Phylogenetic Analysis of *Iliamna* (Malvaceae) Using the Internal Transcribed Spacer Region

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The genus *Iliamna* Greene has a taxonomically complex history. Since its desciption in 1906, the genus was not recognized for some time, several species were initially placed into other genera, and the species status of a few was questioned. Today, eight species of *Iliamna* are recognized. Six species are located in western North America and two are found isolated to the east. Species in *Iliamna* are very similar morphologically with only a few characters distinguishing several as separate entities. The need for systematic study became apparent since all but one species are considered rare or endangered. Also, the differentiation between two endangered species, *I. corei* and *I. remota*, was unclear in a previous study using random amplified polymorphic DNA fragments. Of the western species, four overlap in distribution (*I. crandallii, I grandiflora, I. longisepala,* and *I. rivularis*) and their recognition as separate species has been questioned. The focus of this study was to develop a phylogeny for *Iliamna* using sequences from the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA genes in order to determine its biogeographical and evolutionary history. Cladistic analysis was performed and the resulting phylogeny is presented. The ITS data provide new insights in the origination of the genus and its distribution. In *Iliamna*, the ITS region is 677 base pairs long with 120 sites providing information in the formation of phylogenetic trees. *Iliamna* forms a well-supported clade distinct from related genera and is monophyletic. Three well-supported groups are formed. One contains representatives from the Pacific Northwest. Another contains all of the remaining species with the third clade nested therein. This last clade contains the two eastern species, *I. corei* and *I. remota*, but there is not enough variability to support the divergence of these taxa as distinct species. There is also not sufficient variability in the ITS region to identify the western species *I. crandallii, I. grandiflora, I. longisepala* and *I. rivularis* as distinct entities.

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Chapter 1

Introduction

1.1 Project Summary

The genus *Iliamna* Greene consists of eight species, all native to North America. Two species are located east of the Mississippi River (*I. corei* (Sherff) Sherff and *I. remota* Greene) and the others to the west (*I. bakeri* (Jepson) Wiggins, *I. crandallii* (Rydberg) Wiggins, *I. grandiflora* (Rydberg) Wiggins, *I. latibracteata* Wiggins, *I. longisepala* (Torrey) Wiggins, and *I. rivularis* (Douglas ex Hooker) Greene). The plants in *Iliamna* are herbaceous perennials with maple-like leaves and white to lavendar-pink petals. Most frequently, they are found growing along streamsides in montane habitats. The seeds have a thick coat that requires scarification in order for germination to occur. The decline of several species (*I. bakeri*, *I. corei*, *I. grandiflora*, *I. latibracteata*, *I. longisepala*, and *I. remota*) has been attributed to destruction of habitat and/or fire supression. The rarity of two species, *I. corei* and *I. remota*, has prompted restoration efforts as well as a study of genetic variablity between the two. I was interested in discovering how these two species were related to each other and to the western taxa. Through the use of sequences from the internal transcribed spacer of the nuclear ribosomal DNA, I investigated relationships among the species in *Iliamna*. Species within the genus historically have been placed into other genera and *Iliamna* itself was not recognized for some time. Therefore, I intended to show with the molecular phylogeny that *Iliamna* is a valid genus, and that it contains several species.

1.2 Higher Classification of *Iliamna* (Malvaceae)

There are 110 genera within the Malvaceae, with approximately 65% of these genera being limited to North American (Fryxell, 1997). Most genera of the family are pantropical, with some representatives being temperate. Generally, research on the Malvaceae has focused on:

(1) how this family differs from and is related to other members of the Malvales; and (2) how members of the Malvaceae are related to one another (La Duke and Doebley, 1995). The position of the Malvaceae within the Malvales has been extensively studied within recent years.

The order Malvales lies within the subclass Dilleniidae (Cronquist, 1988). The Malvales include Elaeocarpaceae, Tiliaceae, Sterculiaceae, Bombacaceae and Malvaceae. The Elaeocarpaceae are often combined with Tiliaceae into one family (Cronquist, 1988), while others remove Elaeocarpaceae from the order Malvales and consider it allied with the order Theales (Chase et al., 1993). The Elaeocarpaceae are placed into the Malvales by Cronquist based upon their valvate sepals versus a presumed ancestral state of imbricate sepals. In excluding the Elaeocarpaceae, the remaining four families are very closely allied and are referred to as the core Malvales, but their delimitation as separate families remains questionable (La Duke and Doebley, 1995).

The separation of the Malvales from other members of the Dilleniidae is based mostly on the unique nectary glands (Cronquist, 1988; La Duke and Doebley, 1995). In Malvales, the nectaries consist of densely packed, multicellular, glandular trichomes, which usually grow so close together that they form a cushion-like structure. Within Malvaceae, Bombacaceae, and Sterculiaceae, the glandular trichomes are localized on the sepals, and the nectar is available through openings at the bases of the overlapping petals. Members of the Malvales also share stellate or lepidote pubescence, mucilage cells, cavities, or canals, and a stratified phloem with wedge-shaped rays. Lastly, within the four core Malvales families, the seeds have fatty acids containing a cyclopropenyl group, which appears to be restricted to this order.

The traditional classification of the core Malvales has not been supported in recent studies. Judd and Manchester (1997) concluded the current classification to be artificial, not reflecting the true evolutionary history of the group, based on morphological, anatomical, palynological, and chemical characters. Although the Elaeocarpaceae was included in Cronquist's (1988) grouping of the Malvales, Judd and Manchester (1997) excluded this family. The Tiliaceae, Sterculiaceae, and Bombacaceae appeared to be paraphyletic (Judd and Manchester, 1997). Tiliaceae appears to be ancestral to the others, since it retains numerous primitive features of the core Malvales. Malvaceae, on the other hand, is monophyletic and presents the most derived characters of the order. Bombacaceae was shown to be closely allied to the Malvaceae, both families having monoloculate anthers for example. Thus, the evolutionary history of the core Malvales was summized to be, from most primitive to most derived, Tiliaceae, Sterculiaceae, Bombacaceae and Malvaceae.

Furthermore, Judd and Manchester (1997) proposed that the core Malvales be considered at the familial level, the Malvaceae *s.l.* This broad grouping for the Malvaceae *s.l.* can be diagnosed by several synapomorphies. First, the aforementioned unique nectaries, which are unusual among angiosperms, are found exclusively in the core Malvales. Other synapomorphic characters include stamens lacking a conspicuous connective tissue, a fibrous bark, a valvate calyx aestivation, tile cells in the xylem, palmately veined leaves, the last two being quite homoplasious, and cyclopropenyl fatty acids. Judd and Manchester's (1997) Malvaceae *s.l.* supports a previous notion of Browin in 1818 that the famlies in Malvales were synonomous to tribes in the Rosaceae.

Analysis of chloroplast rbcL gene sequences also provides strong support for the monophyly of a broadened Malvales order grouping (Alverson et al., 1998). Within the core Malvales, of those families traditionally placed into the order, only the Malvaceae appear monophyletic. Based on the rbcL data, the expanded Malvales would include the following families, from ancestral to most derived, respectively: Thymelaeaceae, Elaeocarpaceae, Cistaceae, Dipterocarpaceae, Sarcolaenaceae, Sphaerosepalaceae, Bixaceae, Sterculiaceae, Tiliaceae, Bombacaceae, and Malvaceae. These results also support other studies using the chloroplast genes rbcL and ndhF that the order Malvales should be placed in the subclass Rosidae (Chase et al., 1993), not the Dilleniidae as in the Cronquist system. Of the dicotyledon subclasses, the Rosidae and Dilleniidae are the most difficult to distinguish between morphologically, and several groups of taxa may be placed in either subclass (Cronquist, 1993).

The monophyly of the core Malvales was confirmed by another analysis, this time using sequences from the chloroplast gene ndhF (Alverson et al., 1999). This study also confirmed that the Tiliaceae, Sterculiaceae, and Bombacaceae were not natural forming groups reflecting an evolutionary history; i.e., not monophyletic. Also, the Malvaceae and Bombacaceae were found to be closely allied, sharing a 21 base pair gap, which differentiates them from the other Malvales. The ndhF results and the results of morphological analyses, reveal a lack of resolution within the core Malvales, warranting further study. In the core Malvales, the generalization that as staminal fusion and loss of woodiness increases a more derived state is obtained does not hold true with the ndhF data set. Furthermore, as Alverson et al. (1998) pointed out, the androecium of members of the core Malvales shows a level of plasticity that is unusual among eudicots and should be investigated further.

Another analysis of the Malvales using plastid sequences, this time from both the rbcL and atpB genes, again confirmed the monophyly of the core Malvales (Bayer et al., 1999). Bayer et al., enlarged the Malvaceae *s.l.* to include the Tiliaceae, Sterculiaceae, Bombacaceae and Malvaceae, following Judd and Manchester (1997). Subsequently, Bayer et al. (1999) divided the family into nine subfamilies. The Malvaceae *s.s.*, being found monophyletic, by weak support, was retained in the subfamily Malvoideae. The other traditional families, Bombacaceae, Tiliaceae, and Sterculiaceae, had representatives scattered throughout the phylogeny and eight new subfamilies of Malvaceae *s.l.* were created to contain these taxa. The Malvoideae in conjunction with the Bombacoideae formed a well-supported monophyletic clade, separating these two "families" from the others.

However, the subfamily Bombacoideae, as defined by Bayer et al., did not include all members of the traditional family Bombacaceae (Bayer et al., 1999). The two subfamilies Bombacoideae and Malvoideae would be combined if *Hibiscus* was excluded from the analysis. As Hutchinson said (1967: 538) "Malvaceae without the great genus *Hibiscus* would be like a horse without a tail" (Bayer et al., 1999: 289). Many characters that are used to differentiate the "Malvaceae" fall within the range of those in the Bombacoideae. However, the majority of the Malvoideae genera can be put into the present classification through the traditional combination of characters. The authors did say that an increased taxa sampling was needed to further support their findings, and that their results were not to be treated as the final solution to the Malvales dilemma.

In the present study, the Malvaceae was treated in the traditional sense, Malvaceae *s.s.* Members of this family are either annual, perennial, or biennial herbs or small trees to shrubs (Zomlefer, 1994). As mentioned above, representatives contain a mucilaginous sap and have lepidote or stellate pubescence. The leaves are usually alternate in arrangement, palmately veined, with margins ranging from entire or serrate to variously lobed. Usually, the leaves have stipules at the base of the petiole, which vary in length and shape among the genera. The flowers are actinomorphic, perfect and hypogynous (Zomlefer, 1994). Flowers are either found in determinate cymose inflorescences or as solitary axillary flowers. The corolla is composed of five petals that are distinct, obovate, asymmetrical, and attached to the base of the staminal column. The epicalyx subtending the flower consists of connate or separate bracts. Typical of the family, the stamens are fused into a tube that surrounds the style in the monodelphous stamen arrangement. When the flower opens, the immature stigmas remain inside the staminal tube, while the anthers spead out and dehisce. Later, once the filaments for the opened anthers recurve, the stigmas emerge. The anthers have one locule, dehisce by logitudinal slits, and contain large, spherical, spiney pollen grains. The compound pistil is 2- to many-carpellate, and the stigmatic area is often divided. Locules in the ovary often form a ring, with each compartment containing one to many ovules. Fruits are either schizocarps or loculicidal capsules, and contain seeds that are frequently pubescent.

