	2.7	2.1	2	1	6	17	25	6	1	1	1	1	1	0.3	1	0.32	0.6	1	1	6	30	1	1.7	1	0	0.01
		mean	2.45	3.85	3.55	3	1	6	21	27.5	6	1	1	1	1.5	1.5	0.4	1.05	0.36	0.6	2	1	6	32.5	1	1.7	1	0	0.01
		stdev	1.768	1.626	2.051	1.41	0	0	5.657	3.536	0	0	0	0	0.707	0.707	0.141	0.07	0.06	0	1.41	0	0	3.54	0	0	0	0	0
M. aboriginum	N=20	max	4.2	7	5.4	4	3	6	45	60	6	1	2	2	3	3	0.5	1.27	0.7	1.18	4	2	6	30	1	2	1.3	1	0.1
		min	0.9	0.2	1.5	1	2	4	7	10	5	1	1	1	2	0	0	0.54	0.2	0.22	2	1	4	10	1	0.9	0.75	0	0.02
		mean	1.94	3.73	3.29	3.14	2.33	5.43	26.24	29.71	5.62	1	1.76	1.71	2.29	2.19	0.21	0.91	0.48	0.65	2.95	1.05	4.9	20.1	1	1.44	0.99	0.92	0.04
		stdev	0.95	1.58	1.1	1.01	0.48	0.75	11.46	11.85	0.5	0	0.44	0.46	0.46	0.87	0.14	0.22	0.16	0.26	0.5	0.22	0.62	6.39	0	0.27	0.18	0.28	0.02
M. clementinus	N=10	max	3.5	6	6	3	5	6	9	50	6	1	1	2	3	3	0.65	1.04	0.32	1.18	3	2	6	40	1	2	1.27	1	0.16
		min	0.7	3	2.2	1	2	5	0	10	5	1	1	1	2	0	0	0.55	0.15	0.32	1	1	4	10	1	0.9	0.55	0	0.04
		mean	1.71	4.55	4.05	1.91	3.27	5.09	3.45	24.18	5.09	1	1	1.64	2.64	1.27	0.28	0.75	0.23	0.66	1.45	1.36	5.09	19.3	1	1.37	0.94	0.6	0.1
		stdev	0.75	1.2	1.09	0.94	1.42	0.3	2.5	12.73	0.3	0	0	0.5	0.5	0.79	0.22	0.17	0.06	0.28	0.69	0.5	0.54	10.5	0	0.34	0.25	0.55	0.05
M. davidsonii	N=22	max	4.6	9.5	7.7	4	5	6	45	50	7	1	2	2	3	3	1.18	0.95	0.55	0.6	3	2	6	35	1	2.1	1.1	1	0.08
		min	0.4	1.7	2	1	2	3	5	7	4	1	1	1	1	0	0.1	0.3	0.16	0.12	1	1	4	7	1	0.9	0.4	0	0.02
		mean	2.2	5.33	4.59	2.41	3.18	5.09	21.14	29.27	5.26	1	1.74	1.18	2.05	1.73	0.32	0.65	0.27	0.32	1.82	1.05	5.5	16.1	1	1.38	0.85	0.42	0.05
		stdev	1.17	2.62	1.86	1.22	1.1	0.92	10.15	12.1	0.86	0	0.45	0.39	0.72	0.94	0.28	0.17	0.09	0.13	0.59	0.21	0.74	6.71	0	0.31	0.17	0.51	0.02
M. densiflorus	N=24	max	2.6	6.2	5	4	4	6	50	50	6	4	2	2	3	2	1	1.32	0.52	1.49	3	2	6	20	1	2.6	9	1	0.3
		min	0.5	1.5	1.4	1	1	3	2	10	3	1	1	1	2	0	0	0.54	0.14	0.22	1	1	3	2	1	1	0.6	0	0.01
		mean	1.32	3.19	3.07	1.69	2.62	4.41	12	21.38	4.32	1.08	1.97	1.73	2.56	0.92	0.26	0.82	0.31	0.76	1.62	1.03	4.73	8.76	1	1.43	1.18	0.69	0.13
		stdev	0.56	0.98	1.04	0.83	0.6	0.82	8.34	9.73	0.85	0.49	0.16	0.45	0.5	0.68	0.19	0.17	0.09	0.32	0.68	0.16	1.04	4.57	0	0.31	1.72	0.48	0.07
M. fasciculatus var. catalinensis	N=7	max	2.1	8.3	6.2	1	4	6	50	50	5	1	1	1	3	3	0.45	0.79	0.59	0.5	3	2	6	20	1	2.6	1.3	1	0.05
		min	0.6	2.5	3.2	1	2	2	0	9	2	1	1	1	2	1	0.1	0.68	0.2	0.27	2	1	3	5	1	1.3	0.7	1	0.016
		mean	1.49	5.16	4.81	1	2.57	4	14.43	29.57	3.38	1	1	1	2.13	1.88	0.3	0.72	0.33	0.35	2.25	1.5	4.25	10.1	1	1.78	1.08	1	0.032
		stdev	0.56	1.97	1.12	0	0.79	1.29	18.58	17.58	1.3	0	0	0	0.35	0.99	0.14	0.04	0.12	0.07	0.46	0.53	0.89	5.28	0	0.47	0.25	0	0.017
M. fasciculatus var. nesioticus	N=6	max	2.3	6	5.5	1	5	4	6	45	4	1	1	1	1	2	1.4	0.88	0.36	0.34	3	2	6	30	1	2	1.1	0	0.024
		min	0.8	3.5	3.5	1	2	3	0	10	3	1	1	1	1	0	0.3	0.7	0.25	0.18	1	1	3	5	1	1.3	0.7	0	0.013
		mean	1.62	4.73	4.43	1	2.67	3.83	1.67	22	3.67	1	1	1	1	1.17	0.88	0.75	0.31	0.23	2.17	1.33	4.33	17.5	1	1.7	0.95	0	0.018
		stdev	0.64	0.91	0.71	0	1.21	0.41	2.16	12.08	0.52	0	0	0	0	0.75	0.41	0.07	0.04	0.06	0.75	0.52	1.03	10.4	0	0.28	0.14	0	0.004
M. fasciculatus var. nuttallii	N=10	max	3.2	7.6	7.1	2	3	6	70	70	6	1	1	1	2	3	0.77	1.04	0.4	0.33	3	2	6	30	1	2.7	1.6	0	0.036
		min	0.7	1.6	1.5	1	2	2	5	15	2	1	1	1	1	0	0.1	0.55	0.2	0.11	1	1	4	7	1	1.2	0.5	0	0.006
		mean	2.02	4.9	4.63	1.09	2.36	4.55	46.64	51.27	5.09	1	1	1	1.27	1.36	0.44	0.71	0.27	0.23	1.64	1.45	5.55	22.6	1	1.77	0.99	0	0.015
		stdev	0.72	1.82	1.68	0.3	0.5	1.51	21.27	18.76	1.58	0	0	0	0.47	1.03	0.2	0.16	0.07	0.07	0.67	0.52	0.82	7.23	0	0.49	0.31	0	0.01

Table 5.4, continued.

Species			petiole length	Leaf width	leaf length	leaf texture	leaf base	petiol pubes.	pubes. Upper	pubes. Lower	stem pubes.	stem pubes. Type	pubes. Color	inflores. Type	#FL/ Node	#Bract/I NFL	Pedicel	sepal length	sepal width	bract length	sepal shape	bract color	pubes dens INFL	# Rays	IFL pubes. Type	petal length	stamen length	stipitate	ray length
M. fasciculatus var. fasciculatus	N=64	max	2.6	6.3	6.6	4	5	6	70	60	6	1	2	2	3	3	1.28	1.5	0.53	0.59	4	2	6	30	1	2.4	9	1	0.09
		min	0.3	0.2	1.5	1	1	2	0	5	2	1	1	1	1	0	0.04	0.3	0.15	0.1	1	1	3	6	1	1	0.27	0	0.008
		mean	1.34	3.35	3.29	1.49	2.49	4.21	13.98	26.87	4.33	1	1.18	1.22	1.63	1.08	0.33	0.66	0.29	0.29	2.08	1.14	4.66	14.7	1	1.5	1.02	0.21	0.036
		stdev	0.6	1.08	1.07	0.76	0.74	1.03	12.46	11.93	1.07	0	0.39	0.42	0.63	0.86	0.24	0.15	0.08	0.1	0.67	0.35	0.98	6.47	0	0.3	1.12	0.42	0.019
M. fremontii	N=71	max	4.2	9.2	9.2	6	5	7	60	70	7	1	2	2	3	3	0.83	3.5	0.73	1.2	4	2	7	30	1	2.4	727	1	0.166
		min	0.5	1.5	1.4	1	1	3	5	7	2	1	1	1	1	0	0	0.5	0.1	0.11	1	1	2	7	1	0.6	0.34	0	0.01
		mean	1.82	4.27	3.75	2.52	2.62	5.55	23.52	28.93	5.47	1	1.21	1.26	1.96	1.71	0.31	0.91	0.3	0.62	1.81	1.56	5.58	18.4	1	1.4	13.51	0.72	0.081
		stdev	0.81	1.74	1.59	1.06	1.04	0.75	14.62	14.99	0.8	0	0.41	0.44	0.51	0.86	0.21	0.38	0.12	0.28	0.68	0.5	0.78	6.13	0	0.37	96.19	0.46	0.032
M. hallii	N=33	max	3.2	8	7.1	4	5	6	60	60	6	1	2	1	3	3	1.18	0.8	0.32	0.36	3	2	6	30	1	1.9	1.1	1	0.26
		min	0.6	1.9	1.4	1	1	3	5	12	4	1	1	1	1	0	0.15	0.3	0.1	0.05	1	1	4	6	1	0.73	0.45	0	0.003
		mean	1.51	4.06	3.83	2.6	2.6	4.67	27	35.8	5.33	1	1.2	1	1.73	1.33	0.34	0.55	0.22	0.2	1.8	1.2	5.53	18.5	1	1.31	0.78	0.17	0.04
		stdev	0.68	1.88	1.7	0.99	1.3	0.98	16.69	14.31	0.9	0	0.41	0	0.8	0.98	0.26	0.12	0.06	0.08	0.56	0.41	0.83	7.91	0	0.33	0.2	0.39	0.071
M. jonesii	N=31	max	3	4.7	4.5	4	3	6	80	80	6	1	1	2	2	3	1.5	1	0.41	0.65	2	2	6	32	1	2.5	1.5	1	0.106
		min	0.7	1.1	1.3	1	1	4	5	17	2	1	1	1	1	0	0.16	0.5	0.1	0.2	1	2	4	8	1	1	0.43	0	0.01
		mean	1.36	2.56	2.41	2.19	2.16	5.59	45.16	49.34	5.56	1	1	1.03	1.19	1.69	0.54	0.76	0.27	0.39	1.69	2	5.69	20.2	1	1.58	0.92	0.06	0.041
		stdev	0.52	0.97	0.77	1.03	0.99	0.76	21.61	20.26	1.05	0	0	0.18	0.4	0.82	0.29	0.16	0.07	0.12	0.47	0	0.64	6.53	0	0.3	0.25	0.24	0.028
M. marrubioides	N=8	max	2.8	6	6.2	2	3	6	40	45	6	1	2	2	3	3	0.59	1.27	0.52	1.2	3	1	6	27	1	2	1	1	0.061
		min	1	2	2	1	1	3	10	13	4	1	2	1	1	1	0.14	0.76	0.18	0.6	1	1	3	8	1	1.15	0.4	0	0.033
		mean	1.61	3.59	3.55	1.67	2	4.56	19.67	25	5	1	2	1.33	2.22	2.22	0.34	1.03	0.27	0.89	1.89	1	4.89	14.2	1	1.5	0.73	0.33	0.051
		stdev	0.57	1.26	1.49	0.5	0.87	1.01	10.91	12.13	0.87	0	0	0.5	0.97	0.83	0.16	0.14	0.1	0.2	1.05	0	1.05	5.43	0	0.24	0.25	0.58	0.016
M. orbiculatus	N=30	max	3.6	8	7	4	5	6	36	60	6	1	2	3	3	2	0.95	1.23	0.5	0.9	3	2	6	22	1	2.1	1.4	1	0.4
		min	0.9	2.8	1.7	1	1	3	7	10	4	1	1	1	1	0	0.1	0.55	0.14	0.25	1	1	4	8	1	1.09	0.33	0	0.023
		mean	1.9	4.49	3.66	2.08	3.03	5	16.95	24.62	4.95	1	1.76	1.3	2.11	0.97	0.37	0.84	0.25	0.48	1.41	1.11	5.57	14.4	1	1.52	0.82	0.73	0.084
		stdev	0.72	1.28	1.13	0.92	1.32	0.97	7.45	9.97	0.88	0	0.43	0.52	0.46	0.73	0.18	0.16	0.09	0.15	0.6	0.31	0.6	4.17	0	0.28	0.27	0.45	0.07
M. palmeri var. palmeri	n=34	max	6.5	7.1	8	3	4	6	30	36	6	4	2	3	3	3	0.5	1.7	0.7	2.3	2	1	6	22	6	3.5	1.9	1	0.65
		min	0.4	1.8	1.9	1	1	3	1	4	4	1	1	3	2	0	0	0.6	0.2	0.62	1	1	2	6	1	1.1	0.8	0	0.03
		mean	2.75	4.41	4.8	1.93	2.44	4.78	10.63	20	4.89	2.44	1.96	3	2.7	2.3	0.2	1.26	0.38	1.53	1.19	1	4.81	10.3	3.67	1.9	1.17	0.84	0.16
		stdev	1.51	1.62	1.58	0.78	0.75	0.7	8.49	8.61	0.58	1.53	0.19	0	0.47	0.95	0.15	0.29	0.12	0.44	0.4	0	1.08	3.77	1.86	0.53	0.29	0.37	0.12
M. palmeri var. involucratus	N=20	max	5.2	7	7	4	3	6	6	32	6	4	2	3	3	3	0.9	1.5	0.59	1.7	2	2	6	16	6	3.2	1.7	1	0.156
		min	0.7	1.7	1.9	1	1	3	0	6	3	1	2	3	2	0	0	0.61	0.16	0.5	1	1	3	3	1	0.95	0.6	0	0.04
		mean	1.95	3.7	4.14	2.43	2.24	4.71	2.33	15.86	4.52	2.14	2	3	2.75	2.29	0.21	1.02	0.34	1.08	1.29	1.05	4.62	10.3	2.86	1.81	1.12	0.93	0.107
		stdev	1.08	1.47	1.55	0.87	0.54	0.78	2.13	7.23	0.98	1.49	0	0	0.44	0.78	0.2	0.26	0.1	0.3	0.46	0.22	0.92	3.68	1.59	0.59	0.36	0.27	0.041
M. paniculatus	N=7	max	1.5	5	6	3	3	6	30	30	6	1	2	3	3	3	2.1	1.27	1	0.91	4	2	6	18	1	2.1	1.1	1	0.1
		min	0.5	2.1	2.5	2	1	4	8	12	5	1	1	1	1	2	0.18	0.7	0.36	0.3	3	1	3	10	1	1.3	0.8	1	0.05
		mean	1.06	3.4	3.74	2.38	1.75	5.38	19.25	26.13	5.63	1	1.5	1.5	1.57	2.38	0.84	1.04	0.67	0.6	3.88	1.5	5.13	15.3	1	1.61	0.92	1	0.066
		stdev	0.31	1.09	1.11	0.52	1.04	0.74	9.07	6.47	0.52	0	0.55	0.76	0.79	0.52	0.7	0.17	0.24	0.19	0.35	0.53	1.13	2.76	0	0.25	0.12	0	0.02

Figure 5.1. Map of the distribution of specimens analyzed for morphological variation in *Malacothamnus*. Arrows indicate locations for material included in the analysis of sequence variation of the ITS region.