To distinguish among genera in Malvaceae, the variations in the epicalyx structure are among the most useful characters (Zomlefer, 1994). The whorl of the epicalyx may be an aggregate of bracts or stipules. The segments or lobes comprising the epicalyx can be few to many in number, are often subulate or lanceolate in shape, and vary from broad and foliar to being inconspicuous. Furthermore, the lobes may be any degree from separate to fused into a cup. Lastly, in some genera, the epicalyx is abscent. The method of seed dispersal and fruit dehiscence also varies among the malvaceous genera (Cronquist, 1988; Zomlefer, 1994). Some have loosely coherent carpels, while others have loculicidal capsules. Once the carpels open, seeds are dispersed by apical awns on the carpels or by trichomes on the seeds. In cotton, a member of the Malvaceae, long trichomes on the seeds aid in dispersal.

Within the last forty years, chromosome number has also been used to distinguish genera and sub-families or alliances in the Malvaceae. Chromosome counts have been conducted within the family, resulting in a base chromosome range from five to approximately 135 (Alverson et al., 1998). *Iliamna* has a chromosome number of n=33. Genera at one time believed to closely allied to *Iliamna* include *Sphaeralcea, Malacothamnus,* and *Phymosia* and chromosome numbers for representatives of these are known. *Sphaeralcea* has a base chromosome number x=5. *Phymosia* and *Malacothamnus* have chromosome numbers of n=17, a characteristic of the Malacothamnus alliance discussed below. If either of these genera are ancestral to *Iliamna*, then *Iliamna* would be an allopolyploid that lost one chromosome. A further discussion of chromosome number and morphology of *Iliamna* follows in the next section.

In addition to chromosome number and morphology, molecular techniques are being employed to study the genera within Malvaceae *s.s.*. Recently, LaDuke and Doebley (1995) used restriction enzymes to digest the chloroplast genome in order to investigate relations among genera and alliances in the family. Digestion with twelve restriction enzymes resulted in 236 variable sites, which were analyzed by parsimony analysis. LaDuke and Doebley describe five tribes, which form natural groups, and they include representatives from the tribes Decaschistieae, Gossypieae, Hibisceae, Malvavisceae and Malvaeae, with the last being the largest. *Iliamna* was found to be a member of the Malacothamnus alliance within the tribe Malvaeae. This alliance also contained *Malacothamnus*, and most likely *Phymosia* would be placed here, but it was not included in their study. Six most parsimonious trees were produced. The trees differ only in the positions of *Malacothamnus*, *Iliamna*, and *Urocarpidium. Urocarpidium* is a member of the Sphaeralcea alliance in the tribe Malvaceae. In most of the trees, the Malacothamnus, Sphaeralcea, and Malva alliances form a clade and are not monophyletic. In particular, *Iliamna* and *Malacothamnus* reside in a five-branched polytomy dividing representatives of the Sphaeralcea and Malva alliances. In another most parsimonious tree, the Malacothamnus alliance appears as sister to the Sphaeralcea alliance. The lack of resolution and support in this phylogeny may have improved had *Phymosia* as well as other pertinent taxa been included.

1.3 Taxonomic History of *Iliamna*

In 1830, the type species of the genus, *Malva rivularis* Douglas ex Hooker, was described from the Rocky Mountains. Eight years later, *Sphaeralcea acerifolia* Nuttall ex Torrey and Gray, which was very similar to *Malva rivularis* Douglas ex Hooker, was described. Then in 1849, John Torrey transferred *Malva rivularis* to *Sphaeralcea* as *S. rivularis* (Douglas ex Hooker) Torrey, and 25 years later (1874) he described *Sphaeralcea longisepala* Torrey from Washington state. Not until 1904 were any more species added, at which time P. A. Rydberg described two new species from Colorado, *S. crandalii* Rydberg and *S. grandiflora* Rydberg.

In 1906, E.L. Greene established the genus *Iliamna* based on morphological characters, that set *Malva rivularis* Douglas ex Hooker and *Sphaeralcea acerifolia* Nuttall ex Torrey and Gray apart, along with two new species, *I. angulata* Greene and *I. remota* Greene. Greene did not include *S. longisepala* Torrey nor Rydberg's two species from Colorado in his new genus. Why Greene chose the name *Iliamna* is unknown (Weber, 1987). Since there is a lake in Alaska of the same name, he may have thought it sounded pretty and gave it to these pretty plants. Or, he may have combined two classical languages with *ilyos*, Greek for mud, and amnis, Latin for river, but this explanation is highly unlikely.

Most other botanists, however, did not recognize *Iliamna* as a valid genus, but considered the four species to belong in *Sphaeralcea* (Wiggins, 1936). Greene did not include a thorough description or a key to the genus nor did he include an extensive explanation for the separation of these four species from *Sphaeralcea.* This, in conjuction with his reputation as a "splitter," one who believes in defining species based on a few characters, left doubt as to the validity of the genus in the minds of other botanists.

In 1913, Rydberg removed *S. acerifolia*, *S. crandallii*, *S. grandiflora*, *S. longisepala*, and *S. rivularis* from *Sphaeralcea*, believing them to be distinct, and placed them into the West Indian genus *Phymosia* Hamilton. He included *P. acerifolia* (Nuttall ex Torrey and Gray) Rydberg, *P. rivularis* (Douglas ex Hooker) Rydberg, *P. grandiflora* (Rydberg) Rydberg, *P. crandalii* Rydberg, and *P. longisepala* (Torrey) Rydberg. Later that year, Greene's *I. remota* was transferred to *Phymosia* as well by Rydberg, as *P. remota* (Greene) Rydberg. In 1925, W. L. Jepson described the next addition to this group, *Sphaeralcea bakeri* Jepson from northern California. Rydberg (1932) did not include *S. bakeri* Jepson in *Phymosia.* However, he did reduce *S. acerifolia* Nuttall ex Torrey and Gray to synonomy under *I. rivularis* (Douglas ex Hooker) Greene.

I. L. Wiggins revised *Iliamna* Greene in 1936, to include seven species, *I. rivularis* (Douglas ex Hooker) Wiggins, *I. grandiflora* (Rydberg) Wiggins, *I. remota* (Greene) Wiggins, *I. longisepala* (Torrey) Wiggins, *I. bakeri* (Jepson) Wiggins, *I. crandallii* (Rydberg) Wiggins and a newly described species *I. latibracteata* Wiggins. Furthermore, Wiggins reduced *I. angulata* Greene to synonomy under *I. grandiflora.*

Wiggins based his separation of the genus 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 forty species of *Sphaeralcea* contained in twelve sections. *Iliamna*, in contrast, ranges from southern British Columbia to nothern New Mexico, growing in moist habitats along streams, in meadows, and on mountain hillsides. Species in *Iliamna* often grow at elevations above 7000 feet in mountain habitats (see Figure 1.1). 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. Stipules in *Iliamna* are inconspicuous, as they are in *Sphaeralcea*, but they readily fall off.

Flowers in the two genera differ in color, petal size, and petal margin. There are numerous differences in the morphology of carpels between the two genera. *Sphaeralcea* has small carpels (less than 5 mm long) with two well-defined portions, one portion being a basal, seminiferous, non-dehiscent part and the other an empty, dehiscent, terminal portion. In *Iliamna*, the carpels are larger in diameter (6-10 mm), have obtusely rounded apices with two pubescence types, coarse stellate trichomes along the back margin and long, stiff, simple trichomes covering the rest of the carpel back (Wiggins, 1936). The carpels are not divided into portions in *Iliamna.* Fruits are schizocarps and contain two to four seeds per mericarp

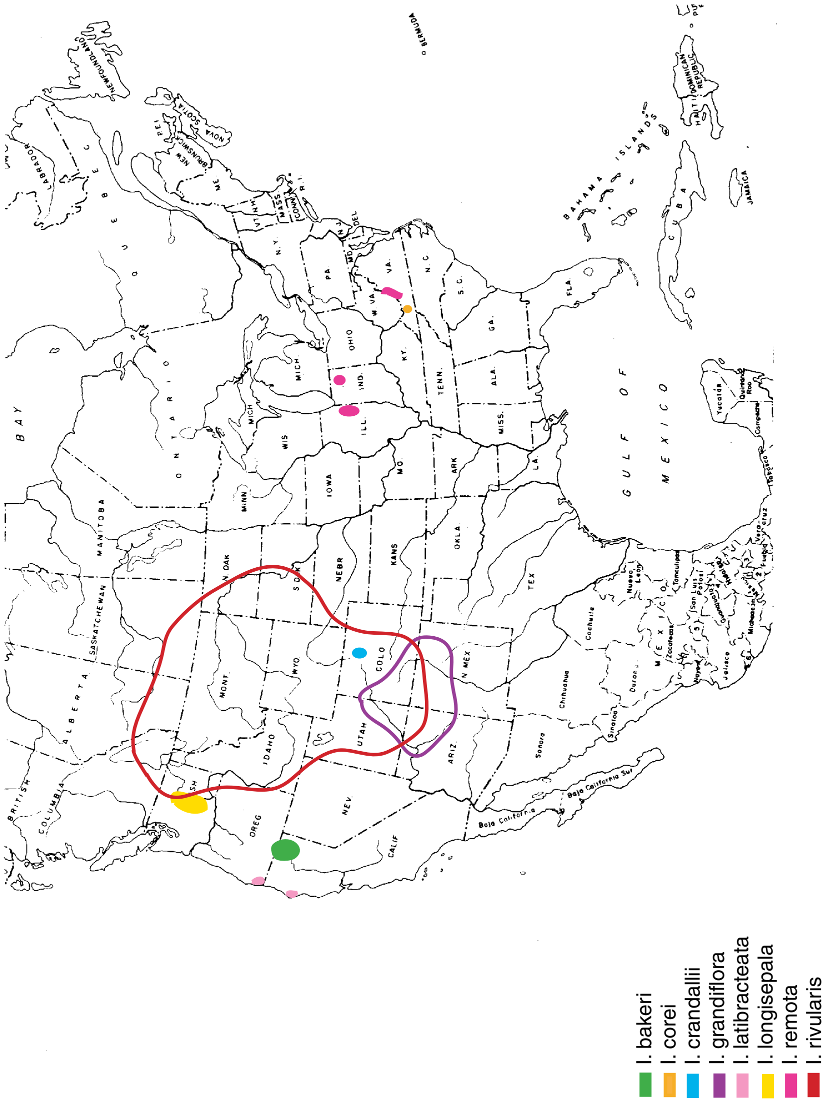


Figure 1.1: Distribution Map for species in *Iliamna*

and they practically fill each mericarp. The seeds are reniform and vary from puberulent to glabrous. In *Sphaeralcea*, the seeds are found one to three per carpel and have very short trichomes (Fryxell, 1997). For dispersal, the thin-walled mericarps in *Iliamna* dehisce longitudinally along the dorsal edge to the ventral margin where a fibrous strand connects the carpel and receptacle (Wiggins, 1936). Lastly, Wiggins points out a difference in chromosome number between *Sphaeralcea* and *Iliamna*. *Sphaeralcea* has chromosomes in multiples of five, while *Iliamna rivularis* and *I. remota* have been found to have 33 pairs of chromosomes (Wiggins, 1936).

The differences between *Phymosia* and *Iliamna* are not as great, but they are sufficient for the separation of these two genera (Wiggins, 1936). *Phymosia* contains eight species distributed in central Mexico, Guatamala, and the Bahamas (Fryxell, 1997). In form, *Phymosia* is shrubby to arborescent, whereas *Iliamna* is a hardy herbaceous perennial dying back to the crown each year. Also, the carpels in *Phymosia* (10-12 mm long) are larger than in *Iliamna*, and the pubescence consists uniformly of stellate trichomes that are often on short stalks, stipitate. Leaves in *Phymosia* are 3-7-lobed, with the central lobe being the largest. They are also thicker and more densely covered in stellate trichomes than those of *Iliamna*. Stipules in *Phymosia* are subulate and retained. The flowers may be solitary or form umbels. The fruits are globose to oblate schizocarps that are tomentose and contain 2-3 seeds per mericarp. The seeds themselves are glabrous and reniform in shape. As with *Sphaeralcea*, chromosome number is different from *Iliamna* with *Phymosia* having 17 pairs ($n=17$) (Fryxell and Stelly, 1993). With *Iliamna* having 33 chromosome pairs, it has been hypothesized that *Iliamna* may be derived from an allotetraploid *Phymosia* that lost one chromosome pair (Wiggins, 1936; Fryxell, 1997).

Lastly, *Malacothamnus* Greene is thought to be closely linked to *Phymosia* and *Iliamna*, as seen by La Duke and Doebley's (1995) study discussed earlier. *Malacothamnus* contains eleven species of spreading or erect shrubs found in California and northern Baja California (Fryxell, 1997). Phyllotaxy varies greatly, with leaf blades being orbicular to ovate, unlobed to 3-5-lobed, and the leaf margin varying between subentire and serrate. As with other members of the Malvaceae, a stellate pubescence is present. The stipules in *Malacothamnus* are subulate to lanceolate or falcate and inconspicuous. Fruits are subglobose or obovate schizocarps with solitary seeds. The seeds are minutely stellate-pubescent or rarely glabrous. As with *Phymosia*, *Malacothamnus* has 17 pairs of chromosomes (Bates and Blanchard, 1970; Fryxell and Stelly, 1993). Morphological and cytological data indicate a strong interrelation between *Iliamna*, *Phymosia*, and *Malacothamnus* (Bates and Blanchard, 1970).

1.4 Species Descriptions and Controversy

Currently, the genus *Iliamna* consists of eight species, *I. bakeri, I. corei, I. crandallii, I. grandiflora, I. latibracteata, I. longisepala, I. remota* and *I. rivularis*. *Iliamna remota*, first described in 1906, was originally found on an island in the Kankakee River in Illinois. During the 1920s, *I. remota* was recognized as being in danger of extinction, and seeds were collected and supposedly dispersed along railways in Illinois, Indiana, and Virginia, and the populations continue to increase (Stewart and Porter, 1995; Stewart et al., 1996). The presence of *I. remota* in Virginia was not noted before that time. *Iliamna corei* was first found in 1932 at the type locality on Peter's Mountain near Narrows in southwest Virginia by Earl Core. At that time though, the Peter's Mountain plants were thought to be another population of *I. remota*. It was not until 1946, that Sherff named the Virginia plants as a different variety, *I. remota var. corei*. Later, Sherff (1949) concluded that these two varieties were indeed separate species.

Iliamna remota differs from *I. corei* in height (to 2 m for *I. remota*), petal color (lighter in *I. remota*), leaf width (wider in *I. remota*), and flower fragrance (non-fragrant in *I. corei*) (Stewart and Porter, 1995). *Iliamna corei* is found in a single population and was state and federally listed as endangered in 1986. The genetic distinction between *I. remota* and *I. corei* was established through RAPD profiling (Stewart and Porter, 1995; Stewart et al., 1996). However, this analysis showed that *I. corei* and *I. remota* are very similar and it was unclear if *I. corei* should be recognized as a separate species from, or a subspecies of, *I. remota*.

The Western species *Iliamna rivularis*, *I. grandiflora*, and *I. crandallii* are morphologically very similar and have overlapping geographical ranges. With the western species, *I. rivularis* has the widest distribution, occurring from southern British Columbia to northern New Mexico and as far west as Washington and east to western South Dakota (Figure 1.1). This distribution overlaps population distributions of *I. crandallii*, *I. grandiflora*, and *I. longisepala*. *Iliamna rivularis* is described as having leaves with 5-7-lobes, ranging in height from 6-20 dm, pedicel and calyx with stellate trichomes and petals being pink to white. This description also holds for *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 and *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).

The remaining species, *I. latibracteata*, *I. longisepala*, and *I. bakeri*, have not been questioned as to their status as 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 10-14 mm in length. *Iliamna bakeri* is found east of the *I. latibracteata* populations and has rose-purple flowers in axillary clusters, with bractlets being 5-8 mm long, and with finely puberulent seeds. *Iliamna longisepala* is located in central Washington overlapping with the western distribution of *I. rivularis*. It has flowers in solitary or few-flowered axillary clusters, and has petals that are densely tomentulose inside, a trait that has not been noted in other species.

In summary, the eight described species of *Iliamna* are very similar morphologically, sometimes with only a few characters differentiating one species from another. Despite the use of molecular methods (RAPDs), a distinction between subspecies and a separation of species is not clear with *I. remota* and *I. corei*. Further work is required in order to resolve questions of species status and of the relationships within *Iliamna*.

1.5 The Internal Transcribed Spacer

The internal transcribed spacer (ITS) has been used in numerous systematic studies at the generic and specific levels of a wide array of plant taxa (Baldwin et al., 1995). The two internal spacers, ITS-1 and ITS-2, are located between genes encoding the 5.8s, 18s and 26s nuclear ribosomal RNA (nrRNA) subunits (Baldwin, 1992) (see Figure 1.2). The ITS-1 and ITS-2 spacers, in addition to the 5.8s nrRNA are referred to as the ITS region (Baldwin et al., 1992). Individually, ITS-1 and ITS-2 are around 300 bp in length and the 5.8s subunit is almost invariant in length within angiosperms (163-164 bp), making the entire ITS region less than 700 bp.

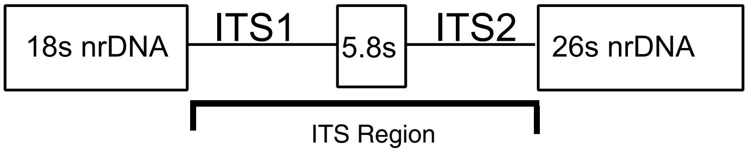


Figure 1.2: The Internal Transcribed Spacer

Even though ITS-1 and ITS-2 are part of the ribosomal transcriptional unit, these sequences are not incorporated into mature ribosomes (Baldwin, 1992). The two ITS sequences, however, do appear to function in the maturation of nuclear ribosomal RNAs as specific 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. Given the short length and the highly conserved nature of the flanking ribosomal subunit genes, the ITS region is easily amplified from small amounts of genomic DNA by the polymerase chain reaction.

1.6 Functional Constraints and Evolution of the rDNA Multi-Gene Family

As described above, the internal transcribed spacer is located within the ribosomal DNA repeat that codes for the ribosomal subunits. In nuclear DNA this region occurs in tandemly repeated arrays at the nucleolar organizer region of specific chromosomes (Vander Stappen et al., 1998). Each complete unit is transcribed as a precursor, which is processed into the mature 18s, 5.8s, and 26s RNA subunits. The ITS regions aid in the maturation process.

Among angiosperms, sequences near the 5' and 3' ends of ITS-1 are highly variable and cannot be unambiguously aligned, but do form similar stem-loop structures (Liu and Scharal, 1994). The stem-loop regions contain a high proportion of guanine (G), cytosine (C), and thiamine (T), indicating that the derived secondary structures would be highly stable. However, a region near the center of ITS-1 is highly conserved and alignable across numerous plant families, even between monocots and dicots (Liu and Scharal, 1994). This small motif of less than 25 base pairs forms a hairpin. The combination of these three regions, the central hairpin and the stem-loop structures near the 5' and 3' ends, suggest that the secondary structure, rather than the primary sequence structure, is under functional constraints within ITS-1. Little has been done to discover similar stem-loop structures within the ITS-2 region, since it is less variable than the first spacer.

Given the similarity in position of the stem-loop regions of ITS among angiosperms, it has been proposed that the ITS regions function as a scaffold for processing the coding regions (Liu and Scharal, 1994). Furthermore, the overall secondary structure of the ITS-1 region may be to serve to present the small central conserved sequence motif, which could be recognized by processing enzymes in ribosomal function.

It is believed that the ribosomal genes exist as a multiple gene family because of the need for large amounts of the products (Ohta, 1991). In addition, the important functions of ribosomes in processing DNA in protein production requires uniformity among copies of the rDNA. The rDNAs often show high levels of homogeneity within species because of gene flow while maintaining varying levels of diversity between species (Polanco et al., 1998). The most common variations among copies of the spacer regions in the rDNA within a species (i.e., polymorphisms) are single base pair substitutions (Baldwin et al., 1995). Other variations include insertion-deletion events (indels), which occur in a small proportion of the spacer regions. Homogeneity among the repeated copies within individuals is the result of concerted evolution through gene conversion and unequal crossing over events (Li, 1997; Hurst and Smith, 1998). Through these mechanisms the copies are converted to being identical (homogenized) and then are distributed throughout a species through gene flow (fixation).

Gene conversion occurs when two sequences interact in such a way that one sequence transforms the other without being altered itself (Li, 1997). This may happen in one of five possible ways, between two alleles at the same locus, through intrachromatid swapping, through sister-chromatid interactions, through non-allelic loci on homologous or non-homologous chromosomes, or through interactions between the gene copies. It is assumed that gene conversion occurs immediately after chromosome replication for these events to take place. The amount of DNA transferred during a single gene-conversion event can vary greatly but does not cause a change in the number of genes. Exchange within and between chromosomes does not require very high sequence similarity either and a preference may be seen in which sequence is retained and copied. It is the repeated cycles of gene conversion that lead to the homogenization of duplicated genes (see Figure 11.8 in Li, 1997).