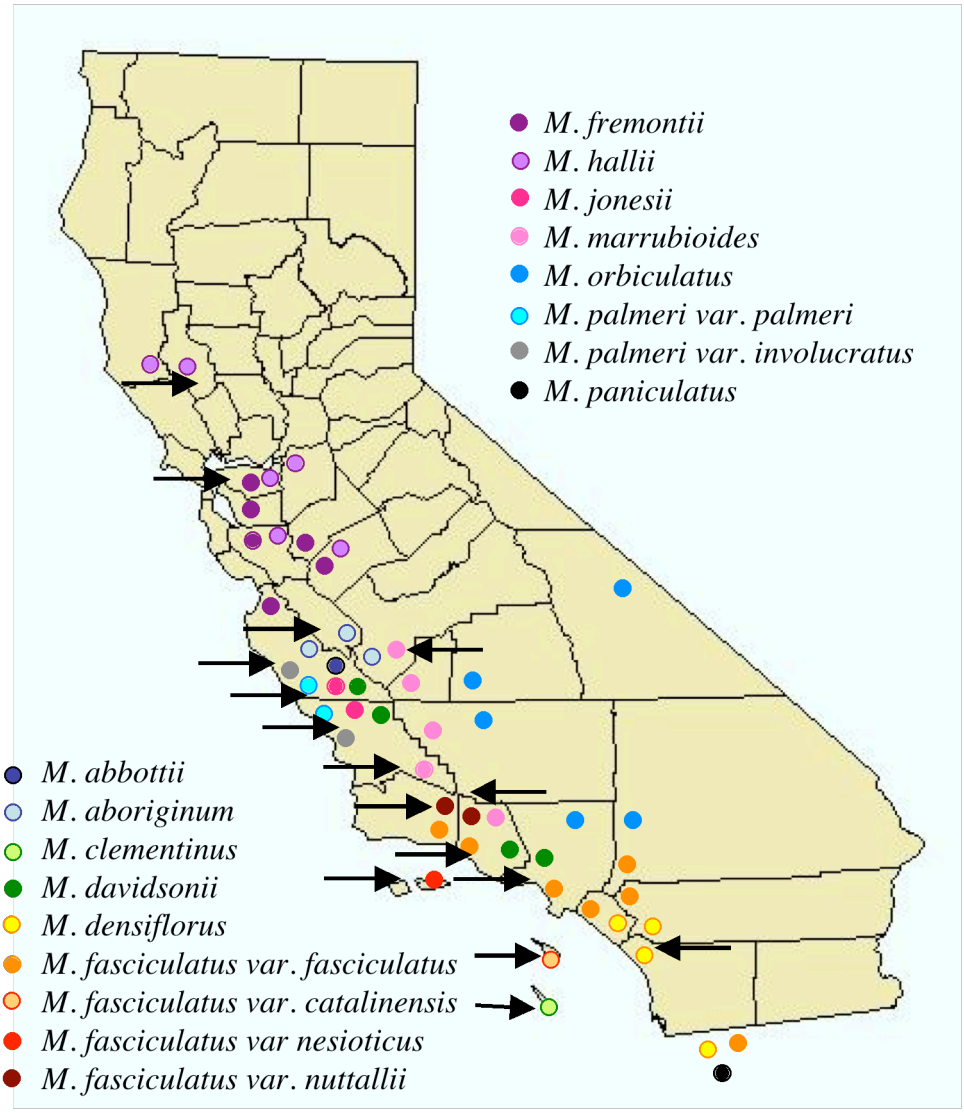


Figure 5.2. Results from multidimensional scaling of all taxa included. Symbols for taxa are as indicated.

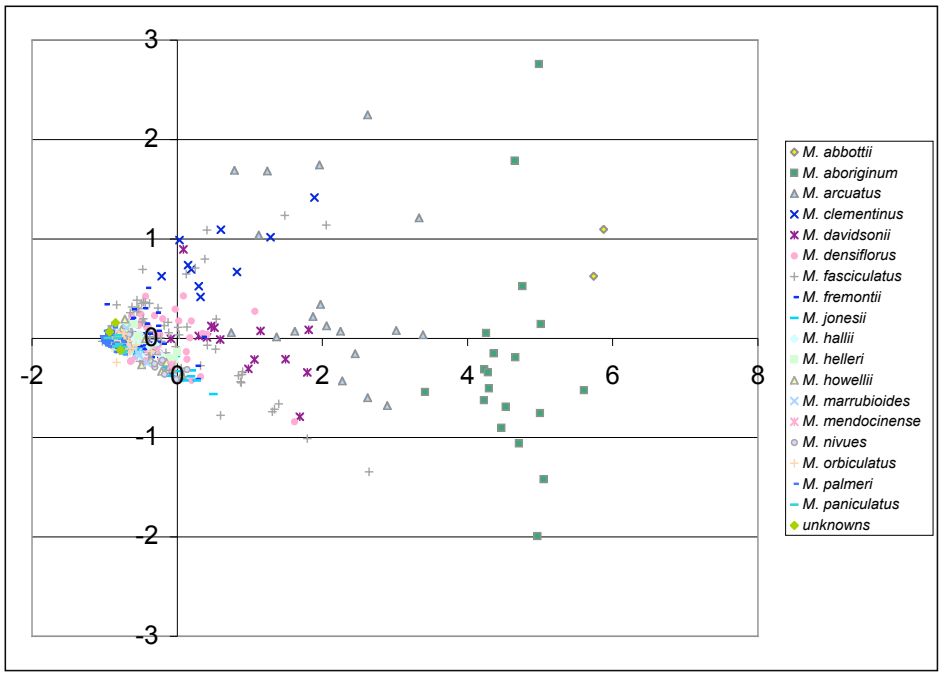


Figure 5.3. Results from multidimensional scaling of selected taxa. Symbols for taxa are as indicated.

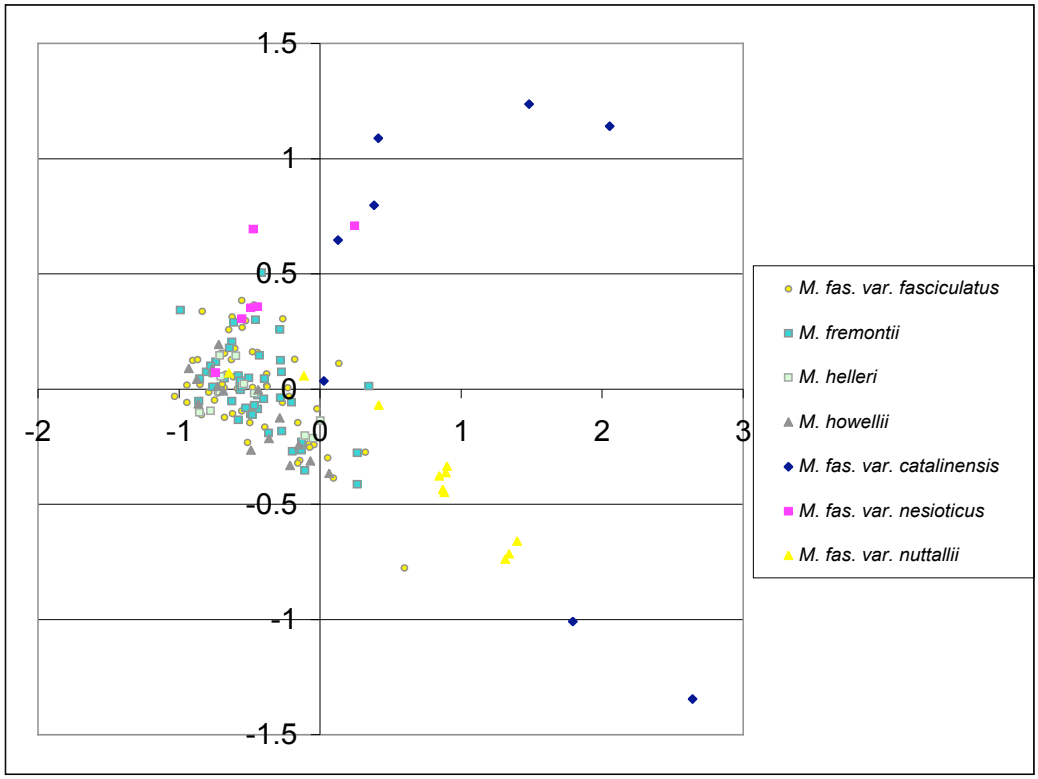


Figure 5.4. Results from multidimensional scaling of selected taxa. Symbols for taxa are as indicated.

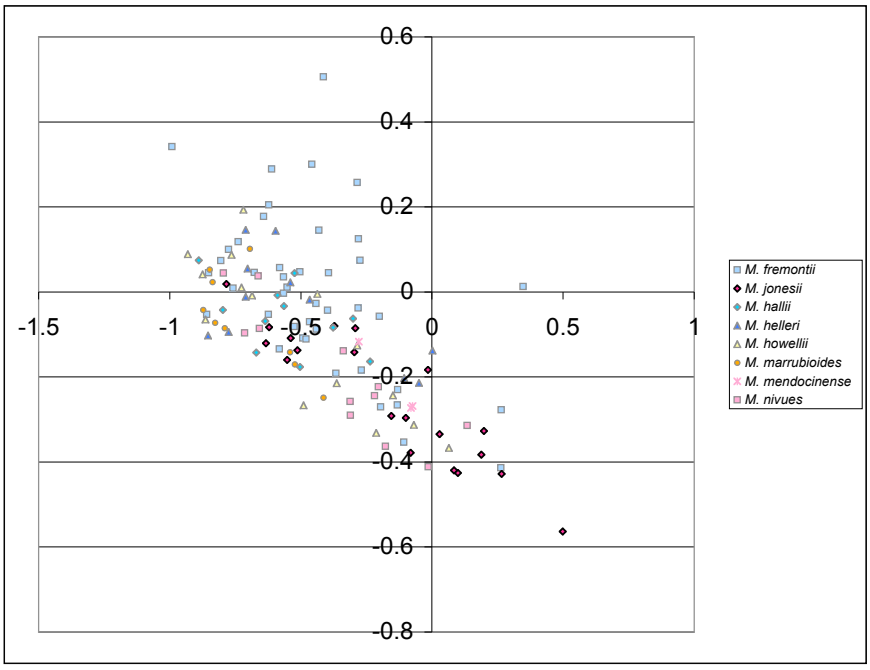


Figure 5.5. Results from multidimensional scaling of selected taxa. Symbols for taxa are as indicated.

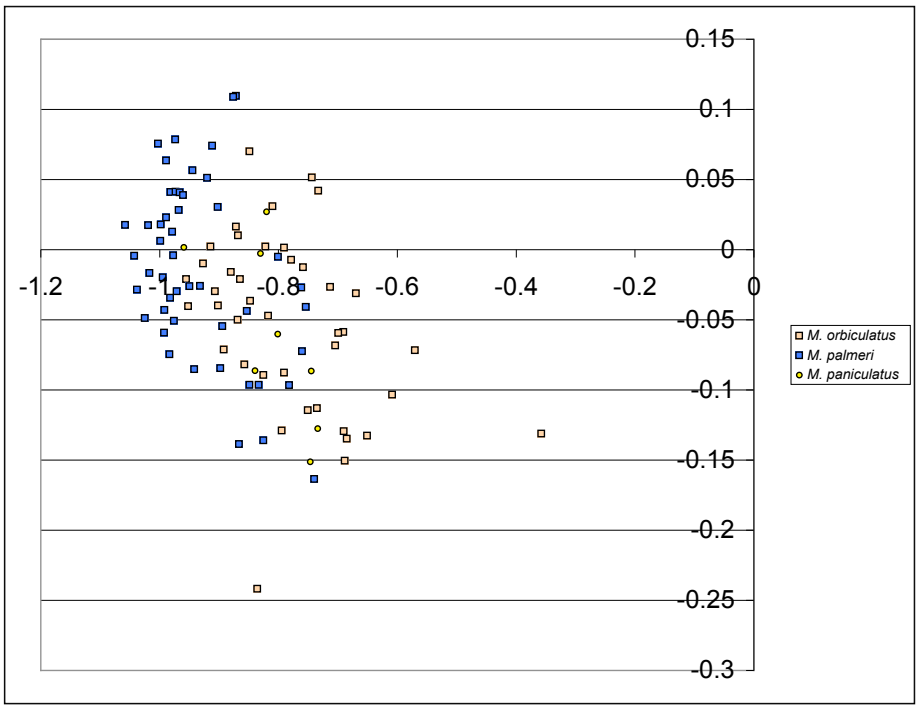


Figure 5.6. UPGMA phylogram of the *M. fasciculatus* group. Taxa representing the majority of branches in a group are indicated.