In unequal crossing-over, a sequence duplication occurs in chromosome, or chromatid, and a deletion is created in the homologous chromosome (Li, 1997). As a result, unequal crossover can produce fluctuations in the number of repeats in a gene family (see Figure 11.3 in Li, 1997). This type of event may occur between two sister chromatids or between two homologous chromosomes at meiosis, the latter occurring more frequently. As a result of unequal crossing-over, each of the daughter chromosomes is more similar to the other than to the parental chromosome. Eventually, one type of repeat will become dominant in the gene family and homogenization results. This type of concerted evolution creates shifts in the position of repeats on the chomosome.

In large gene families with tandem repeats, as is the case for nrDNA, unequal crossing-over may be more important than gene conversion in the concerted evolution process (Li, 1997). For example, the number of repeats can fluctuate without having any adverse effects. With a larger number of repeats being exchanged, the rate of concerted evolution increases as well. Correspondingly, homogeneity increases as the number of repeats increases. Rate then increases as homogeneity among the copies increases, leading to a self-feeding repetition. As a result of this process, it is believed that nrDNA is found in up to thousands of copies in the nuclear genome (Baldwin et al., 1995).

1.7 Phylogenetic Utility of the Internal Transcribed Spacer

Several factors make the ITS region valuable for use in phylogenetic analyses (Baldwin et al., 1995). First, the ITS region is highly repeated in plant nuclear genomes, along with other components of the nrDNA multigene family including a highly variable region between the ribosomal repeat, the intergenic spacer. The high copy number of the nrDNA repeat facilitates the amplification and sequencing of the nrDNA.

Secondly, the nrDNA multigene family undergoes rapid concerted evolution as described above (Baldwin et al., 1995). This property of the ITS region is most important from a phylogenetic standpoint and promotes accurate reconstruction of species relationships from sequencing. However, non-homologous copies are occasionally present with point mutations and/or insertion/deletion events, causing small variation among the copies within a species.

Lastly, the ITS region is relatively small (ca. 700 bp) and is flanked by highly conserved sequences, the 18s and 26s nrDNA genes (Baldwin et al., 1995). Because of this, universal primers can be used to amplify and sequence the ITS region. Primers were originally designed for amplification of fungal rRNA and derived from sequences of fungi (*Saccha-*

romyces), animals (*Drosophila*), and plants (*Oryza sativa* and *Hordeum vulgaris*) (White et al., 1990). These primers have been used successfully with members of the Liliaceae, Asteraceae, Rosaceae, and Araliaceae.

The ITS-1 region is longer than ITS-2 in most angiosperms, and as a result the percentage of potentially informative and alignable nucleotide sites is greater in ITS-1 (Baldwin et al., 1995). Phylogenetically, ITS-1 is more informative than ITS-2 on average, in having 29% more variable nucleotides than ITS-2 and providing more synapomorphies (Baldwin, 1992). As expected, resolution of phylogenies based on ITS-1 or ITS-2 alone is comparable to the extent of variation in each spacer, with ITS-1 data usually providing more complete phylogenetic resolution. Phylogenetic trees that are based on either ITS-1 or ITS-2 alone may indicate relationships that were not represented by the other spacer. By combining data sets from ITS-1 and ITS-2, more robust and complete trees result. Therefore, the ITS-1 and ITS-2 regions were used in conjunction in this study in order to provide maximal phylogenetic resolution and support.

In recent literature, the ITS region is frequently used for resolving phylogenies from a variety of genera. Sang et al. (1995) used sequences from the ITS region to construct a phylogeny for the genus *Robinsonia* (Asteraceae). *Robinsonia* is the second largest endemic genus of flowering plants on the Juan Fernandes Islands and has seven recognized species. With considerable morphological divergence among the species, the ITS region was chosen to establish a molecular phylogeny. Six of the seven species were analyzed in addition to two outgroup species. A completely resolved phylogeny was produced once data from ITS-1 and ITS-2 were combined, which showed *Robinsonia* to be monophyletic with two subgenera. Furthermore, the tree produced with ITS was fully concordant with those produced using morphology.

The ITS region was also been used in reconstructing a phylogeny for *Panax* (Araliaceae) (Wen and Zimmer, 1996). The genus is economically important because of the medicinal uses of its members. Phylogenetic analysis of the ITS sequences obtained for the twelve species of *Panax* suggested that one of the two eastern North American species was most closely related to the eastern Asian species and showed the genus to be monophyletic. Also, the three medicinally important species did not form a monophyletic clade as was previously thought. Lastly, low ITS sequence divergence in conjunction with 8 of 12 *Panax* species occurring in the Himalayas and central and western China, the authors suggested that a rapid evolutionary radiation occurred in China, and that these areas are the current centers for diversity of *Panax*.

Another example of the utility of ITS in phylogenetic analysis of plants is with *Rubus* (Rosaceae) (Alice and Campbell, 1999). In this case, the entire ITS region was used (ITS-1, 5.8S rDNA, and ITS-2) to construct a phylogeny comprised of fifty-seven taxa, including representatives of the subgenus *Rubus*, the eleven remaining subgenera, and a closely related species. Length and G+C content of the ITS in *Rubus* were consistent with those reported previously in other angiosperms. The ITS phylogeny was generally consistent with biogeography and ploidy level of *Rubus*. However, the ITS phylogeny was not consistent with traditional morphological characters used in *Rubus* taxonomy.

The ITS region does not always produce a phylogeny with complete resolution. Often polytomies result where there is not enough variation between taxa to generate dichotymously branching clades. An example occurs with *Lupinus* (Ainouche and Bayer, 1999). Forty-four taxa within this genus were examined along with five outgroup taxa. The resulting phylogeny indicates the genus to be monophyletic and to contain five main clades with a division between the eastern and western New World lupines. However, there is a lack of resolution within three of the five main clades, with the formation of several polytomies in the New World taxa. Furthermore, the ITS data do not resolve relationships at the base of the genus – the five main clades form a polytomy – indicating an initial rapid radiation of lupines following dispersal of the common ancestor.

1.8 Project Overview and Objectives

All but one species of *Iliamna* (*I. rivularis*) is considered rare or endangered and little has been done to determine how these species are related. Work on the Eastern species *I. corei* and *I. remota* was unable to conclude whether these two were separate species or subspecies of one another (Stewart and Porter, 1995; Stewart et al., 1996). Since there is a lack of knowledge about the relationships within *Iliamna* and most species are rare, this study used molecular systematics in order to determine a phylogeny for the genus.

The investigation had four specific objectives:

1. To develop a phylogeny of the genus *Iliamna*.
2. To determine if *I. corei* is a separate species from, or a subspecies of, *I. remota*.
3. To decide conclusively whether or not *I. rivularis* is the ancestor of *I. remota* or any other *Iliamna* species.
4. To use the resulting phylogenetic tree to develop a biogeographical history of *Iliamna*.

The resulting phylogeny of *Iliamna* may provide information about whether the two disjunct populations of *I. remota*, Illinois-Indiana and Virginia, are genetically distinct and represent two subspecies. If this result is obtained, the case for *I. remota* in Illinois to be listed as federally endangered, as well as state endangered, would be further supported. On the other hand, data may show the opposite to be true, indicating *I. remota* is successfully founding new populations outside its historic distribution and is not in danger of extinction. Also, has *I. remota* been in Virginia prior to the suspected dispersal by the Chicago Wildflower Society in the 1920s (Stewart and Porter, 1995)?

This study may also determine whether *I. rivularis*, *I. crandallii*, and *I. grandiflora* have been interbreeding and are a single species as suspected by others (Harrington, 1964; Weber, 1987). These taxa are very similar morphologically, with only a few characters to distinguish them (see discussion above), and overlap in distribution. It may also be possible to determine whether there is gene flow between populations of *I. longisepala* and *I. rivularis* since these are in close proximity.

Chapter 2

Materials and Methods

2.1 Plant Materials

Representatives from the eight species of *Iliamna* were used in this study as well as a representative from *Sphaeralcea* to serve as an outgroup (Table 2.1). Plant material already available was obtained by T.F. Wieboldt and N. Stewart from 1994 to 1995 for *I. latibracteata, I. longisepala, I. remota,* and *I. rivularis.* Material of *Sphaeralcea incana, I. bakeri, I. corei, I. crandallii*, and *I. grandiflora* was collected by the author in 1999, at which time latitude and longitude were recorded using the Global Positioning System. Voucher specimens for all were placed in the Massey Herbarium at Virginia Tech.

2.2 Plant 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) for use in amplification and sequencing. The available samples were frozen at -80°C upon arrival . Tissue collected during the summer of 1999 was dried in silica gel at a ratio of 50 g of silica gel to no more than 5 g of leaf tissue. During DNA isolation, a mucilage high in polysaccharides prevented the precipitation of a high quantity of DNA. To improve upon the procedure, 2% PVP (polyvinyl pyrolidone) was added to the 2% CTAB (hexadecyltrimethylammonium bromide), and a phenol:chloroform:isoamyl alcohol (24:24:1) step was incorporated to aid in the removal of phenolics. To prevent precipitation of the polysaccharides, 2 M sodium chloride was mixed with the isolated DNA [eluted in TE (10 mM Tris, 1 mM EDTA)] and 100% ethanol in order to precipitate the genomic DNA (Fang et al., 1992). The DNA was then resuspended in TE for storage. Isolated genomic DNA was quantified on a 1% agarose TAE (Tris, Acetate, and EDTA) gel containing 0.5 μg/L of ethidium bromide and examined under ultra-violet light.

Table 2.1: Plant Materials Used; Voucher Specimens Deposited at Virginia Tech (VPI)

Species	Collection Location	Voucher Number	Genbank Accession
I. bakeri	McCloud, CA	TBS 99-10	AF271165 to AF271167
	Mt. Shasta, CA	TBS 99-9	AF271168 to AF271169
I. crandallii	Buffalo Pass, CO	TBS 99-6	AF271176 to AF271178
	Fish Creek Falls, CO	TBS 99-3	AF271173 to AF271175
I. corei	Research Station (VPI)	TFW 6761	AF271170 to AF271172
I. grandiflora	Grand Canyon, AZ	TBS 99-11	AF271179 to AF271182
I. latibracteata	Agness, OR	VS s.n. 7/6/1994	AF271183 to AF271186
I. longisepala	Swakane Canyon, WA	KK s.n. 6/1994	AF271190 to AF271192
	Rocky Reach, WA	KK s.n. 7/19/1994	AF271187 to AF271189
I. remota	Kankakee River, Altorf, IL	From Type Locality	AF271196 to AF271197
	James River, Amherst Co., VA	TFW 5577	AF271193 to AF271195
I. rivularis	Clearwater NF, Idaho CO., ID	DS s.n. 7/1994	AF271198 to AF271200
	Cache Co., UT	TFW 1704	AF271201 to AF271203
Sphaeralcea incana	Painted Desert, AZ	TBS 99-13	AF271204 to AF271205

Total genomic DNA was used in the amplification of the ITS region by the polymerase chain reaction (PCR). The ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGT AAAAGTCGTAACAAGG-3') primers, designed by White et al. (1990), were used in the amplification using a thermocycler program of 1 min. 97° C; 1 min. 48° C; 45 sec. 72° C adding 4 sec. per cycle to the extension step for 40 cycles followed by 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 μM oligonucleotide primer (Gibco, BRL), 1.9 nM $MgCl_2$, 200 μM each deoxynucleotide triphosphate, 10 mM Tris-HCl, 0.1% Tridon X, 50 mM KCl, and 1 unit Taq Polymerase (Promega, Corp. Madison, WI). The amplified products were purified by gel electrophoresis and cleaned with the Qiagen Gel Extraction kit to remove excess template, primer-dimers, and oligonucleotides for most samples (Qiagen Corp.). For some samples, amplification products were cleaned by an ethanol precipitation and used in a subsequent amplification to generate a fragment for cloning.