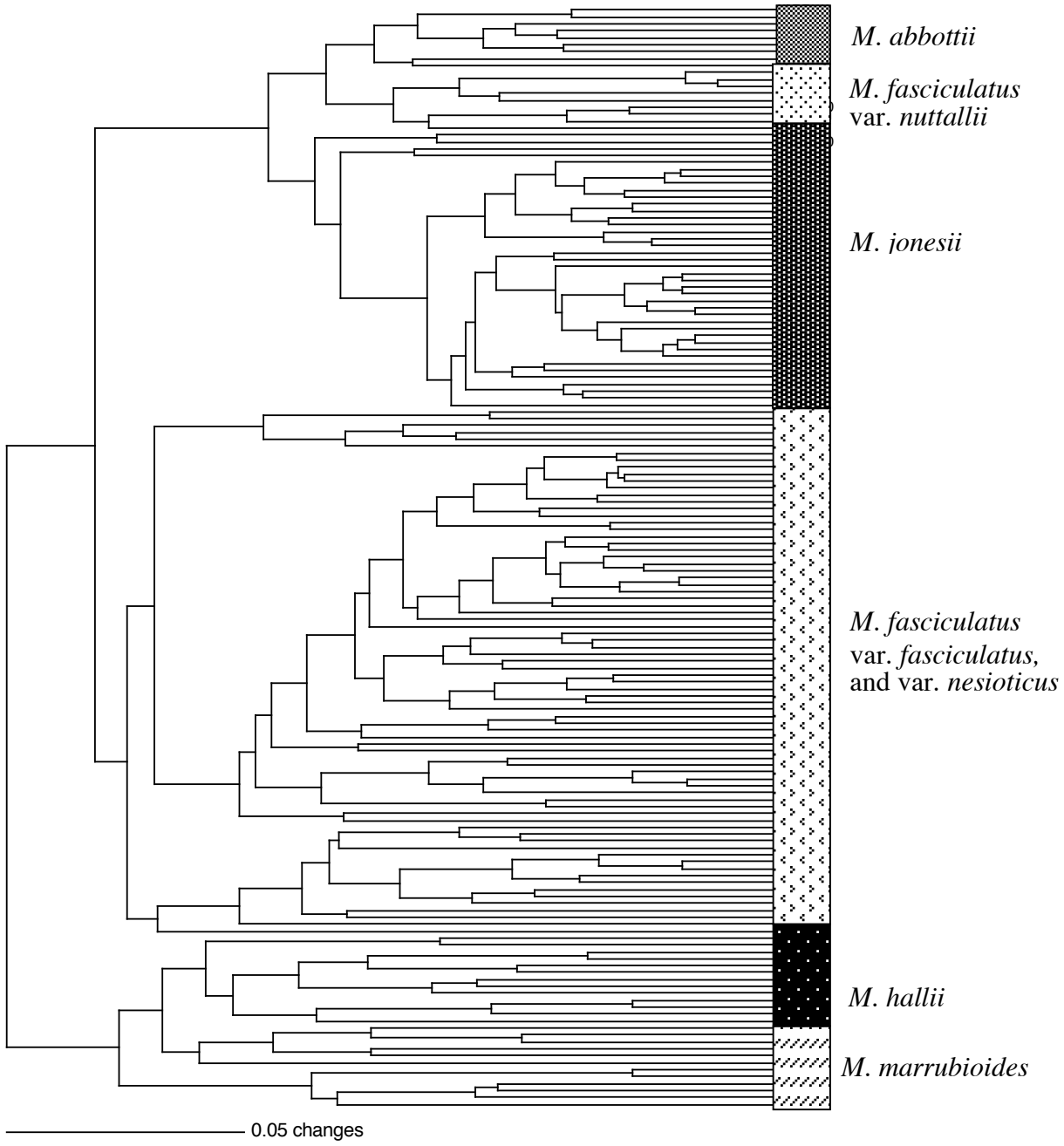


Figure 5.7. UPGMA phylogram of the *M. fremontii* group. Taxa representing the majority of branches in a group are indicated.

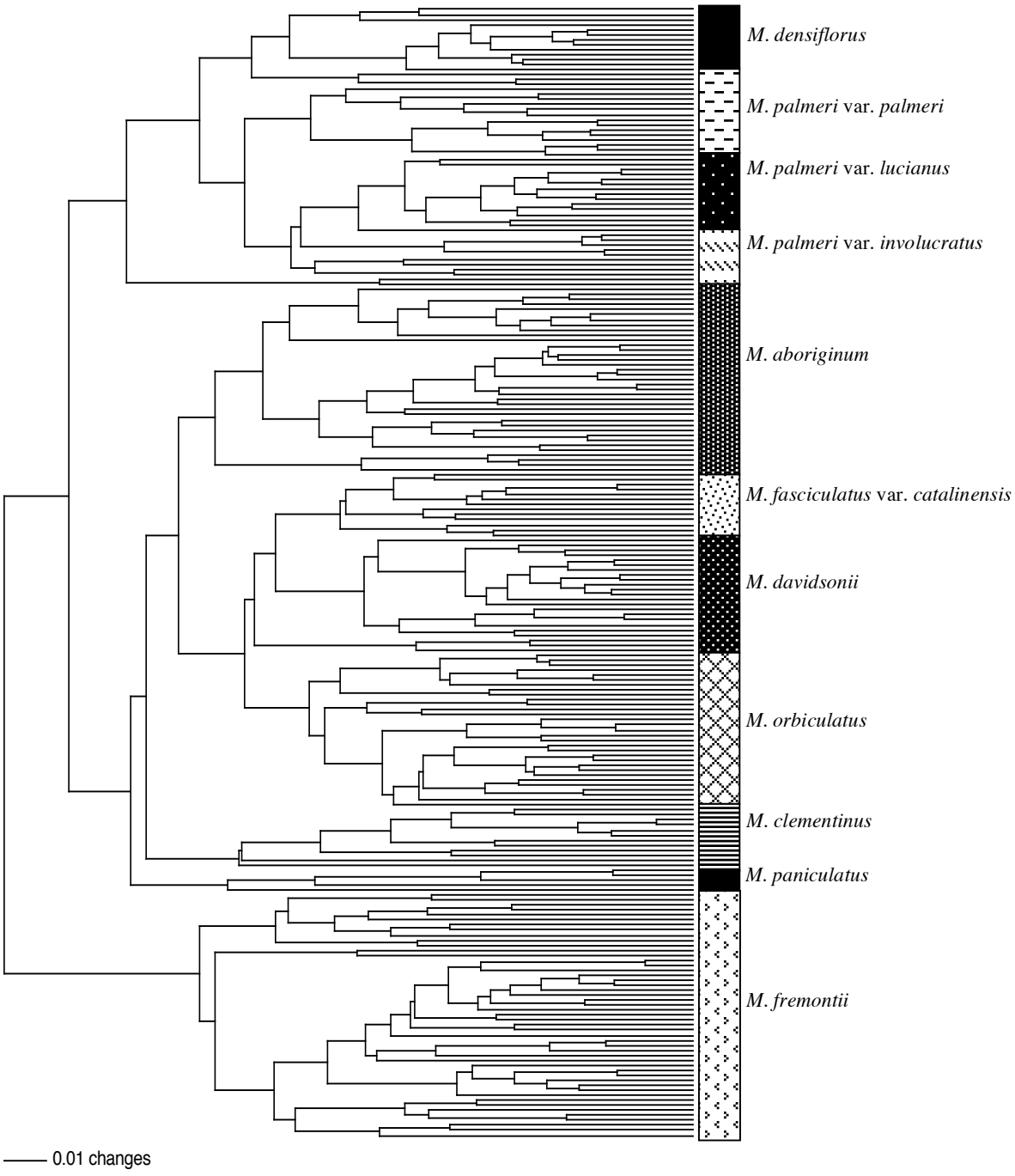
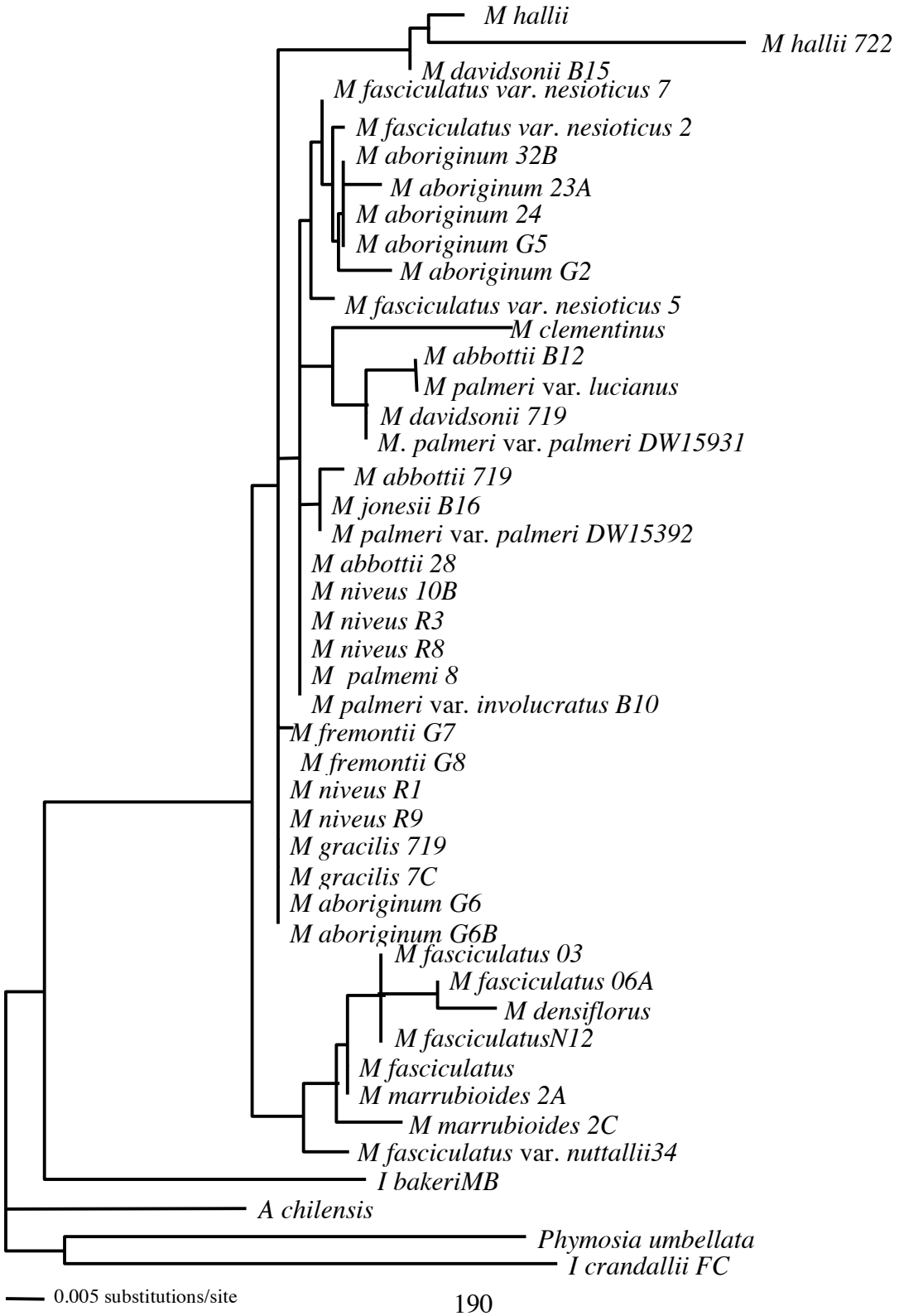


Figure 5.8. Maximum likelihood analysis of relationships in *Malacothamnus* based on sequences of the ITS region. Abbreviations for genera are as follows: *A* = *Andeimalva*, *I* = *Iliamna*, and *M* = *Malacothamnus*. Numbers (and letters) after the species name indicate the sample identification.



Chapter 6

Genetic variation within and between *Iliamna corei* and *Iliamna remota* (Malvaceae): implications for species delimitation

Abstract

Iliamna corei and *I. remota* are classified as endangered species; however, their designation as separate species has been questioned. To address this problem inter-simple sequence repeat (ISSR) data were generated to examine patterns of genetic differentiation within and between these two taxa. ISSRs were used to screen individuals for genetic diversity of *I. corei* from the single known natural population and two garden populations, and individuals of *I. remota* representing six natural populations and four garden populations. Using ten primers, 140 informative markers were generated. Ninety-four percent of loci detected revealed polymorphisms. Cluster analysis using Neighbor Joining (NJ), ordination with Principle Coordinate Analysis (PCoA), and Analysis of Molecular Variance (AMOVA) were used. NJ analysis showed the two species to be genetically distinct. In addition, the Illinois populations of *I. remota* were genetically distinct from the Virginia populations of *I. remota*. Principle Coordinate Analysis supports the findings of the NJ, with a separation of *I. corei* and *I. remota*. In the AMOVA, the majority of variation detected was within the populations, which is consistent with other self-incompatible plants. The results indicate a correlation between the geographic distributions of the species and gene flow.

Introduction

Iliamna Greene (Malvaceae) includes eight species distributed across temperate North America (Wiggins 1936). *Iliamna corei* (Sherff) Sherff and *Iliamna remota* (Greene) Wiggins

represent the easternmost distribution of the genus. *Iliamna remota* was first described in1906 from specimens collected on the Kankakee River in eastern Illinois (Greene 1906). During the 1910s, *I. remota* was recognized as being in danger of extinction and seeds were collected by the Chicago Wildflower Society and reportedly dispersed along railways in Illinois, Indiana, and Virginia (Sherff 1949). The presence of *I. remota* in Virginia was not reported before this time. Earl Core first found *Iliamna corei* atop Peters Mountain in southwestern Virginia in 1932 although, at that time, it was believed to represent a disjunct population of *I. remota* (Sherff 1949). It was not until 1946 that Sherff named the Virginia plants as a different variety, *I. remota* var. *corei* (Sherff 1946). He later concluded that these two varieties were indeed separate species, citing floral scent, habitat, and leaf morphology among the differences (Sherff 1949).

Iliamna corei is known from one natural population located near Narrows, Virginia on Peters Mountain (Stewart and Porter 1995). Because of recent droughts, increased herbivory, and winter mortality, the population has declined from approximately 40 plants in the late 1980s to 16 plants in the fall of 2002 (Thompson 1988; Edwards and Allen 2002). This species is listed as federally and state endangered. Flowers in *I. corei* have been shown to be protandrous and self-incompatible (Stewart and Porter 1995), and the seeds require scarification in order for germination to occur (Baskin and Baskin 1997).

At the type locality near Altorf, Illinois, the population of *I. remota* has disappeared and reappeared several times depending upon flooding of the Kankakee River (Sherff 1949; Swinehart and Jacobs 1998). In Indiana, the species has been found in several scattered populations near railways and/or small streams; some of these have been eradicated by human

activities. *Iliamna remota* is listed as state endangered in Illinois, Indiana, and Virginia. Few morphological differences have been noted that distinguish *I. corei* from *I. remota* (see Table 6.1), although differences in habitat and success in colonization are the most striking. Populations of *I. remota* in Virginia have been considered to be of anthropogenic origin (Porter 1991), with plants occurring along railways and streams.

In a previous study using RAPDs (Random Amplified Polymorphic DNA), Stewart and Porter (1995) and Stewart et al. (1996) determined that *I. corei* and *I. remota* were not genetically distinct. No statistical significance was found between the species using RAPD data with an analysis of molecular variance. An UPGMA (Unweighted Pair Group Method using Arithmetic Averaging) analysis did indicate *I. corei* to be derived from within the *I. remota* populations of Virginia examined. However, the taxonomic relationship of *I. corei* to *I. remota* was not determined. The four clumps of *I. corei* from the natural population on Peters Mountain are believed to be four genets that are very similar genetically (Stewart and Porter 1995). Populations of *I. remota* from Illinois and Indiana were not included in the study using RAPDs.

In the present study, inter-simple sequence repeats (ISSRs) were chosen to assess the genetic variation of *I. corei* and *I. remota* and to explore the utility of this type of data for assessing genetic variation in rare and/or endangered plants. Samples were included across both species' ranges in order to examine genetic variability within and between species in comparison to their geographic distributions. Furthermore, the data were used to assess whether or not *I. corei* and *I. remota* are distinct entities.

The ISSR markers have been used in numerous studies to examine variability among hybrid lines of cultivated plants, natural hybrid complexes, and variability in population genetics (Wolfe and Liston 1998; Wolfe et al. 1998; Culley and Wolfe 2001; Wolfe and Randle 2001). In ISSRs, a single primer is used in DNA amplification, resulting in multiple fragments of varying lengths. The pattern of fragments can be compared in assessing genetic variability. Studies using ISSRs are increasing, especially where RAPDs or allozymes were uninformative (Esselman et al. 1999). Primers used in an ISSR reaction consist of short repeated sequences, di-, tri-, or tetranucleotides, with either a small 5í or 3í anchor (Wolfe et al. 1998) (Table 6.3). The anchor generally consists of one to three degenerate nucleotides (e.g., C/G or T/A) and serves to prevent strand-slippage artifacts from forming during the reaction. Either by varying the number of repeats or by changing the short anchoring sequence, additional DNA banding patterns are produced and can be analyzed independently.

An advantage of ISSRs is that no prior sequence knowledge is needed, since primers are based upon repetitive elements in the DNA. It has been found that in plants the (AT)n and (CA)n microsattelites are the most abundant. Microsatellites and the ISSR markers are interspersed throughout the genome (Moreno et al. 1998). The use of ISSRs has expanded to not only study genetic diversity, but to distinguish among cultivars and to construct linkage maps, as well (Arcade et al. 2000).

The ISSR markers are inherited in a dominant Mendelian fashion and interpreted similar to RAPDs. In contrast to ISSRs, primers used in RAPDs are based upon anonymous genomic markers (Huff et al. 1993). RAPDs are traditionally used in assessing plant population genetics,

in constructing genetic maps, to identify hybrids, and to identify germplasms of crop plants. Several disadvantages of RAPDs have been noted, including template-primer mismatches, low levels of polymorphism, and reproducibility (Wolfe et al. 1998). ISSRs not only avoid these difficulties, but also have a higher cost-effectiveness per polymorphism (Wolfe and Randle 2001). In comparison to DNA sequence data, results from analysis of ISSRs in Raoulia were consistent in their value to infer genetic diversity (Smissen et al. 2003).

The presence of an ISSR marker may represent the dominant homozygote or heterozygote state, whereas the absence of a marker may not be due to a homozygous recessive state (Culley and Wolfe 2001). The absence may be the result of (a) a lost annealing site, (b) an insertion or deletion in the fragment between the primer sites, or (c) data scoring, which can be minimized by running multiple gels or PCR sets. Furthermore, the absence of bands may outweigh the signal from positive band matches (presence), since the absence of a band may not be due to identical mutations. Only ISSR marker presence is used for assessing genetic variation.

The objectives of this study were to (1) examine genetic variation of rare plants found in disjunct populations, (2) assess the utility of ISSRs in studying rare and endangered plants, and (3) assess the taxonomic relationship of *I. corei* to *I. remota*.

Materials and Methods

Sampling Strategy

All known populations of *I. corei* and *I. remota* were sampled (Figure 6.1 and Table 6.2). Species in *Iliamna* are rhizomatous and the number of stems emerging from the crown does not reflect the true number of individuals (Stewart and Porter 1995). Seven clumps were sampled from the natural population of *I. corei*, with one representative from each. Of these samples, five consistently amplified with the ISSR PCR reactions. The garden population of *I. corei* was started from seeds collected from the seed bank on Peters Mountain and has been maintained in cultivation for approximately 10 years. From the garden population, seven individuals produced banding patterns and were used in this study. Additional *I. corei* seeds were collected from fruits in the native population, germinated, and grown in the greenhouse. Ten individuals were successfully out-planted to a common garden with ten individuals of *I. remota* and of *I. rivularis*.

The largest population of *I. remota* sampled occurs along Interstate Highway 64 near mile marker 16 in Alleghany County, Virginia. As with *I. corei*, the population exists as several clumps of stems. In sampling the population, a transect was used in order to sample genets throughout the population, a total of five genets being sampled. The other Virginia populations sampled included all of the individuals present. Population size varies greatly from year to year, due to the amount of rainfall and herbivory (K. Heffernan, Virginia Natural Heritage Program, pers. comm.). The four Illinois garden populations sampled were started from seeds obtained from the native population on the Kankakee River. All members of these populations were sampled. From the type locality, four individuals of *I. remota* were sampled, representing the entire population. One population of *I. remota* from Indiana was sampled. The plant was grown

from seed obtained from an extirpated population on the Walbash Railroad (M. Jacobs, Goshen College, pers. comm.). One sample from *Iliamna rivularis* (Douglas ex Hooker) Wiggins was included in the study. It represents the southernmost population of *I. rivularis*, which is found from Idaho to Utah, Arizona, and New Mexico. The sample is included as an outgroup and not for direct comparison of genetic diversity.

ISSR Profiling

Total genomic DNA was isolated using the DNEasy Extraction kit (Qiagen Corp. Valencia, California). Tissue samples were first ground in liquid nitrogen, then placed in the appropriate buffer and heated at 65(C for 30 min. Following an extended incubation, the extraction continued following the manufacturer's directions. After isolation, samples were quantified using a 1% agarose gel, and the DNA visualized under UV light with ethidium bromide (0.5%) staining.

Amplification reactions consisted of 2.5 units of Taq Polymerase (Promega Corp., Madison, Wisconsin), 20 to 30 ng DNA, 3.0 mM MgCl2, 2.5 μM dNTPs, 1X reaction buffer, and 0.5 μM of primer. The amplification program was as follows: 40 cycles of 30 sec. at 94°C, and 45 sec. at 45°C, 1.5 min. at 72°C. A total of sixteen primers were initially examined for variability. Ten of those primers (see Table 6.3) showed variability between samples and populations. The resulting reactions were analyzed by electrophoresis in a 1.5% agarose gel stained as indicated above. Negative controls, those that contained no template DNA, were included for each reaction set to verify the purity of reagents. Samples for electrophoresis included 3 μl of blue-orange loading dye (Promega Corp.) and 10 μl of the 25 μl ISSR reaction.

The electrophoresis was stopped when the Orange G dye ran 6 cm from the wells, as an indication that sufficient separation between fragments could be seen.

Data Analysis

Bands were scored manually and verified using the AlphaImager software package (AlphaInnotech, Miami, Florida). A 100 bp ladder with sizes ranging from 1500 bp to 100 bp (Promega Corp.) was used to estimate sizes of the ISSR products. The total number, number of polymorphic, population-specific, and species-specific bands were noted. Data were entered into a spreadsheet as presence/absence data (1/0) and analyzed using NTSYS version 2.1 (Exeter Software Corp., Setauket, New York) to generate the similarity matrix using Dice's coefficient. Dice's coefficient takes into account the presence of shared and unique bands present in samples, but not the shared absence of bands (Sneath and Sokal 1973). The similarity matrix from NTSYS was implemented in PAUP4.0b10 to generate the Neighbor Joining dendrogram (Swofford 2003). A principle coordinate analysis (PCoA) was also performed in NTSYS. An analysis of molecular variance (AMOVA) was conducted to determine the significance of differences between populations and species (Excoffier et al. 1992). Variation was partitioned among populations and individuals within populations, among and within populations, and among groups. Significance levels for variance estimates were computed by non-parametic permutational procedures using non-Euclidean distances (Huff et al. 1993). AMOVA provides three statistics □st, □sc, and □ct. The □st is equivalent to the correlation of random haplotypes between populations relative to all of the samples, □sc as random haplotypes within a population relative to all of the samples, and □ct as the molecular diversity of random haplotypes within a

population relative to those from the groups (Excoffier et al. 1992). AMOVA was conducted to test if no genetic subdivision exists among the populations or species. A high □st value would indicate high population subdivision (Wright 1978).

Results

A total of 140 loci were detected with an average of 14 loci per primer (Table 6.3). Bands scored varied in size from 300bp to 1700bp. Replicate reactions and gels were completed to verify the repeatability of the amplifications and scoring. Variability between samples within the same population was detected, and several loci were specific for individual populations and geographic regions. Over all samples, 94% of loci were variable. Three loci were specific for samples from Illinois, three for *I. corei*, and eight for *I. remota* samples from the vicinity of Covington, Virginia. Also, eight loci were found only in plants sampled in Virginia, including representatives of both *I. corei* and *I. remota*. Geographic and population specific loci obtained were not primer specific.

The Neighbor joining (NJ) analysis results in two groups with four smaller clusters (Figure 6.2), one that represents *I. corei*, one for *I. remota* from Indiana and Illinois, and two of *I. remota* from Virginia. Within the Illinois group, a cluster forms with four samples from the Kankakee River population (ReILx), but the remaining samples do not represent any population-based pattern. On the NJ dendrogram, the cluster containing the Kankakee River population (ReIL) is on a branch indicating a higher number of changes. The *I. rivularis* sample was used as an outlier. This RiUT sample is located on a branch exterior to the Virginia and Illinois populations sampled.

Within the Virginia samples, three groups resolved. One group contained representatives from the *I. corei* populations on Peters Mountain (CoPMx), from the research garden (CoPFx), and the common garden (CoGx). Two additional groups of *I. remota* samples formed. One group, with samples from the Covington area (ReCCx and ReLRx), and one with the Bedford County area (ReVAx) and Clifton Forge samples (ReLMx) resulted. As indicated in the NJ dendrogram, the two species, *I. corei* and *I. remota*, in Virginia were genetically isolated. Populations sampled did cluster in this analysis as well.

The Principle Coordiante Analysis (PCoA) (Fig. 6.3) also resolved two main groups based on geography (Virginia and Illinois/Indiana). Within the plot of the Illinois populations, the natural population (ReILx) formed a loose group. Within the Virginia group, a separation of *I. corei* and *I. remota* resulted. Populations formed sub-groups within these larger clusters.

In performing the AMOVA (Table 6.4), samples were partitioned by species (level 1) and by state (level 2) in order to examine genetic variation between species and by geographic distance. Four groups were defined to represent 13 populations and 47 individuals. The four groups were the Illinois samples, the Indiana sample, *I. remota* from Virginia, and the *I. corei* samples. Within the nested variance, 14.03% of the genetic variation can be attributed to differences among groups (state versus state), 26.61% of genetic diversity to populational differences, and 59.36% to differences between individuals within a population. The Φst value was 0.406 ($P < 0.0099$), the Φsc value was 0.310 ($P < 0.0099$), and the Φct value was 0.140 ($P < 0.0099$).

Discussion

ISSRs provided a better estimate of gene flow and genetic variability within and between *I. corei* and *I. remota* than RAPDs. The degree of polymorphism with ISSR markers (94%) in *Iliamna* was greater than that of RAPD data (85%) (Stewart and Porter 1995). The study using RAPDs indicated significant variation within and among the four Virginia populations sampled (□st=0.51 and □sc=0.43). The high □st value obtained indicated extreme population subdivision among the Virginia *Iliamna* species (Stewart and Porter 1995).

In the present study, ISSR markers, again, indicated a high amount of genetic subdivision within the populations. When □st is less than one (and greater than 0), the genetic variation within populations is significant (Sales et al. 2001). The ISSR markers resulted in a □st of 0.406. The statistics also indicate a high degree of genetic diversity within the populations (see Table 6.4), since the majority of variation detected was found within the populations (59.36%). This high degree of genetic diversity within populations may be due to the self-incompatibility of the plants. In years when the different clumps of *I. corei* do not flower simultaneously, there is a marked decrease in seed production (T. Wieboldt and R. Edwards, Virginia Tech, pers. comm.). We would expect to see a higher level of genetic diversity in cross-pollinated plants than with self-pollination (Culley and Wolfe 2001). With out-crossing species, a higher level of genetic recombination and maintenance of diversity results (Huff et al. 1993). Roman et al. (2002) found *Orobanche crenata* populations in southern Spain to have 94.29% genetic diversity among individuals. As with *Iliamna*, *O. renata* is cross-pollinated and produces seeds that have a prolonged viability in the soil.

The high level of polymorphism found in *Iliamna* using ISSR markers is consistent with the results of other population genetic studies using ISSRs. With *Viola*, the mean proportion of polymorphic loci was 77.1% (Culley and Wolfe 2001), in *Cicer* 87% of loci were polymorphic (Rajesh et al. 2002), and among four species of *Hyrobanche* 64 to 96% of loci were polymorphic per primer (Wolfe and Randle 2001). As in these studies, unique banding patterns were found in populations of *I. remota* and *I. corei* and could be used to distinguish between populations. Population structuring of ISSRs in *Iliamna* was evident. Unique loci were detected based on location (Illinois versus Virginia) and from populations (Table 6.2).

The greatest correlation with genetic variation is seen in locations of populations, suggesting isolation by distance. The NJ dendrogram clusters plants by state, populations, and species. In Illinois, there is only one natural population of *I. remota*, and this was the seed source for the garden populations. The natural population may represent a remnant of a larger midwestern prairie *Iliamna remota* distribution. The Indiana population of *I. remota* may be a remnant of such as well. The Indiana sample included here is from the Pumpkin Vine Nature Trail, which was grown from seed obtained from a population in a nearby field (M. Jacobs, Goshen College, pers. comm.), although this field population has been eradicated through farming activity. Near this locality, however, were additional populations consisting of a few plants each (Swinehart and Jacobs 1998). The plants are or were located adjacent to the Wabash Railroad or downstream from it on the Elkhart River (Elkhart Co., Indiana). The position of the Indiana sample (ReGO) in the cluster analysis (Figure 6.2) and the PCoA suggest that the Illinois plants must have been the seed source for the Indiana populations.