2.3 Cloning and Sequencing of the ITS Region

Initially, the sequences of double stranded DNA 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 (Perkin-Elmer) with 5 pmol of either the ITS4 or ITS5 primer and 100 to 200 ng of DNA. The reactions were fractionated by gel electrophoresis in an ABI 373 automated sequencer at the University of Maine Sequencing Facility. The resulting sequences showed multiple overlapping sequences of varying lengths. This was due to several insertion-deletion (indel) mutations with the ITS-1 region within a species. Since the direct sequencing of PCR product resulted in unreadable ITS sequences, cloning was conducted in order to obtain sequences for individual copies of the ITS region.

First, the already amplified ITS regions were re-amplified using modified ITS4 and ITS5 primers to add restriction enzyme recognition sites to the fragment, HindIII to the 5' end and EcoRI to the 3' end. Amplification followed the method above with approximately 20 ng of amplified DNA in place of the genomic DNA. After amplification, the product was purified by an ethanol precipitation. The cleaned product was digested, as was the vector (50 ng), with 10 units of HindIII and EcoRI each, in a buffer containing 29 mM Tris-HCl, 58 mM potassium acetate, 9 mM magnesium acetate, 6.2 mM Spermidine, and 0.1 mM dithiothreitol. Reactions were incubated at 37° C overnight and stopped with a phenol:chloroform (1:1) extraction. Products were cleaned using Qiaquick Gel Extraction (Qiagen Corp.). In order to place the ITS fragment into the plasmid p-Bluescript (Stratagene), the T4 ligase enzyme (Promega Corp., Madison, WI) was used with a 5:1 insert to plasmid ratio. The ligation reaction followed the protocol supplied by Promega Corp. with a 10 μl reaction volume.

The ligation reaction was used in an electroporation to transform bacterial cells, *E. coli* strain

DH10B. Electrocompetent cells were prepared ahead of time and held at -80° C (Dower, 1989; Smith et al., 1990). Electroporation reactions contained 1 μl of the ligation reaction and 40 μl of DH10B cells in 0.1 cm cuvettes and were pulsed at 1.8 kV and 200 Ω. After the treatment, cells were placed in 1 ml of SOC media for one hour at 37° C. Then, 200 μl of the culture was used inoculate solid LB medium containing 100 mg/ml of Ampicillin (LBAmp_{100}) coated with 20 μl of both 20 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-GAL) and 20 mg/ml isopropyl thio-β-D-galactoside.

After an overnight incubation colonies containing recombinant plasmids were identified by blue/white screening. If the colony was white, this indicated that the bacteria contained a plasmid that had a fragment inserted into the multiple cloning site. If the colony was blue, then the plasmid in the bacteria did not have a fragment interrupting the β-galactosidase gene at the multiple cloning site. Individual white colonies were used to inoculate 5 ml LBAmp_{100} media and incubated overnight at 37° C. Plasmid DNA was isolated following an alkaline lysase miniprep (Birnboim and Doly, 1979). The plasmid miniprep was isolated from 4.5 ml of culture using an alkaline lysase miniprep procedure (Binboim and Doly, 1979) and resuspended in 50 μl of TE. A 2 μl sample of the plasmid DNA was digested as above with EcoRI and HindIII in a 50 μl reaction to verify the presence of an ITS fragment insertion before sequencing. Digestion products were fractionated by agarose gel electrophoresis and quantified by comparison to a Lambda HindIII standard (Figure 2.1).

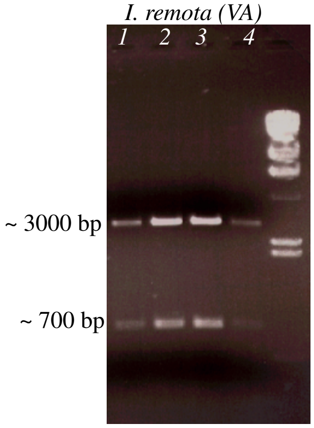


Figure 2.1: Agarose gel analysis of the recombinant plasmid after the EcoRI and HindIII digestion. Bands at 3000 bp are the plasmid and at ca. 700 bp are the ITS fragment. A lambda HindIII standard was used for size and quantity approximation

The double stranded plasmid DNA was also used in sequencing reactions following the procedure mentioned above. Two to four independent clones for each species sample were sequenced. Either the ITS4 and ITS5 primers or the T7 and T3 primers that anneal to vector sequences flanking the multiple cloning site were used in sequencing reactions at 5 pmol per reaction with 200 ng of plasmid DNA. Sequencing reactions were either cleaned by an ethanol-salt precipitation (Perkin-Elmer) or by chromotography through sephadex at the Veterinary Medicine Sequencing Facility at Virginia Tech. Samples were sequenced at either the University of Maine Sequencing Facility (ethanol precipitated samples) or at the Veterinary Medicine Sequencing Facility at Virginia Tech. Both locations used an ABI 373A automated sequencer (Applied Biosystems, Inc., Foster City, CA). Each primer pair provided at least 65% overlap in sequence data. Chromatograms were manually edited using Edit View (Applied Biosystems, Inc., Foster City, CA). The derived sequences (41 total) were deposited in GenBank (Table 2.1).

Sequences were aligned visually using Sequence Navigator (Applied Biosystems, Inc., Foster City, CA). Boundaries for the ITS-1, ITS-2, and 5.8s rDNA regions in *Iliamna* were determined by comparing sequences available in GenBank of other representatives of Malvaceae (*Anotea, Hibiscus,* and *Gossypium*). Two insertion-deletion mutations in the first ITS spacer (ITS-1) required gaps of one to five bases to be added in most sequences during the alignment. Within ITS-2, one to three-base gaps were needed for the alignment.

2.4 Outgroup Selection

The choice of *Sphaeralcea* for the outgroup was based upon several factors. The placement of *Sphaeralcea* representatives as sister to the Malacothamnus alliance in LaDuke and Doebley's (1995) study of chloroplast restriction digest affirms its closeness to the genus of study. Also, species in *Sphaeralcea* resemble those of *Iliamna* morphologically. Third, representatives of these two genera occur in roughly the same geographical area. Historical treatments of *Iliamna* had placed these species into *Sphaeralcea* based upon the previous two criteria. Lastly, *Sphaeralcea* was more readily available for collection compared to the other related genera, *Phymosia* and *Malacothamnus.*

2.5 Phylogenetic Analysis

Phylogenies were generated using PAUP* 4.0 (Swofford, 2000). The RANDOM TREES option was used to infer the presence of phylogenetic signal (Hillis and Huelsenbeck, 1992). The g1 value for the distribution of 100,000 random trees was calculated using the critial value (at $\alpha = 0.05$) for 250 variable characters and 25 taxa.

Using FITCH parsimony, a heuristic search was performed by RANDOM stepwise addition (100 replicates) following tree bisection-reconstruction (TBR) branch swapping in PAUP* 4.0

(Swofford, 2000). The resulting trees were saved and used as the starting trees in RANDOM addition following the nearest neighbor intersection (NNI) branch swapping. From these resulting trees, the strict consensus was composed. Searches were conducted using each spacer individually, the 5.8s nrDNA individually, and the complete data set. Gaps were treated as missing data.

Robustness and topology of the trees were evaluated using the consistency index (CI), retention index (RI), bootstrap, decay values, and taxon jackkniffing. The consistency index measures homoplasy by dividing the minimum amount of evolutionary steps by the actual tree length (Judd et al., 1999). The lower the CI value, the more characters contradict the evolutionary tree. The CI may decrease, however, as more taxa are added since the tree length will increase. The retention index differs in that it acounts for multiple independent origination events for derived characters (Judd et al., 1999). This index equals the maximum tree length minus the actual tree length, divided by the maximum length minus the minimum length. Both of these indices are indicators of the validity of the tree produced.

Robustness of the phylogeny was measured by bootstrap, decay, and taxon jackknifing analyses (Judd et al., 1999). In bootstrap analysis, multiple randomized matrices are constructed from the data by a random sampling of characters with replacement of the characters. From these matrices, most-parsimonious trees are constructed and used to form a consensus tree. The bootstrap values given indicate a 50% or higher representation of a branch in the parsimonious trees produced. Only those branches that receive a 50% or higher value are retained in the consensus tree, all others are collapsed. The decay value indicates how many extra steps are required to find a tree without a particular branch or clade. So, the higher the decay value, the more robust a particular clade is. In taxon jackkniffing, character matrices are generated as in the bootstrap analysis, but once randomly selected a character is not replaced. Again, the multiple matrices are used to produce most-parsimonious trees that are compiled into a consensus tree. And, if a clade is maintained in more than 50% of the parsimonious trees, then it is retained. Bootstrap and taxon jackknife values are usually similar and higher values indicate the stability of a clade.

Chapter 3

Results

3.1 ITS Sequence Analysis

Sequences were obtained for two to four independent clones of the ITS region of the eight species of *Iliamna* and *Sphaeralcea incana* (see Appendix A). Replicate clones were sequenced until all indel mutations from the two spacers were represented, all of the various ITS copies within a species were not sequenced. Within *Iliamna*, representatives from two populations were analyzed for each species, with the exception of *I. corei, I grandiflora*, and *I. latibracteata.* In a previous study using RAPDs, it was found that individuals of *I. corei* were genetically identical (Stewart and Porter, 1995; Stewart et al., 1996) and because of the endangered status of this species, only one representative was used.

A summary of the results is shown in Table 3.1. The length of ITS-1 varies from 292 to 296 bp and the length of ITS-2 ranges from 215 to 218 bp (Table 3.1). Length variation within a species is due to two insertion-deletion events (indels) in the ITS-1 region and one indel in ITS-2. The length of the 5.8s rDNA was invariable among the species at 163 bp. Intraspecific variation differs between the ITS-1 and ITS-2 regions. The number of polymorphisms in ITS-1 ranges from 10 in *I. rivularis* to 1 in *I. latibracteata.* In ITS-2, the number of polymorphisms range from 8 in *I. crandallii* and *I. grandiflora* to 2 in *I. corei.* Within a species, pairwise difference ranges from 0.1% in most species to a high of 3.5% in *I. latibracteata.* In comparison to the outgroup *Sphaeralcea*, *I. corei* has a pairwise sequence difference of 11% followed by *I. remota* (VA) at 10.9%. *Iliamna bakeri* and *I. latibracteata* are the most similar to *Sphaeralcea*, differing, by 7% and 8% (see Appendix B for complete pairwise distance data).

Total G+C content for the entire spacer region is 52.7%, the ITS-1 region 50.7%, and the ITS-2 region 54.4%, and the 5.8s rDNA 53.7%. Within the entire region, 29.7% nucleotide sites are variable. ITS-1 provides a greater number of variable sites, 105, than ITS-2, 76 sites. Of the variable nucleotide sites in the entire region, 120, 14.9%, are phylogenetically informative. ITS-1 contributes a greater number of informative sites (67 sites) than does ITS-2 (47 sites).

3.2 Phylogenetic Analyses

Phylogenetic signal of the ITS data set is significant ($P > 0.01$) based on the value of the g1 statistic (-0.76742). The heuristic search of the ITS-1 and ITS-2 data resulted in 50 most-parsimonious trees requiring 276 evolutionary steps (Figure 3.1). The adjusted consistency index (CI) excludes homoplastic characters and was 0.8331, the retention index (RI) was 0.9270. When the ITS-1 data were analyzed alone, 96 equally parsimonious trees were produced with 139 steps, a CI=0.8417 and RI=0.9353 (Figure 3.2). The ITS-2 data resulted in 9 equally parsimonious trees with 106 steps, CI=0.8585, and RI=0.9442 (see Figure 3.3). The 5.8s nrDNA data alone did not produce a phylogenetically resolved tree, only three clades were generated and the tree was only 23 evolutionary steps long.

The combined data set produced the most resolved tree (Figure 3.1). All ingroup taxa, the *Iliamna* species, are contained in Clade A with 100% bootstrap support. Clade B contains the Pacific Northwestern species and is well-supported with 100% bootstrap support and by numerous synapomorphies distinguishing this clade. The remaining six species fall within Clade C with 100% bootstrap support. The polytomy in Clade C contains the western species plus the last group, Clade D, which is comprised of the eastern species. Clade D contains *I. remota* and *I. corei* and has 100% bootstrap support. *Iliamna remota* from Illinois is ancestral to both *I. remota* (VA) and *I. corei* with 69% bootstrap and 53% jackknife support.

Table 3.1: Summary of variation within the ITS region.

Region	Length	G+C Content	Average Number of Polymorphisms	Number of Variable Sites	Phylogenetically Informative Sites	Percent Phylogenetically Informative
ITS-1	292-296	50.7%	5	105	67	22.6%
5.8s nrDNA	163	53.7%	1	20	6	3.7%
ITS-2	215-218	54.4%	6	76	47	21.6%
Entire Region	670-677	52.7%	12	201	120	14.9%

3.3 Conserved Regions in ITS

In the ITS-1 region, Liu and Schardl (1994) reported a conserved sequence that can be aligned across many flowering plant families. In *Iliamna*, the conserved motif is 21 basepairs long, is found 183 bp into ITS-1 and is the same for all eight species. This position places the motif in a similar location to that in other angiosperms (Liu and Schardl, 1994). Table 3.2 shows the sequence motif in *Iliamna* in comparison to other representative angiosperms. The conserved sequence motif of *Iliamna* also forms the predicted hairpin loop (Liu and Schardl, 1994) as in the other sequences (Figure 3.4).

Table 3.2: Conserved sequence motif within ITS-1.

Arabidopsis thaliana	`GGCACG-AAAAGTGTCAAGGAA`
Nicotiana rustica	`GGCGTGGAAAAGCGCCAAGGAA`
Oryza sativa	`GGCGCC-GACGGCGTCAAGGAA`
Triticum aestivum	`GGCGCC-GAAGGCGTCAAGGAA`
Gossypium longicalyx	`GGCGTG-AATTGCGCCAAGGAA`
Gossypium robinsonii	`GGCGCG-AATCGCGCCAAGGAA`
Iliamna spp.	`GGCGCG-AATTGCGCCAAGGAA`

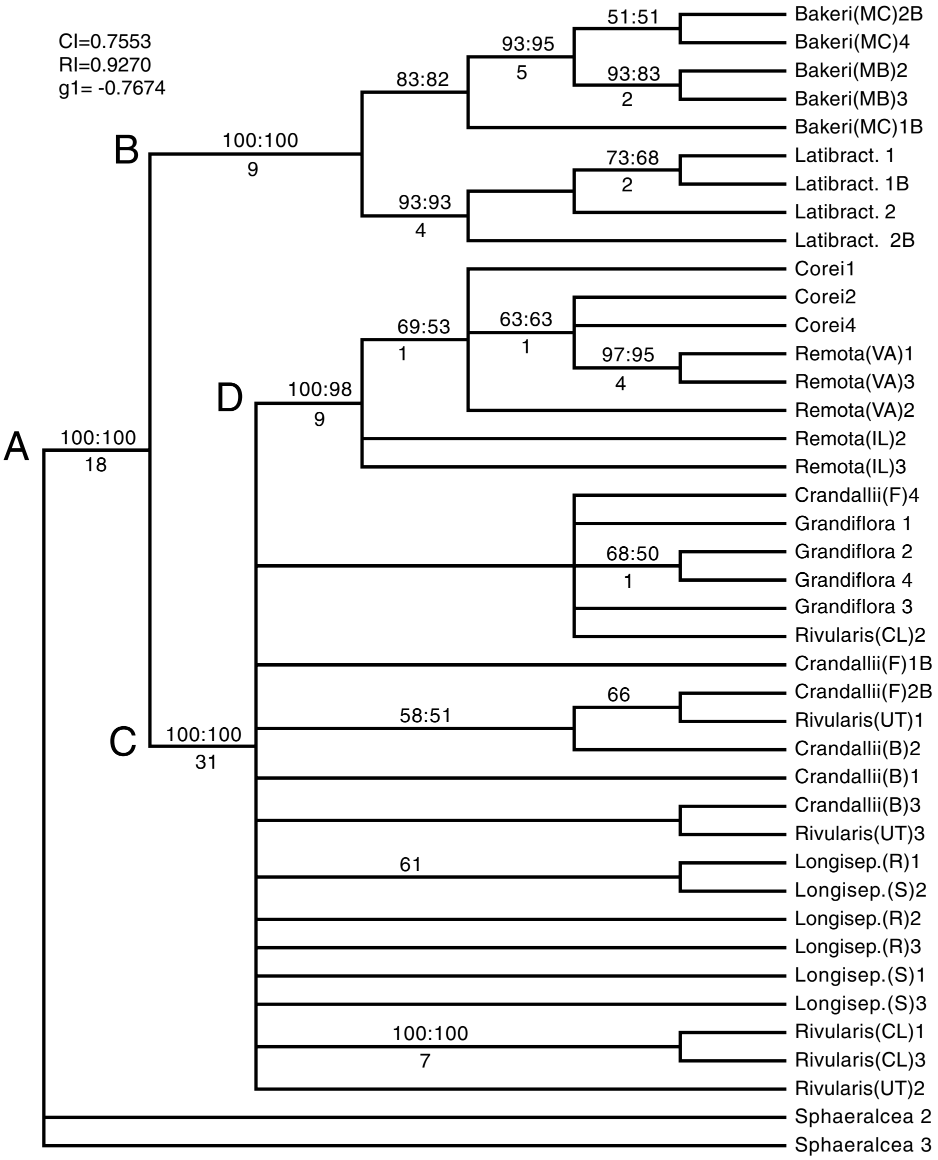


Figure 3.1: Strict consensus tree for the combined ITS-1, 5.8s, and ITS-2 data sets. Numbers above are bootstrap values and jackknife, numbers below, decay values. Bakeri(MC)=*I. bakeri* McCloud,Bakeri(MB)=*I. bakeri* Mt. Shasta, Latibract.=*I. latibracteata*, Corei=*I. corei*, Remota(VA) *I. remota* Virginia, Remota(IL)=*I. remota* Illinois, Grandiflora=*I. grandiflora*, Crandallii(F)=*I. crandallii* Fish Creek Falls, Crandall(B)=*I. crandallii* from Buffalo Pass, Rivularis(CL)=*I. rivularis* from Clearwater N.F., Rivularis(UT)=*I. rivularis* from Cache Co., Longisep.(R)=*I. longisepala* from Rocky Reach, Longisep.(S)=*I. longisepala* from Swakane Canyon, and Sphaeralcea=*Sphaeralcea incana var. incana.* Numbers after taxa label represent the numbered clone.

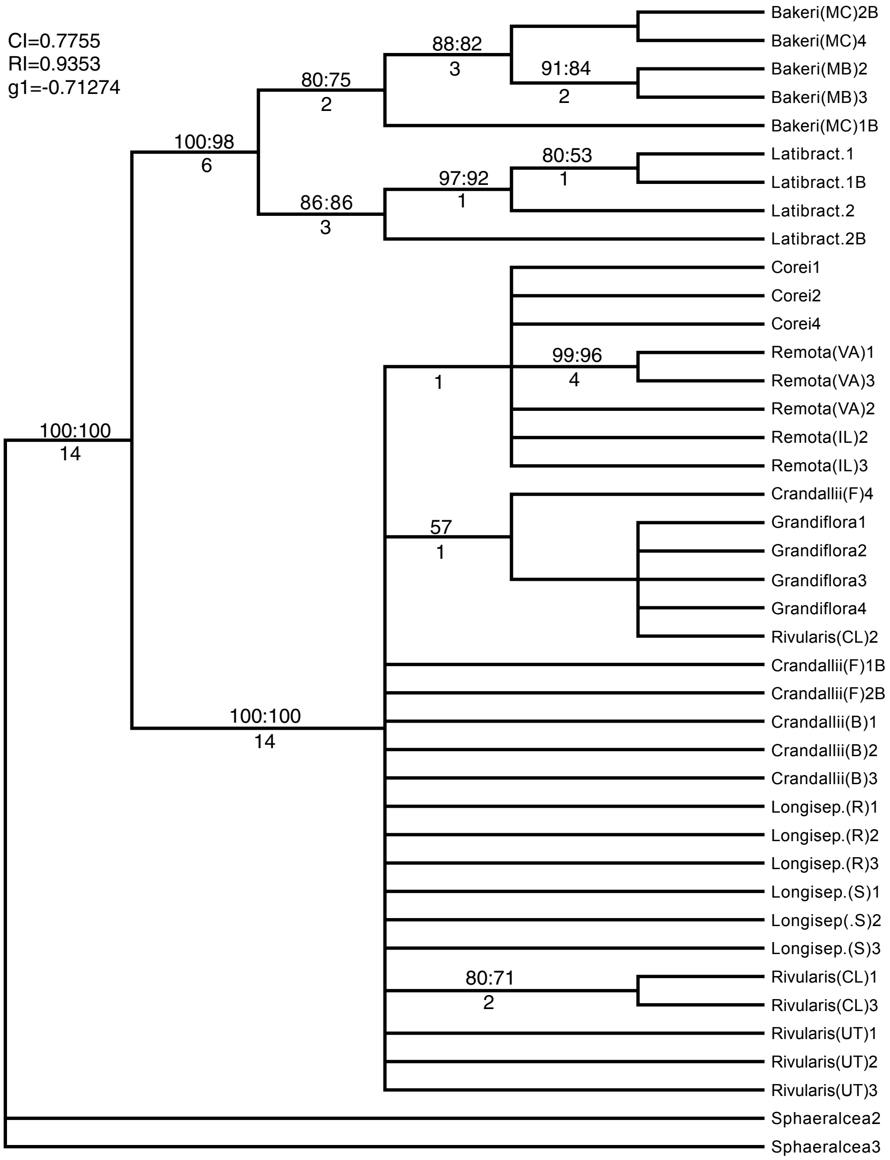


Figure 3.2: Strict consensus tree for the ITS-1 data set. Numbers above are bootstrap values and jackknife, numbers below, decay values. See Figure 3.1 for taxa labels.