Two scenarios can be proposed for the distribution of *Iliamna* in Virginia. First, multiple introductions of *Iliamna* to Virginia may have occurred. One event could have led to the establishment of the Peters Mountain Mallow (*I. corei*) in Giles County, a second introduction giving rise to those populations along the James River near Bedford, and a third leading to populations near Covington. The second scenario involves only a single introduction with subsequent migration and later diversification of *Iliamna* populations in Virginia. Seed dispersal by members of a native plant society or by railway is the hypothesized method of the introduction of *I. remota* to Virginia (Porter 1991). In pairwise comparisons of ISSR markers for the Covington to Bedford populations, 66-79% difference is observed. Yet, in comparing the *I. remota* samples from Covington to the *I. corei* samples, a 50-70% difference is observed. This difference may reflect the differing habitats and dispersal mechanisms of the populations. The discrepancy between the genetic profiles of the groups of *I. remota* in Virginia leads us to believe that these populations are the result of separate introductions. These individuals have been able to establish stable populations in highly disturbed sites with little variation in population sizes. The segregation of *I. corei* and *I. remota* populations within Virginia indicates multiple events of seed dispersal. The three clusters of *I. corei* in the NJ phylogram (Figure 6.2) reflect the three seed sources, with the CoGx garden plants being derived from the source CoPM population and the individuals established from the seedbank (CoPFx) being genetically distant.

Plants in Illinois and Indiana are genetically distinct from the Virginia plants. The western populations are older, some are in greater numbers, and all show a greater genetic diversity than the plants in Virginia. In the NJ phylogram, the greater genetic diversity in the

western population is evident with the longer branches indicating a greater number of changes from the nearest node.

Iliamna corei in Virginia and *I. remota* in Illinois are genetically distinct and significant variation was found between the species. The ISSR technique has produced sufficient evidence to warrant their continued taxonomic separation. The results of this study also support the supposition that *Iliamna corei* is self-incompatible, with a higher within versus between population variance. In examining genetic variance with ISSRs, genetic drift does not appear to be a factor in the taxa. If genetic drift were occurring, we would see a loss of variability within populations and an increase in variance between populations (Schaal, 2002). The populations are able to persist through flood and drought years through maintenance of genotype variance. The maintenance of various genotypes is indicated with 59.36% within population variance and is further evidence that inbreeding depression has not occurred.

The utility of ISSRs in assessing genetic variation in rare plants with small populations has been established here. This technique can provide information on the overall level of genetic variation in populations and be used to infer the health of the populations, whether or not a population is undergoing drift, founder effects, or inbreeding depression by examining the level of genetic variation within a population or between multiple populations. In using ISSRs, loci can be detected to identify individuals from populations or geographic regions. In the future, this study can be expanded to include the remaining species of *Iliamna* to better understand how the easternmost species are related to the western taxa. Additional molecular and morphological

analyses of the genus are underway to elucidate relationships within the genus and the origin of the taxa within it.

Literature Cited

Arcade, A., F. Anselin, P. Faivre Rampant, M.C. Lesage, L.E. Paques, and D. Prat. 2000. Application of AFLP, RAPD, and ISSR markers to genetic mapping of European and Japanese Larch. *Theoretical and Applied Genetics* 100: 299-307.

Baskin, J. M. and C. C. Baskin. 1997 Methods of breaking seed dormancy in the endangered species *Iliamna corei* (Sherff) Sherff (Malvaceae) with special attention to heating. *Natural Areas Journal* 17: 313-323.

Culley, T.M., and A.D. Wolfe. 2001. Population genetic structure of the cleistogamous plant species *Viola pubescens*, as indicated by isozyme and ISSR molecular markers. *Heredity* 86(5): 545-556.

Edwards, R. and J. C. Allen. 2002. *Population monitoring and management of Peters Mountain Mallow* (*Iliamna corei*). The Nature Conservancy, VAFO Technical Report 03-01. The Nature Conservancy of Virginia, Charlottesville, Virginia.

Esselman, E.J., L. Jianqiang, D.J. Crawford, J.L. Winduss and A.D. Wolfe. 1999. Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers. *Molecular Ecology* 8: 443-451.

Excoffier, L., P. E. Smouse, and L. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Greene, E. L. 1906. Certain malvaceous types. *Leaflets of Botanical Observations and Criticism*. Pp. 205-209.

Huff, D. R., R. Peakall, and P.E. Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.). *Theoretical and Applied Genetics* 86: 927-934.

Moreno, S., J.P. Martin, and J.M. Ortiz. 1998. Intersimple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica* 101: 117-125.

Porter, D. M. 1991. Peters Mountain Mallow. In K. Terwilliger, comp, *Virginia's endangered species: Proceedings of a symposium*. Blacksburg, VA: The McDonald and Woodward Publishing Co., 130-133.

Rajesh, P. N., V. J. Sant, V. S. Gupta, F. J. Muehlbauer, and P. K. Ranjekar. 2002. Genetic relationships among annual and perennial wild species of *Cicer* using inter simple sequence repeat (ISSR) polymorphisms. *Euphytica* 129:15-23.

Roman, B. D. Rubiales, A. M. Torres, J. I. Cubero, and Z. Satovic. 2002. Genetic diversity in *Orobanche crenata* populations from southern Spain. *Theoretical and Applied Genetics* 103 (6-7): 1108-1114.

Sales, E. S. G. Nebauer, M. Mus, and J. Segura. 2001. Population genetic study in the Balearic endemic plant species *Digitalis minor* (Scrophulariaceae) using RAPD markers. *American Journal of Botany* 88 (10): 1750-1759 OCT 2001

Schaal, B. 2002. Conservation genetics of natural and introduced plant populations. Missouri Botanical Garden 49th Annual Systematics Symposium Presentation.

Sherff, E. E. 1946. Notes on certain plants in Gray's manual range. *Rhodora* 48: 89-96.

Sherff, E. E. 1949. Further notes on *Iliamna*. *American Journal of Botany* 36: 502-503.

Smissen, R. D., I. Breitwieser, J. M. Ward, P. A. McLenachan, and P. J. Lockhart. 2003. Use of ISSR profiles and ITS-sequences to study the biogeography of alpine cushion plants in the genus *Raoulia* (Asteraceae). *Plant Systematics and Evolution* 239: 79-94.

Sneath, P. H. A. and P. R. Sokal. 1973. *Numerical Taxonomy*. San Francisco: W. H. Freeman and Company.

Stewart, C. N. and D. M. Porter. 1995. RAPD Profiling in Biological Conservation: An Application to estimating Clonal Variation in Rare and Endangered *Iliamna* in Virginia. *Biological Conservation* 74: 135-142.

Stewart, C. N. , G. Rosson, B. W. Shirley, and D. M. Porter. 1996. Population genetic variation in rare and endangered *Iliamna* (Malvaceae) in Virginia. *Biological Journal of the Linnean Society* 58: 357-369.

Swinehart, A. L. and M. E. Jacobs. 1998. Rediscovery, status and preservation of the endangered Kankakee Globe Mallow (*Iliamna remota*) in Indiana. *Rhodora* 100: 82-87.

Swofford, D. 2003. PAUP: Phylogenetic analysis using parsimony, version 4.10b. Sinauer and Associates.

Thompson, P. J. 1988. Tale of two mallows: Peters Mountain Mallow. *Plant Conservation* 3: 7.

Wiggins, I. L. 1936. A resurrection and revision of the genus *Iliamna* Greene. *Contributions from the Dudley Herbarium, Stanford University* 1: 213-229

Wolfe, A. D., and A. Liston. 1998. Contributions of PCR-based methods to plant systematics and evolutionary biology. In: D. E. Soltis, P. S. Soltis and J. J. Doyle, *Plant Molecular Systematics II*. Boston: Kluwer, 43-86.

Wolfe, A. D., Q.-Y. Xiang, and S. R. Kephart. 1998. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. *Molecular Ecology* 7: 1107-1125.

Wolfe A. D. and C. P. Randle. 2001 Relationships within and among species of the holoparasitic genus *Hyobanche* (Orobanchaceae) inferred from ISSR banding patterns and nucleotide sequences. *Systematic Botany* 26 (1): 120-130.

Wright, S. 1978. *Evolution and the Genetics of Populations. Vol. 4. Variability within and among natural populations.* Chicago: University of Chicago Press.

Table 6.1. Morphological Variation in *Iliamna corei* and *Iliamna remota*.

	Iliamna corei	***Iliamna remota***
Type locality	Peters Mountain, Narrows Virginia	Kankakee River, Altorf, Illinois
Distribution	Giles County, Virginia	Kankakee County, Illinois; Goshen County, Indiana; Bedford and Alleghany Counties, Virginia
Morphological apomorphies	Terminal leaf lobe elongate, deep leaf sinuses, several flowering stalks per plant, unscented flowers (?)	Terminal leaf lobe not longer than others, shallow leaf sinuses, terminal raceme, flowers fragrant
Plant height	0.5 to 1 meter	1.5 to 2 meters
Habitat	Shallow forest soils	Fluvial, disturbed soils

Table 6.2. Populations of *Iliamna corei* and *I. remota* sampled. The first two letters of the abbreviations indicate the species; the second two, the population sampled; and the last letter or number, the individual within that population. When possible numerous samples within a population were chosen along a transect of the population. In such instances, not all plants were sampled, since clumps of stems are the result of the species' rhizomatous habit. In the remaining populations, all members present in the population at the time of collection were sampled, as indicated by *.

Species	State	Population	Samples
Iliamna rivularis	Utah	Cache County	RiUT (voucher: VPI T. Slotta 99-14)
Iliamna corei	Virginia	Garden 1 (Montgomery County)	CoPF1, CoPF2, CoPF3, CoPF4, CoPF5, CoPF6, CoPF7 (voucher: VPI T. F. Wieboldt 6761)
		Garden 2 (Montgomery County)	CoG1, CoG2, CoG3, CoG4, CoG6, CoG7, CoG8, CoG9, CoG10 (voucher: VPI T. Slotta 2003-14)
		Type Locality (Giles County)	CoPM1, CoPM2, CoPM3, CoPM5, CoPM6 (voucher: VPI G. P. Frank 443)
Iliamna remota	Virginia	Karne's Creek* (Alleghany County)	ReCC1, ReCC2 (voucher: VPI T. Slotta 2001-2)
		Lick Run Preserve* (Alleghany County)	ReLR1, ReLR2, ReLR2B, ReLR3 (voucher: VPI T. Slotta 2001-4)
		Exit 16, Interstate 64 (Alleghany County)	ReLM1, ReLM2, ReLM3, ReLM4, ReLM5 (voucher: VPI T. Slotta 2001-3)
		Bedford* (Bedford County)	ReVA1, ReVA2 (voucher: VPI C. E. Stevens 15,733)
	Indiana	Goshen* (Goshen County)	ReGO (voucher: VPI R. C. Friesner s.n. 7/14/1945)
	Illinois	Garden 1*	ReRO1, ReRO2
		Garden 2*	ReJT
		Garden 3*	ReHiA, ReHiB, ReHiC
		Garden 4*	ReNRB1
		Type locality* Altorf Island (Kankakee County)	ReILA, ReILB, ReILC, ReILD

Table 6.3. Primers used in ISSR amplification, written in the 5' to 3' orientation. Degenerative nucleotides were often used as the 5' or 3' anchor. Maximum number of bands produced per primer per template is indicated.

Primer Name and Sequence	Number of Bands
AGC4: $(AGC)_4$-GY	15
AGAC4: $(AGAC)_4$	16
CA8: $(CA)_8$-RG	14
Doug: $(TC)_7$-YC	12
GA7: GCCA-$(GA)_7$	20
GAC4: $(GAC)_4$-RC	9
Hans: $(AC)_7$-RG	20
TBS1: $(GT)_6$-RA	13
807: $(AG)_8$-TG	9
899: $(CA)_6$ RG	12

Table 6.4. Analysis of Molecular Variance (AMOVA) for 46 individuals using 140 ISSR loci. Statistics included for the nested analysis: degrees of freedom (df), sum of squares (SSDs), mean squared deviations (MSDs), variance component estimates, and the percentage of total variance contributed by each component.

	df	SSD	MSD	Variance	% Total
Among Groups	3	258.478	85.159	4.167	14.03
Among populations within groups	11	451.908	41.083	7.900	26.61
Within populations	32	563.979	17.624	17.624	59.36

Figure 6.1. Distribution of *Iliamna corei* (single county in Virginia, indicated by county filled in black) and *Iliamna remota* (four counties in Virginia, one in Indiana, and one in Illinois, indicated by counties filled in gray)

Figure 6.2. Unrooted Neighbor joining dendrogram. Abbreviations for samples are as listed in Table 6.1. Circles around branches indicate species boundaries, solid line for *I. remota* and dashed line for *I. corei*.

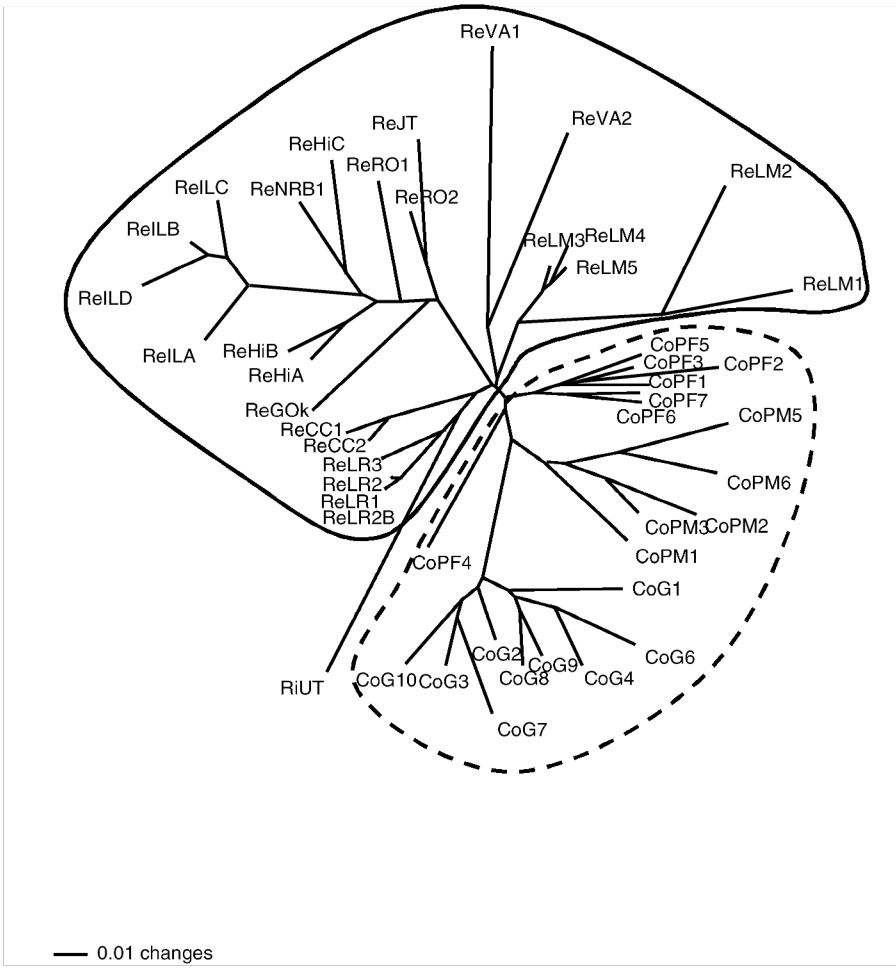
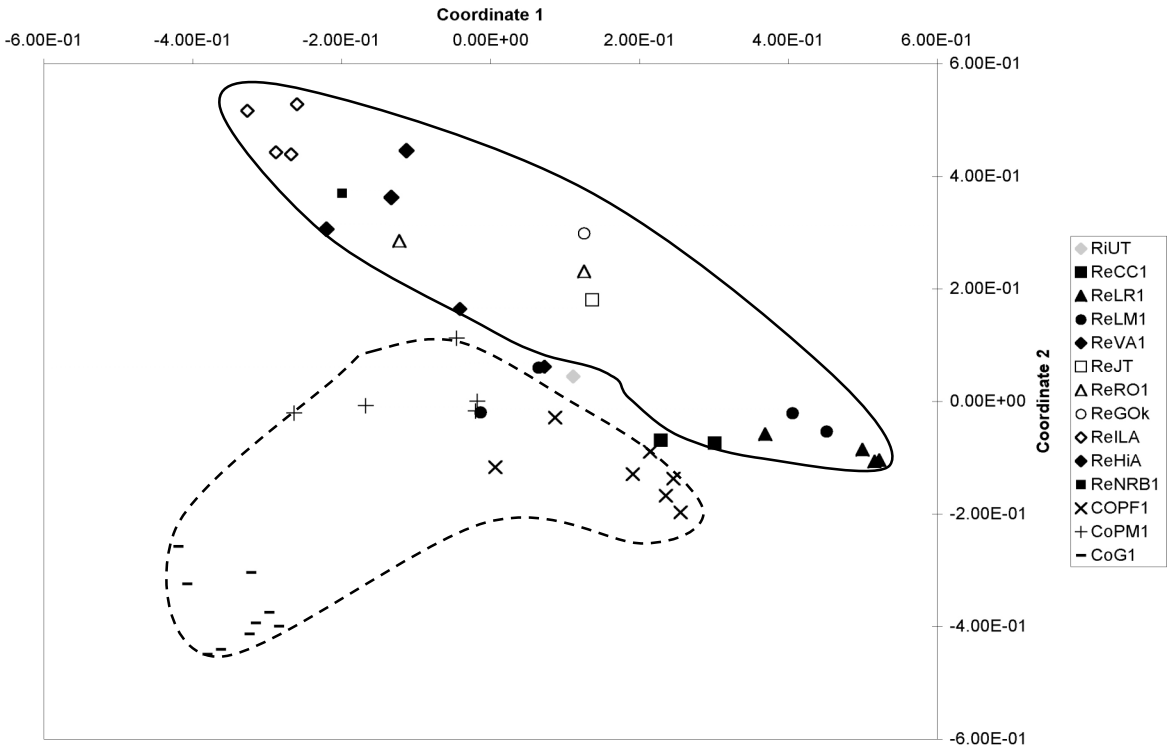


Figure 6.3. Principle coordinate analysis of ISSR data. Abbreviations for samples are as listed in Table 1. Solid line encompasses *I. remota* populations and the dashed line *I. corei*. Symbols for populations provided in figure legend.



Chapter 7

Hybridization in *Iliamna*

Abstract

Iliamna corei and *I. remota* represent two endangered species in a North American endemic genus of Malvaceae. They occur in disjunct populations in Virginia (both species) and Illinois and Indiana (*I. remota*). Prior studies have shown the two to be closely related and it was not clear if they warranted status as separate species. The next closest species, geographically, is *I. rivularis*, which is found throughout the Rocky Mountains. The objectives of this study were to (a) determine whether *I. corei* and *I. remota* are morphologically distinct, and (b) determine the viability and fecundity of hybrids of *I. corei*, *I. remota*, and *I. rivularis*. To study hybridization, crosses were conducted in the greenhouse to produce an F_1 generation that was examined for hybrid success and morphological variation. Hybridization between *Iliamna* species can occur where they grow in proximity to one another. Hybrids produced between *I. corei* and *I. remota* had greater seed viability and plant fecundity than crosses with *I. rivularis*. The relative success of interspecific hybridization between *I. corei* and *I. remota* in the controlled experiment indicates that hybridization is a threat to the preservation of the species. *Iliamna rivularis* does not appear to be a threat to the genetic integrity of *I. corei* and *I. remota* since the populations do not overlap and seed production and viability was reduced.

Introduction

Speciation and the Role of Hybridization

Hybridization of natural populations can have a significant impact on genetic and morphological variation of species, which in turn can affect the taxonomy of a group (Stebbins 1969). Lineages that have diverged may undergo hybridization if speciation is incomplete and the lineages come into contact. The resulting hybrids may have attributes that better equip them for micro-environmental conditions the parent species were not adapted to, such as disturbed habitats. Hybrids may present novel adaptations or intermediate phenotypes that are better suited for environmental changes.

Hybridization between *Asclepias syriaca* and *A. exaltata* in Michigan and Virginia has occurred independently and resulted in intermediate phenotypes of the milkweed congeners in both locations (Kephart et al. 1988). Natural hybrids of *A. syriaca* and *A. exaltata* result from pollen transfer by non-specific pollinators (*Apis* and *Bombus*). Hybrids of these milkweeds are often found at the margins of their native habitats or in disturbed areas near by. *Asclepias syriaca* is often associated with open pastures, riverbanks, dry hillsides, and openings in wooded areas associated with disturbance. In contrast, *A. exaltata* occurs in wooded areas dominated by oak, aspen, and pine in the Appalachians. The increased gene flow between hybrids of *Asclepias* provided an advantage in adapting to new environments via natural selection, especially in *A. exaltata*. Despite repeated backcrosses between the F_1 and subsequent generations and the parents, species have maintained the morphological distinctions of *A. syriaca* and *A. exaltata*.

While hybridization between lineages can increase total variation of the gene pool, as in *Asclepias*, the mingling of the separate gene pools can also degrade the integretiy of the parent species (Stebbins 1969). The effects of hybridization on gene pools can be exacerbated in rare species (Levin et al. 1996). Hybrid offspring may promote the demise of rare species by replacing rare congeners with hybrids or contaminating congener gene pools. Even if hybrids are not viable, the production of hybrids reduces resources for the production of congener offspring (e.g., pollen and eggs). Other biological factors contribute to the occurrence of hybridization as well. Species that are self-incompatible, as is *Iliamna corei*, are at a greater risk to losing integrity of their gene pools through hybridization (Levin et al. 1996). Through out-crossing with an introduced congener, such as *I. remota*, the purity of each parent species is compromised. Through several generations of hybrid seed production, prezygotic barriers between congeners could decrease (Levin et al. 1996). Parent populations could potentially have lowered survivorship and fecundity as hybrids infiltrate their distributions. Continued or recurrent contact between congeners and hybrids can lead to multiple origins of polyploid hybrids as well (Soltis et al. 1995). In *Tragopogon* (Asteraceae), the tetraploid species *T. mirus* and *T. miscellus* have developed in several locations within the last century and are maintained through differences in habitat requirement.

Success of hybrids is highly dependent on environmental factors (Stebbins 1969). The demise of a rare species through hybridization would require all of its populations to be invaded by a congener (Levin et al 1996), and habitat preference to be overcome (Riesberg and Carney 1998). Isolating mechanisms and prezygotic, gametic, and postzygotic barriers would need to be

surpassed for an F_1 generation to be produced and become established in order for hybrid generations to form (Riesberg and Carney 1998). Either spatial or ecological isolation or considerable genetic recombination in hybrid offspring would need to occur in order to completely degrade the parental species. *Iliamna corei* is at a great risk to infiltration since it occurs as a single population, and a second species, *I. remota*, occurs in nearby counties in Virginia (Porter 1991). The greatest risk to *Iliamna corei* and other rare species for potential hybridization lies in the disintegration of ecological barriers (Levin et al. 1996). Through human activities, barriers that used to separate closely related taxa are being broken, and the potential for gene flow between rare plants and common congeners has greatly increased.

The objectives of this study were to determine whether *I. corei* and *I. remota* are morphologically distinct and determine the viability and fecundity of hybrids resulting from crosses between *I. corei*, *I. remota*, and *I. rivularis*. Specifically, representatives of each species were grown in a common garden to determine whether morphological variation is genetically or ecologically based. To study hybridization, crosses were conducted in the greenhouse to produce an F_1 generation. The seeds from this cross were germinated and the F_1 individuals grown in the greenhouse to examine hybrid success and morphological variation.

Methods

Populations Sampled

Seeds collected previously for *I. corei* on Peters Mountain (Narrows, Giles County, Virginia), *I. remota* seeds collected from the Iron Gate population (Alleghany County, Virginia), and *I. rivularis* seeds (Cache County, Utah) were germinated. Germination was enhanced by scarifying the seed coat with a razor blade, while avoiding the embryo (Baskin and Baskin 1998). The scarified seeds were placed onto sterilized moist filter paper in petri dishes. Once the cotyledons emerged, germination rate was calculated and the seedlings were transferred to a peat-vermiculite mixture (Gro-Mix) and placed onto a mist bench to provide constant moisture. After a minimum of four true leaves was present, the plants were transferred to six-inch pots with peat-vermiculite potting mix (Gro-Mix). Plants were maintained in the greenhouse with 12 hours of light per day for the duration of the project.

Hybridization

Hybridization experiments between *I. corei*, *I. remota*, and *I. rivularis* took place in the greenhouse. Upon flowering, anthers were removed before they dehisced in order to prevent self-pollination. Pollen was then transferred from a different plant (see Table 7.1), and pollination bags were used to prevent uncontrolled pollinations. Approximately three weeks after pollination, fruits were collected and were placed at 40°C to dry them. Dried fruits were stored at room temperature. After the fruits were collected, the parent plants were transplanted to a common garden in order to assess morphological variation under identical field conditions.

To examine success of hybridization, seeds of the F_1 generation were germinated as described above. Seedlings were grown in the greenhouse to maturity (5-6 months). Upon

flowering, morphological characters of leaves, plant height, and floral characters were measured (see Tables 7.2 and 7.4). With the F_1 generation, self-pollination, out-crossing with their siblings, and backcrosses to *I. rivularis* were conducted. In addition to the generated hybrid individuals, two individuals of each parent species were grown in the greenhouse. Several individuals of *I. corei* and *I. remota* did not flower, and one individual of *I. rivularis* produced only three flowers. Morphological characters, excluding floral traits, were measured for the remaining individuals in the study that did not flower or that produced flower buds that aborted.

The data matrix for all plants, hybrids and congeners, was analyzed using the PC-Ord program to conduct Principal Components Analysis (PCA) using Eigen vectors (McCune and Mefford 1997). Characters included in this first analysis included all vegetative characters except plant height. Pearson's coefficient of correlation between variables and the axes were calculated as r^2 values. A second PCA was conducted using only those plants that produced flowers. Characters used in this analysis included petal length, staminal column length, sepal length, sepal width, and bract length, all of which had r2 values greater than 0.200 for Axis 1.

Results

Eleven plants of *I. corei*, ten of *I. rivularis*, and twelve of *I. remota* were used in producing artificial hybrids in the greenhouse, which were then transplanted into a garden plot in early fall after fruits were collected. The following summer, morphological characters (see chapter 5 for descriptions) were measured and the health of individuals noted. Three individuals of *I. rivularis* did not survive the winter. The surviving *I. rivularis* plants produced leaves in

mid-April and were in flower by early June. All *I. corei* and *I. remota* plants survived the winter and emerged two to three weeks after *I. rivularis*. Likewise, flowering occurred in *I. corei* and *I. remota* several weeks after *I. rivularis*. Table 7.2 summarizes morphological variation among the individuals in the garden and Figure 7.1 illustrates the results of principle component analysis (PCA) of the morphological data.

In the hybridization experiments, self-pollination was unsuccessful in all attempts in *I. corei*, *I. remota*, and *I. rivularis* (Table 7.1). All out-crossed individuals produced seed that was germinated for an F_1 generation. Two plants of *I. corei* (egg donor) and two of *I. remota* (egg donor) were crossed with *I. rivularis*. Four individuals of *I. corei* (egg donor) were crossed with *I. remota*, and five of *I. remota* (egg donor) were crossed with *I. corei*. Crosses of *I. rivularis* (egg donor) with *I. corei* were not as successful in seed production (Table 7.3). Seed set was lower in crosses of *I. rivularis* (egg donor) and *I. remota* (pollen donor).

Germination rates varied among the various F_1 groups (Table 7.3). From the germinated seeds, 133 individuals were grown for six months in the greenhouse in order to permit flowering. The hybrid individuals were characterized for success, summarized as measurements of growth in Table 7.4. In general, individuals resulting from crosses with *I. rivularis* did not grow as well and did not appear as healthy, many stems were twisted, and most flower buds aborted. Multivariate analysis with PCA revealed no clusters when vegetative characters were examined. Examination of only the floral characters (Fig. 7.2) revealed slightly varying phenotypes in crosses with *I. rivularis* (pollen donor) versus crosses with *I. remota* and *I. corei* as the pollen donor. Some hybrids represented novel phenotypes as well.

Discussion

Morphologically, *I. corei* and *I. remota* are distinct and maintain unique attributes when grown under identical conditions. Individuals of *I. rivularis* had reduced fecundity in comparison to the Virginia species' performances in the common garden. *Iliamna rivularis* is not adapted for the hot, humid summers in Virginia. Individuals of *I. remota* grew to be much taller than either of the other species and flowered for a longer period of time. In the PCA, individuals grown in the garden resembled their conspecific representatives from herbarium specimens (Fig. 7.1). Therefore, the morphological variation seen in this experiment reflects that in the native populations. The morphology of *I. remota* grown for this project, from seeds collected in Virginia, was also consistent with herbarium specimens from the type locality in Illinois.