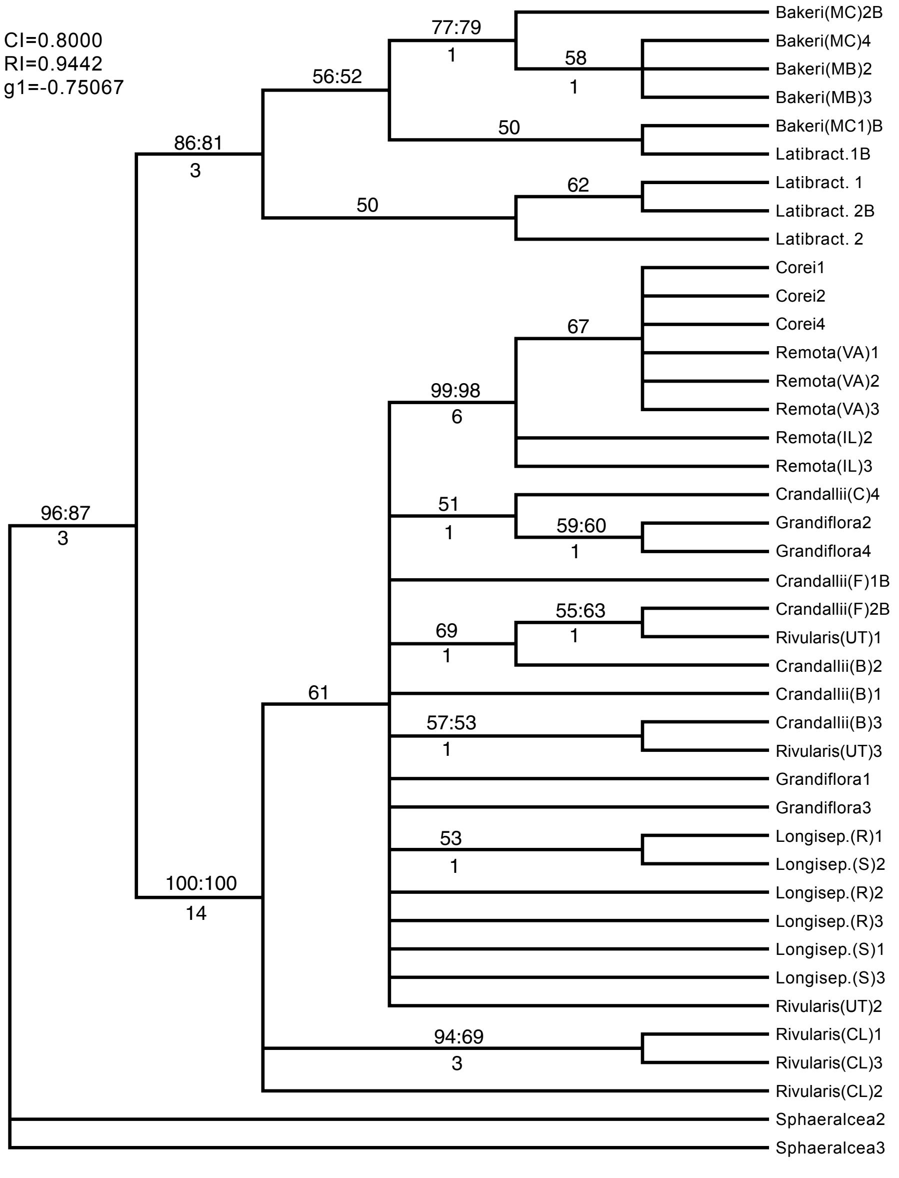
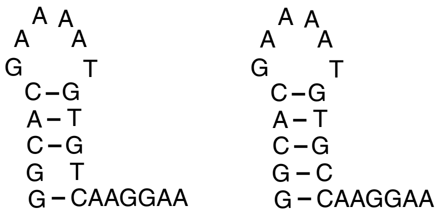


Figure 3.3: Strict consensus tree for the ITS-2 data set. Numbers above are bootstrap values and jackknife, numbers below, decay values. See Figure 3.1 for taxa labels.



Arabidopsis thaliana *Iliamna spp.*

Figure 3.4: Structure of the conserved region in ITS-1

Chapter 4

Discussion

Iliamna is comprised of eight species that are distributed throughout temperate North America. Since its designation by E. L. Greene in 1906, the species in *Iliamna* have been shifted to other genera and several have been questioned as to their status as species. Seven species are classified as rare or endangered and their populations continue to decline. Due to their rarity and questionable classification based on morphological characters and preliminary molecular analysis using RAPDs, a phylogenetic study was conducted using a highly variable region of nuclear ribosomal DNA already defined above. The ITS region has previously been used to generate phylogenies at and below the generic level for a wide array of angiosperm taxa (Baldwin, 1992).

4.1 Phylogenetic Relationships in *Iliamna*

Phylogenetic analysis of the ITS region for *Iliamna* has generated a well-supported cladistic hypothesis for the genus (see Figure 4.1). *Iliamna* is monoplyletic with 100% support. Within the genus, however, the relationships are not as well-defined.

In the Pacific Northwest, *I. bakeri* and *I. latibracteata* form a separate clade from the others with 100% bootstrap and jackknife support. These two species have a 4% to 5% pairwise sequence difference (see Appendix B), suggesting an extened period of divergence. In addition, there is only a 7% to 8% pairwise difference between these species and the outgroup *Sphaeralcea*, whereas there is a pairwise difference greater than 10% between these two and the remaining *Iliamna* species. This implies that either (a) *I. bakeri* and *I. latibracteata* have maintained an ancient copy of the ITS-region not seen in the remaining taxa, or (b) *I. bakeri* and *I. latibracteata* have evolved separately from other *Iliamna* species and belong in a different genus from the others. *Iliamna bakeri* and *I. latibracteata* are found west of the other species and are not believed to interbreed with other *Iliamna* taxa.

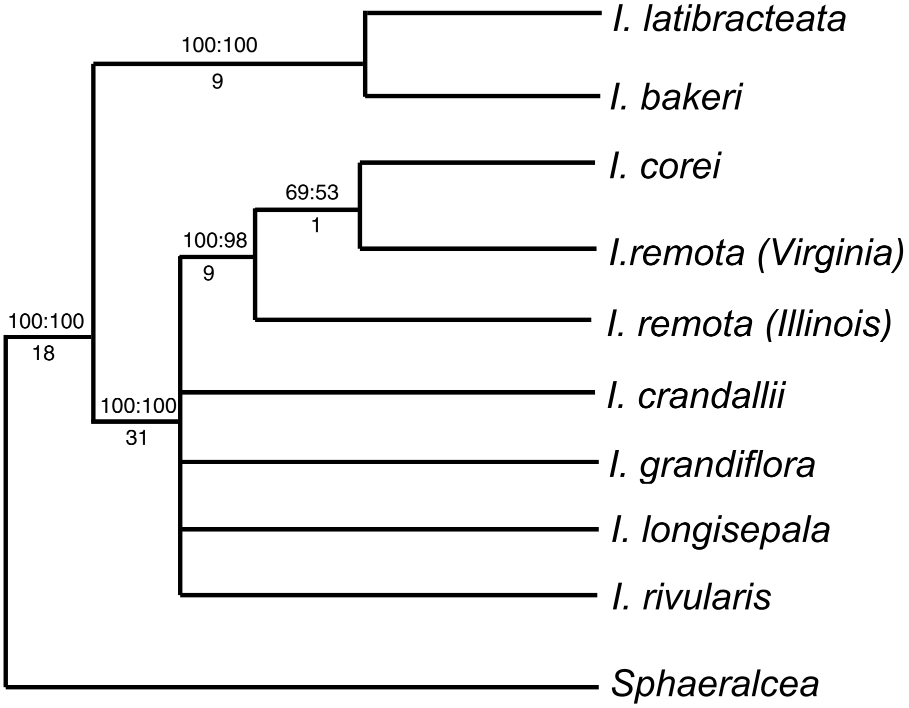


Figure 4.1: Phylogeny of *Iliamna* showing the general relationships between species as seen in the combined analysis of the ITS-1, ITS-2 and 5.8s nrDNA regions. Numbers above are bootstrap values and jackknife, numbers below, decay values.