Hybridization within *Iliamna* could occur if the species were to grow in proximity. The degree of success of interspecific hybridization is not surprising, given the results of molecular phylogenetic studies (see Chapters 2 and 3). The molecular phylogenetic survey of ITS, *trn*L-F, and *rpL*16 (see Chapter 2) indicated *I. corei* and *I. remota* to be sister taxa, and the individual species in Virginia to be more closely related than *I. remota* in Illinois and Virginia. Hybrids produced between *I. corei* and *I. remota* had greater seed viability and plant fecundity than those with *I. rivularis* (Table 7.3).

One general rule of hybrid speciation is that the vigor of hybrids of closely related species tends to exceed the vigor of the parents (Riesberg and Carney 1998). This phenomenon is not well understood genetically, but does explain the success of natural allopolyploids and clonal hybrids. The evolutionary consequences of hybrid vigor, if natural hybrids of *I. corei* and *I. remota* were to form, could lead to the persistence of hybrids in mixed populations. In the greenhouse experiment, the majority of the *I. corei* x *I. remota* F_1 individuals developed healthy vegetation and flowered profusely. Since the success of interspecific hybrids is reported to diminish with subsequent generations, further studies in *Iliamna* are needed to confirm this. Likewise, the performance of hybrids in a native setting was not tested and may not be identical to results obtained in the greenhouse study.

Character expression of the hybrids generated included intermediate and mosaic phenotypes. The size range of characters measured overlapped with those of the parent plants. As seen in the PCA (Fig. 7.2), the majority of hybrids measured for flowering characters fall intermediately between or adjacent to the parent plants. However, several individuals (Re5F, 5J, 10B, and 11C, and C5I) are more similar to the parent plants than to others in their hybrid lines. Other individuals may be expressing novel traits, which place them as outliers in the PCA (Re10F, Ri5F, C11H, and C8D). These findings are consistent with other studies of hybrids, in that parental traits are expressed in hybrid offspring (mosaics) in approximately 40% of individuals and another 40% of individuals have an intermediate morphology (Riesberg and Carney 1998). The presence of mosaics could result from dominant expression patterns from the parental gene pools. The appearance of novel phenotypes usually occurs nearly 10% of the time in other studies, with over 30% of these traits expressed in later hybrid generations. In the

current study, novel phenotypes observed included a compact inflorescence (Re5 representatives) and the development of a basal rosette of leaves (a few individuals in *I. corei* and *I. remota*).

Implications for Preservation

The relative success of interspecific hybridization between *I. corei* and *I. remota* in the controlled experiment indicated that hybridization is a threat to the preservation of the species. However, *Iliamna rivularis* does not appear to be a threat to the genetic integrity of *I. corei* and *I. remota,* since the species are not sympatric and hybrid seed production and viability was reduced. The greatest threat appeared to be the invasion of the *I. corei* gene pool by *I. remota*. Since subsequent crosses of F_1 individuals resulted in a large seed set in this study, the mingling of genomes in natural hybrids could persist as well. Therefore, to maintain the genetic integrity of *I. corei* and *I. remota*, all efforts should be taken to prevent infiltration of their respective populations. The threat of hybridization with *I. rivularis* is low and unlikely since the easternmost populations of this species are in Colorado. If a hybrid zone were to form between the Virginia populations of *I. corei* and *I. remota*, the genetical and morphological distinctness of *I. corei* would be at a great risk of being lost.

Future Directions

This study represents a brief survey of the potential for hybridization in *Iliamna*. Of special concern is the extremely small population size of *I. corei* and the associated risk to a compromised gene pool of the species. The current study should be continued to include examination of subsequent hybrid generations and a greater attempt at conducting backcrosses with multiple parent plants. As stated above, only one individual of *I. rivularis* flowered and was used in the production of backcrossed seeds. To study the role of environmental conditions on the performance in hybrids, multiple generations could be planted in field plots in conditions mimicking native populations. Quantitative trait loci (QTLs) should also be examined to identify regions that are important to adaptation and that differentiate hybrid lines.

Literature Cited

Baskin, C.C. and J.M. Baskin 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego, CA: Academic Press.

Kephart, S.R., R. Wyatt, and D. Parrella. 1988. Hybridization in North American *Asclepias*. I. Morphological evidence. *Systematic Botany* 13: 456-473.

Levin, D.A., J. Francisco-Ortega, and R.K. Jansen. 1996. Hybridization and the extinction of rare plant species. *Conservation Biology* 10: 10-16.

McCune, B. and M.J. Mefford. 1997. *Multivariate Analysis of Ecological Data, ver. 3.0*. Gleneden Beach, OR: MiJM Software.

Porter, D.M. 1991. Peters Mountain Mallow. In *Virginia's Endangered Species*. K. Terwilliger, comp. Blacksburg, VA: The McDonald and Woodward Publishing Company. Pp. 130-133.

Rieseberg, L.H. and S.E. Carney. 1998. Plant hybridization. *New Phytologist* 140: 599-624.

Soltis, P.S., G.M. Plunkett, S.J. Novak, and D.E. Soltis. 1995. Genetic variation in *Tragopogon* species: Additional origins of the allotetraploids *T. mirus* and *T. miscellus* (Compositae). *American Journal of Botany* 82: 1329-1341.

Stebbins, G.L. 1969. The significance of hybridization for plant taxonomy and evolution. *Taxon* 18: 26-35.

Table 7.1. Individuals used in interspecific crosses and success of fruit production for an F_1 Generation.

Individuals	Pollen Source	Fruit Production
I. corei 1 and 2	Not Pollinated	None
I. corei 3 and 4	Self	None
I. corei 5 and 6	*I. rivularis*	5-1fruit; 6-None
I. corei 7, 8, 9, 10, and 11	*I. remota*	7-none; 8-1 fruit; 9-None; 10-3 fruits; 11-1 fruit
I. remota 1, 2, and 12	Not Pollinated	None
I. remota 3 and 4	Self	None
I. remota 5 and 6	*I. rivularis*	5-5 fruits; 6-2 fruits
I. remota 7, 8, 9, 10, and 11	*I. corei*	7-1 fruit (17 seeds); 8-2 fruits; 9-4 fruits (107 seeds); 10-2 fruits; 11-2 fruits
I. rivularis 1 and 2	Not Pollinated	1-1 fruit, no seeds
I. rivularis 3 and 4	Self	None
I. rivularis 5, 6, and 7	*I. remota*	5-3 fruits; 6-None; 7-None
I. rivularis 8, 9, and 10	*I. corei*	None

Table 7.2. Morphological variation assessed in garden grown individuals of *I. corei, I. remota,* and *I. rivularis*. The maximum, minimum, mean, and standard deviation for each species is presented. Units for all quantitative measurements are centimeters.

		Petiole Length	Leaf Width	Leaf Length	Pubescence Upper	Pubescence Lower	Leaf Base Shape	Sepal Length	Sepal Width	Pedicel Length	Bract Length	Bract Width	Sepal Pubescence	Petal Base Width	Petal Margin Width	Staminal Column	Stem Pubescence
I. corei	mean	7.59	8.88	8.41	3	3.13	2	1.43	0.64	1.01	0.83	0.08	5.88	0.3	1.89	0.97	4.38
	max	12	10.66	11	4	4	3	1.86	0.78	1.23	1.15	0.14	6	0.39	2.3	1.1	5
	min	5.43	6.25	6.75	2	2	1	1	0.5	0.77	0.6	0.03	5	0.2	1.6	0.55	1
I. remota	mean	7.25	9.27	17.54	1.67	2	2.44	1.3	0.56	0.44	1.45	0.08	4	0.36	2.1	0.52	2.33
	max	9.52	12.9	83.9	3	3	3	1.75	0.77	0.68	6.9	0.11	4	0.5	2.54	0.58	4
	min	4	4	6.05	1	1	1	0.42	0.45	0.24	0.64	0.03	4	0.3	1.7	0.48	1
I. rivularis	mean	5.65	10.11	9.4	1.17	1.33	2.5	0.95	0.45	0.55	0.52	0.06	2	0.65	1.48	0.57	1.5
	max	9.3	14.32	12	2	2	3	1.85	0.58	0.73	0.82	0.12	2	2	2.14	0.65	3
	min	2.41	6	7	1	1	2	0.62	0.34	0.39	0.34	0.03	2	0.16	0.7	0.43	1

Table 7.3. F_1 seeds produced and germinated. Plants grown to maturity and successful in seed production of an F_2 generation are indicated by an asterisk (*), those used in backcrosses to *I. rivularis* are indicated by a plus symbol (+), and those which resulted in seed when self pollinated by a dash (-).

Maternal Parent	Paternal Parent	Number of Seeds			Number Planted	Fruit Production		
		Collected	Scarified	Germinated		Out(*)	Self(-)	Back(+)
Rem5*+-	*I.rivularis*	169	33	32 (97%)	16	4	3	4
Rem6*+-	*I.rivularis*	51	25	24 (96%)	16	11	2	8
Rem8	*I.corei*	20	3	3 (100%)	3	0	0	0
Rem10*-	*I.corei*	64	45	38 (84%)	16	13	1	0
Rem11*-	*I.corei*	67	45	43 (96%)	13	15	1	0
Riv5*+-	*I.remota*	49	47	25 (56%)	15	8	1	2
Cor5*	*I.rivularis*	27	27	21 (78%)	16	6	0	0
Cor8*	*I.remota*	26	26	25 (96%)	8	9	0	0
Cor10*	*I.remota*	95	44	30 (68%)	16	32	0	0
Cor11*	*I.remota*	45	45	43 (96%)	8	10	0	0

Table 7.4. Morphological variation detected in the production of F_1 hybrids in *Iliamna*. The maximum, minimum, mean, and standard deviation for each species is presented.

Plant ID		Plant Height	Leaf Width	Leaf Length	Terminal Lobe Length	Leaf Upper Pubescence	Petiol e Length	Petal Length	Petal Margin Width	Petal Base Width	Staminal Tube Length	Number of Stigmas	Stigma Color	Pedicel Length	Sepal Length	Sepal Width	Bract Length
*I. corei*10	mean	89.9	10.6	10.4	5.5	3.5	9.6	2.4	2.2	0.3	1.1	12	1.2	1.3	1.8	0.8	1
Number of individuals: 14	max	133	13	12	7	5	19	3.1	2.7	0.5	1.4	14	2	2	2.2	1	1.3
Number of flowering individuals: 13	min	65	7.7	8.5	4.2	1	5.5	2	1.9	0.2	0.9	10	1	0.9	1.3	0.6	0.6
Number of individuals set fruit: 13																	
I.corei11	mean	90	10.4	9.8	5.4	3.5	9.5	3.9	2.4	0.3	1.3	12.1	1.3	1.1	1.9	0.8	1
Number of individuals: 8	max	115	12	11.5	6.5	5	14	12.7	3.1	0.4	1.5	16	2	2	2.3	1	1.2
Number of flowering individuals: 8	min	74	5.9	5.5	4	2	3	2	1.7	0.3	1	10	1	0.5	1.7	0.6	0.5
Number of individuals set fruit: 8																	
I.corei5	mean	60.2	12.3	11.8	7.8	3.1	8.7	2.8	2.6	0.3	1	11	1.5	0.8	1.2	0.5	0.6
Number of individuals: 15	max	86	16.4	16.1	11	4	13.5	3	3.1	0.4	1.2	14	2	1.6	1.5	0.6	1
Number of flowering individuals: 9	min	24	6.2	5.5	3.6	2	3.2	2.5	2.2	0.2	0.9	8	1	0.4	1	0.3	0.4
Number of individuals set fruit: 9																	
*I. corei*8	mean	76.1	6.7	6.8	3.7	2.2	5.6	2.5	2.6	0.3	1.2	11.5	1.3	0.8	1.5	0.7	0.7
Number of individuals: 9	max	88	12	13	6.5	3	12.5	3	3.7	0.4	1.5	14	2	1.2	1.9	0.8	1
Number of flowering individuals: 7	min	59	4.3	3.7	2.4	1	1.5	2	2	0.1	1	8	1	0.3	1.1	0.6	0.5
Number of individuals set fruit: 6																	
*I. remota*10	mean	80.3	15.3	15.5	8.5	2	10.8	2.7	2.1	0.3	1.2	10.7	1.4	0.6	1.4	0.6	0.7
Number of individuals: 17	max	111	75	75	41	3	15.1	3.6	3.6	0.4	1.6	14	2	1.1	1.8	0.8	1.1
Number of flowering individuals: 13	min	6	8.2	9.5	4.6	1	6.5	2	1.1	0.2	0.9	8	1	0.3	0.9	0.3	0.4
Number of individuals set fruit: 13																	
*I. remota*11	mean	90.2	10.5	9.8	5.8	2.3	10	2.7	2.4	0.3	1.1	12.8	1.5	0.6	1.6	0.7	0.8
Number of individuals: 17	max	100	13	11.5	9.2	4	13	3.2	3.1	0.5	1.2	16	3	0.9	2	0.9	1.2
Number of flowering individuals: 13	min	67	5.5	5.9	3.9	0	4.5	2.1	2.1	0.2	0.9	8	1	0.2	0.9	0.3	0.4
Number of individuals set fruit: 13																	
*I. remota*5	mean	73.2	14.1	13.9	8.3	1.6	9.6	2.7	2.7	0.3	1	11.4	2	0.8	1.2	0.5	0.6
Number of individuals: 16	max	105.7	17.7	16.5	10.5	3	15.3	3.3	3	0.3	1.1	14	3	1.2	1.4	0.7	0.8
Number of flowering individuals: 11	min	51	10.6	10.2	5.5	0	6	2.1	2.1	0.2	1	7	1	0.4	0.9	0.4	0.3
Number of individuals set fruit: 11																	
*I. remota*6	mean	72.1	13.5	14.8	8.4	1.1	10	2.9	2.4	0.3	1	13	1.3	0.9	1.1	0.5	0.6
Number of individuals: 16	max	111	17.5	17.3	11.2	2	14.5	3.1	2.6	0.5	1.3	16	2	2.2	1.1	0.5	0.9
Number of flowering individuals: 9	min	43	10	10.5	5	0	6.5	2.7	2.1	0.2	0.8	10	1	0.2	1	0.4	0.3
Number of individuals set fruit: 9																	
*I. remota*8	mean	75.9	6.4	6.3	3.4	2.7	4.7	2.7	2.8	0.2	1.4	10.5	2	1.1	1.8	0.6	0.6
Number of individuals: 7	max	100	10.5	9.5	5	3	8.5	3.1	3.6	0.3	1.6	12	3	1.7	2.2	0.8	0.8
Number of flowering individuals: 4	min	59	4.6	4.6	2.1	2	2.4	2.4	2.2	0.2	1.2	8	1	0.6	1.5	0.4	0.4
Number of individuals set fruit: 4																	
I. rivularis 5	mean	73.9	11.8	10.8	6	1.3	9.6	2.9	2.9	0.3	1.1	11.7	1.3	1	1.3	0.6	0.8
Number of individuals: 15	max	126	17	15.5	9	2	18	3.4	3.5	0.3	1.5	14	2	2	1.9	0.7	1.7
Number of flowering individuals: 7	min	6	5.9	5	3.5	0	1.6	2.5	2	0.2	0.9	8	1	0.2	1	0.4	0.5
Number of individuals set fruit: 7																	

Figure 7.1. Principle Components Analysis of *I. corei, I. remota,* and *I. rivularis*. Garden and herbarium specimens are shown below. Individuals of *I. rivularis* are indicated in yellow, *I. remota* in blue, and *I. corei* in red.

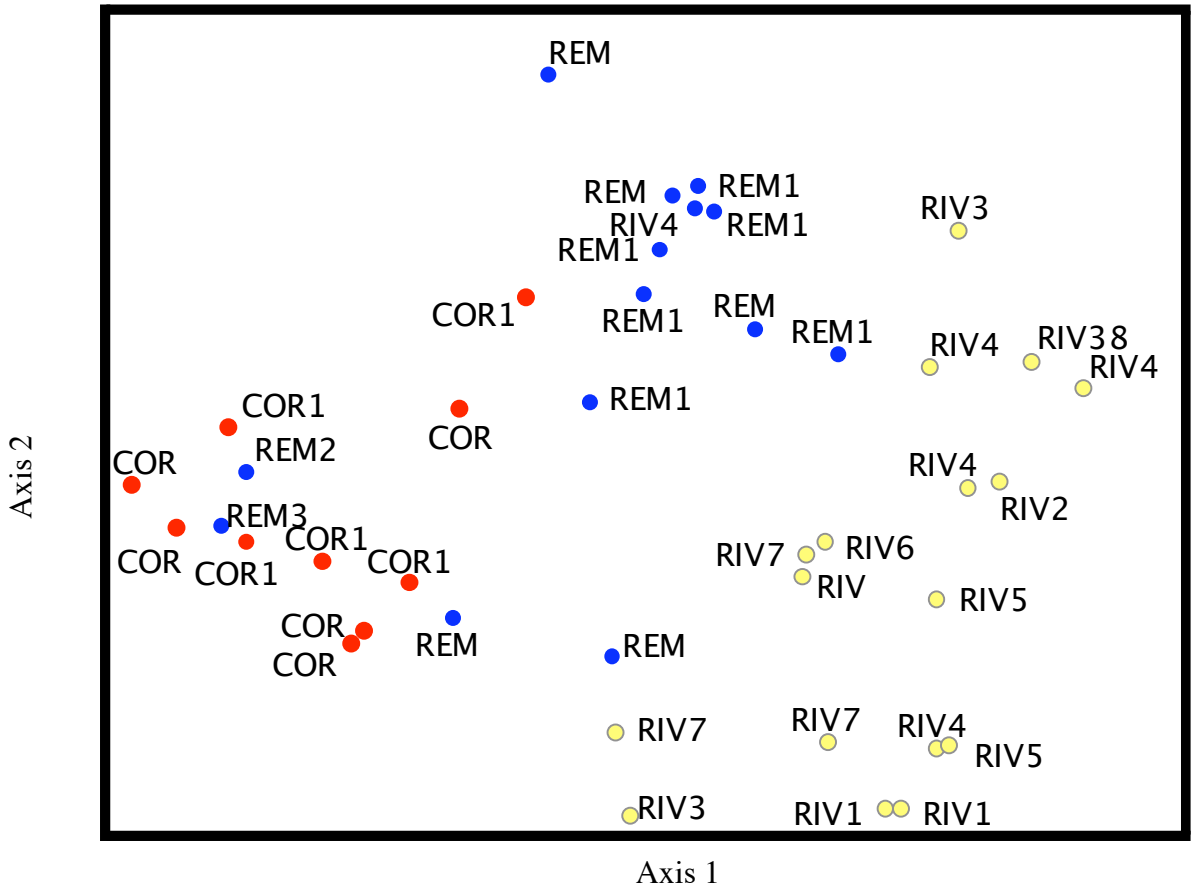
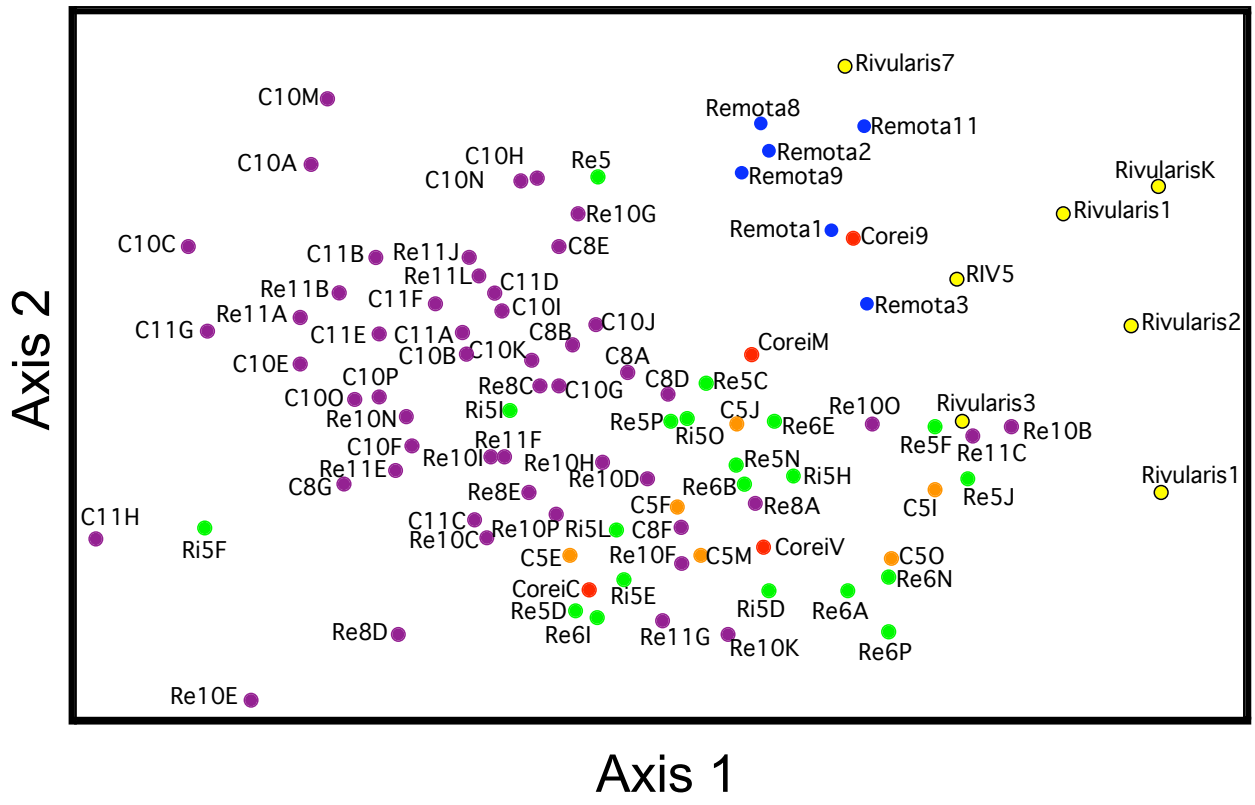


Figure 7.2. Principle Components Analysis of the F_1 generation and conspecifics using the first two axes, which represents 53.392% (Axis 1) and 21.132% (Axis 2) of the total variation. Representatives of pure species of *I. rivularis* are indicated in yellow, *I. remota* in blue, and *I. corei* in red. Representatives of F_1 hybrids of *I. rivularis* and *I. remota* are indicated in green. Representatives of F_1 hybrids of *I. remota* and *I. corei* are indicated in purple. Labels for points indicate the plant identification name.



Chapter 8

Conclusions

Relationships within the *Malacothamnus* alliance were examined through molecular, morphological and garden studies. Sequences from one nuclear (ITS) and two chloroplast (rpL16 and trnL-F) regions confirmed that *Iliamna*, *Malacothamnus*, and *Phymosia* are closely related and can loosely be grouped as the *Malacothamnus* alliance. The *Malacothamnus* alliance is nested within the tribe Malveae and is closely related to other North American members of the tribe surveyed.

In comparing the results from the ITS region to the plastid regions, origins of the genera in the *Malacothamnus* alliance can be inferred. *Iliamna*, which has a haploid chromosome number of n=33, has been thought to be a polyploid derivative of either *Malacothamnus* (n=17) or *Phymosia* (n=17) or both. The ITS results indicate the western *Iliamna* species (*I. bakeri* and *I. latibracteata*) to have either retained an ancestral copy of the region or have introgressed with Malvaceae taxa other than *Malacothamnus* or *Phymosia*. The chloroplast regions, however, produced a phylogenetic hypothesis that disagreed with those of the ITS region. First, the *Malacothamnus* alliance did not form a monophyletic group. Taxa in *Iliamna* were more closely related to *Eremalche* and *Sidalcea* than to either *Malacothamnus* or *Phymosia*. Secondly, the eastern *Iliamna* species (*I. corei* and *I. remota*) were more closely related to *Phymosia* species than to other species in *Iliamna*. This implies that two separate introduction of *Iliamna* to North America took place or that the eastern *Iliamna* species have retained an ancestral copy of the chloroplast genome shared with *Phymosia*. In examination of the GBSSI-1 gene family, alleles shared between *Iliamna* and *Malacothamnus* support the hypothesis of a common ancestor for the *Malacothamnus* alliance. In comparing the multiple gene hypotheses, several conclusions may be inferred. First, *Iliamna* resulted from a single introduction event to the Rocky Mountains, which represents the current center of diversity with the greatest species representation. From there, populations expanded to the north, east and

west. As populations became isolated, allopatric speciation led to the development of distinct taxa in the western and eastern periphery. Populations at these margins had limited gene pools, because of founder effects, and retained similar phenotypes (e.g., *I. bakeri* and *I. corei*) (see Chapter 4). Local adaptations within the Rocky Mountains have resulted in three distinct species, one of which has developed two geographical subspecies.

A single introduction event also accounts for the origin of *Malacothamnus* from Mexico into the Central Valley and Coast Range of California. The current center of diversity for *Malacothamnus* in San Luis Obispo and Santa Barbara Counties has led to the proliferation of isolated populations throughout the chaparral ecosystem. The genus is most likely recently formed, since gene flow between populations and species persists, as can be noted by the lack of genetically distinct taxa (see Chapters 2 and 3). However, morphologically distinct entities can be identified throughout California (see Chapter 5). Taxa have been able to maintain unique phenotypes throughout the isolated populations.

To address the feasibility of hybridization in *Iliamna*, a preliminary study was conducted to assess genetic variation between populations of *I. corei* and *I. remota* and the potential for hybridization (Chapters 6 and 7). The results showed, given the opportunity, offspring of crosses between *I. corei* and *I. remota* would be viable and could proliferate. In assessing genetic variation in populations of *I. corei* and *I. remota*, the species were found to be genetically unique. Hybridization of endangered plants, such as *I. corei*, could lead to the loss of the species. Gene flow between the species would result in a homogeneous gene pool and a loss of the genetic integrity of the species. Hybridization in *Malacothamnus* has resulted in such a loss and in the appearance of intermediate phenotypes in populations (Chapter 6). Future efforts need to critically examine the effects of hybridization on speciation within the *Malacothamnus* alliance.

Vitae

Tracey Ann Bodo Slotta

Education

2000- 2004: Virginia Tech, Blacksburg, VA. Doctoral program in Biology. Dissertation entitled: "Phylogenetics of the *Malacothamnus* alliance (Malvaceae): Assessing the role of hybridization and molecular and morphological variation in species delineation" Advisor: Dr. Duncan M. Porter

1997-2000: Virginia Tech, Blacksburg, VA. Master of Science in Biology. Thesis: Phylogenetic Analysis of *Iliamna* (Malvaceae) Greene Using the Internal Transcribed Spacer. Advisor: Dr. Duncan M. Porter

1995-1997: Bowling Green State University, Bowling Green, Ohio. Bachelor of Science in Biological Sciences, minor in Geological Sciences.

1993-1995: University of Dayton, Dayton, Ohio. Majored in Environmental Biology.

Professional Experience

2004: Research Assistant Fellowship. The Correspondence of Charles Darwin Project, Cambridge University Library and the American Council of Learned Societies.

Spring 2004: Coordinator for Plant Taxonomy Labs. Virginia Tech.

1997-2003: Graduate Teaching Assistant. Biology Department, Virginia Tech.

2003: Instructor. Biology Department, Virginia Tech. Taught Introductory Biology lecture for non-science majors.

Summer 2002: Instructor. Biology Department, Virginia Tech. Taught Introductory Biology lecture for non-science majors.

2000: Summer Internship. Sybron Biochemicals, Inc. Genetically labeled bacterial strains to improve viability testing.

Grants Received

Dec. 2003	Graduate Research Development Program Grant, Virginia Tech ($250)
October 2003	Virginia Native Plant Society Travel Award ($250)
August 2002	Deep Gene Research Group Travel Award, Madison, Wisconsin ($200)
June 2002	American Society of Plant Taxonomists Graduate Student Research Grant ($300)
April 2002	Virginia Academy of Science Small Research Project Grant ($1200)
March 2002	G. Ledyard Stebbins Award, California Native Plant Society ($1440)
October 2001	Travel Fund Grant, Graduate Student Assembly, Virginia Tech ($200)
August 2001	The Nature Conservancyís Peters Mountain Mallow Project ($250)
August 2000	Deep Green Plant Phylogeny Group Travel Award, Portland, Oregon ($420)
June 2000	Center for Innovative Technology with Sybron Biochemicals, Salem, VA. ($2000)
April 2000	Travel Fund Program Grant, Graduate Student Assembly, Virginia Tech ($100)
January 2000	Graduate Research Development Program Grant, Virginia Tech ($300)
May 1999	Virginia Academy of Science Small Research Project Grant ($1120)
January 1998	Graduate Research Development Program Grant, Virginia Tech ($500)