Among the others, the eastern species *I. corei* and *I. remota* form a well-supported clade (the Eastern Clade), with 100% support dividing them from the Rocky Mountain group. However, the remaining four species (*I. crandallii, I. grandiflora, I. longisepala,* and *I.rivularis*) form an unresolved polytomy, herein referred to as the Rocky Mountain Group (RMG). In this group, only the sequences of clones from *I. grandiflora* group together in a clade in the neighbor-joining (NJ) phylogram (Figure 4.2). The neighbor-joining method finds pairs of taxa that minimize the branch length for each cluster of taxa (Saitou and Masatoshi, 1987). The NJ method does not necessarily produce the minimum-evolution tree, as does a heuristic search using maximum parsimony. The NJ method was employed here to examine clustering of taxa based on the evolutionary distance of sequences and to visualize the number of differences between taxa (Swofford, 1993). Also in the NJ phylogram, two cloned sequences of *I. rivularis* from the Clearwater National Forest (ID) appear as ancestral to the remaining members of the RMG and to the Eastern Clade. A similar topology does not result in the parsimony analysis, due to a lack of statistical support and to the relatively low variability between ITS sequences among the RMG. The pairwise distances between *I. rivularis* (CL) and the other Rocky Mountain representatives is only 0.74% to 2.5%. The small divergence values among

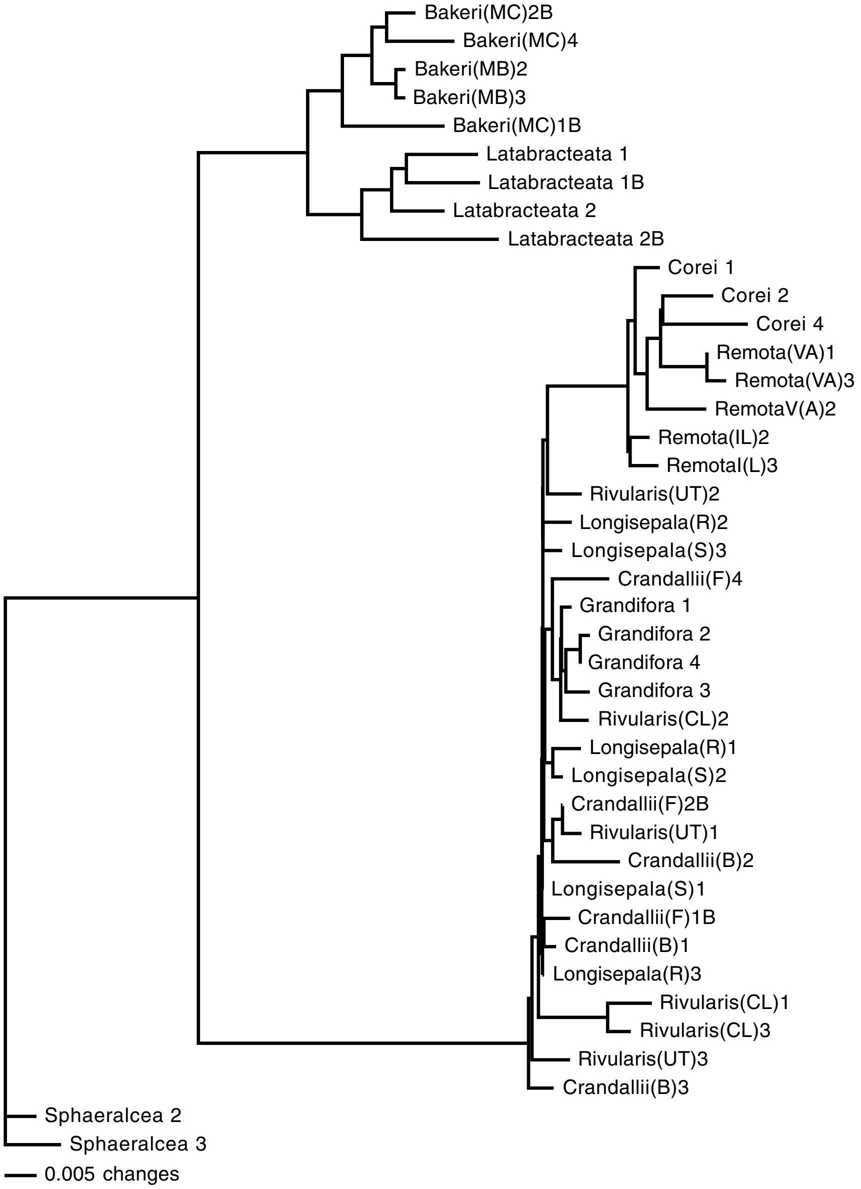


Figure 4.2: Neighbor-joining tree showing evolutionary distance relationships between taxa.

the RMG indicate that the sequences have been evolving independently for a short period of time and/or that there is gene flow between the species. The four species of the RMG do overlap geographically in distribution (see Figure 1.1) and *I. crandallii, I. grandiflora,* and *I. longisepala* are found in localities where *I. rivularis* is rare. 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 a rapid diversification of *I. rivularis* took place in response to novel ecological or morphological adaptations. Either one of these situations is probable for *I. crandallii, I. grandiflora, I. longisepala,* and *I. rivularis*. Among these taxa, *I. longisepala* is found growing along mountain streams in mid- to eastern-Washington and is morphologically most similar to *I. crandallii* (Wiggins, 1936). *Iliamna crandallii* is restricted to the vicinity of Steamboat Springs, CO, where it grows in rich, moist forest soils in open areas. *Iliamna grandflora*, however, is found in lush forests of aspen (*Populus tremuloides*) and Engelmann spruce (*Picea engelmannii*) to drier habitats with Pinon Pine (*Pinus edulis*) along the Colorado River drainage basin into Arizona and New Mexico. As Harrington (1964) pointed out, *I. crandallii* and *I. grandiflora* "are separated mainly on relative size of parts but so much integration occurs in this respect in Colorado plants" (p. 373) that he suggested reducing them to synonomy. Whether or not integration occurs between these taxa has not been determined.

With the easternmost species, similar morphology had led systematists to classify *I. corei* as a variety of *I. remota* in the past (*I. remota var. corei* Sherff) (Sherff, 1946). The two were later designated as species based upon their geographical isolation and morphological differences (Sherff, 1949). In a recent study using RAPDs (Stewart and Porter, 1995), it was impossible to determine which classification is correct. The resulting phylogeny here does little to resolve this question. The sequences from clones of *I. corei* do cluster separately from those of the *I. remota* of Illinois, but are interwined in a clade with *I. remota* from Virginia. Morphologically, *I. corei* and *I. remota* are distinct (Sherff, 1949; Stewart and Porter, 1995; Swinehart and Jacobs, 1998). With increased sampling and examination of additional genes and morphology, the relationship of *I. corei* to *I. remota* may be resolved.

Interestingly, *I. remota* growing in Virginia has diverged from plants growing in Illinois, with a 1.5% to 1.9% pairwise difference between representatives from these states. Whether or not this difference is significant enough to warrant subspecies classification is unclear. Expanded sampling to include sequences from the ITS region for additional plants in Illinois, Indiana, and Virginia may help to answer this question. Also, pollination experiments to determine outcrossing ability could shed some light on the taxonomic status of the *I. remota* populations in addition to the relationships within the Rocky Mountain Group.

4.2 Gene Trees versus Species Trees

The hypothetical cladistic trees resulting from this study are a reflection of the evolution of the ITS region in *Iliamna*. The molecular-based tree may not reflect the evolution of the species (i.e., the species tree) and incongruence between the two trees may appear (Doyle, 1992). Although the ITS region does not code for a protein, it is a continuous block of nucleotides (including the 5.8s nrDNA) that is being used to infer a phylogeny and therefore generates a gene tree (Slowinski and Page, 1999). In a gene tree, the nucleotides are the characters and the terminal taxa are the sequences used, not the species they represent.

Incongruence between a gene tree and a species tree may result from homoplasy, introgression, lineage sorting or noncoalescence, and gene duplication (Doyle, 1992; Wendel and Doyle, 1999). In non-molecular studies, homoplasy is believed to be higher than in molecular analyses because of morphological convergence. Introgression results from a permanent transfer of genes from one species to another. Hybridization is similar in that it is a source of novel gene combinations and a mechanism of speciation (Judd et al., 1999). In introgression, however, the transfer of genetic material occurs without a merging of species. Both introgression and hybridization result in gene flow between species (Small and Wendel, 2000). After hybridization, one of the parent copies of a gene may be lost or fixed depending on drift and selection pressures (Doyle, 1992). If a copy is fixed in the daughter genome and homogenization of alleles does not occur, then polymorphisms result. Polymorphisms may also arise with introgression if the introduced allele is maintained in a multi-gene family; this is referred to as non-coalescence. Maintenance of ancient polymorphisms results in alleles that are phylogenetically closer to those of other species, are exaggerated in species that favor high levels of polymorphism, such as in self-incompatibility, and should be apparent if one or more ancient polymorphisms are shared by two or more species (Small and Wendel, 2000). Introgression and non-coalescence between nuclear genes of plant populations that have not shown recent hybridization are rare, but have been found to occur between New and Old World cotton species (*Gossypium spp.*) (Wendel and Doyle, 1999).

The gene tree produced may also reflect the evolution of paralogous and orthologous copies of genes in a genome (Wendel and Doyle, 1999; Small and Wendel, 2000). A duplicated gene within a species is referred to as a paralogous copy. If a gene evolves and is associated with speciation, it is referred to as an orthologous copy. In phylogenetic reconstruction, only orthologous genes can be used to derive a phylogeny for the organism. In concerted evolution, paralogous copies generally homogenize, limiting intraspecific variation. With tandemly repeated gene copies, as is the case with nrDNA, concerted evolutionary forces are stronger and complete homogenization should occur. Yet, incomplete concerted evolution is present when homogenization varies between loci, and as a result paralogous copies will be maintained (Wendel and Doyle, 1999). Phylogenies generated under such instances will analyze both paralogs and orthologs; therefore, the gene tree generated may not reflect the species tree (Wendel and Doyle, 1999; Small and Wendel, 2000).

In the gene tree produced for *Iliamna*, introgression and/or hybridization, non-coalescence, and gene duplication influence the tree topology. The polytomy resulting in the Rocky Mountain taxa (*I. crandallii, I. grandiflora, I. longisepala,* and *I. rivularis*) suggests that gene flow is occurring between these taxa, since there is a high degree of similarity in the ITS sequences. Gene flow between these taxa may be due to hybridization, since their distributions overlap. The lack of variability between sequences of the RMG may also represent a recent radiation of these taxa in the formation of ecotypes.

Non-coalescence, the maintenance of ancient polymorphisms in a multigene family, could explain why *I. bakeri* and *I. latibracteata* ITS sequences have a greater similarity to the chosen outgroup (*Sphaeralcea*) than to ITS sequences of other species from the same genus. It is possible that expanded sampling of these species and of related genera could further elucidate the relationships of *I. bakeri* and *I. latibracteata* to other members of *Iliamna*.

Lastly, homogenization of the multiple copies of the nrDNA has not occurred in this recently evolved genus. This is evident in the length variation of the ITS sequences obtained from individual species. As a result both paralogous and orthologous copies are included in the analysis. It is impossible to distinguish between the two because of the low variability of the sequences. Therefore, the gene tree produced for *Iliamna* serves as just a starting point in understanding relationships within the genus.

4.3 Geological History and *Iliamna*

The present day distributions of species in *Iliamna* do not illustrate their historic ranges. Based upon changes in climate and topography, populations of *Iliamna* migrated, speciated, and faced extinction. Regional changes of climate in the continental United States affected plant distributions and speciation events to varying degrees. By examining the geological history of North America, inferences about migration, speciation, and extinction can be made for *Iliamna* species.

4.3.1 Northwestern United States

During the Late Pleistocene, the Cordilleran ice sheet extended southward into northern Washington, Idaho, and Montana, reaching a peak glaciation 18,000 years before present (YBP) (Delcourt and Delcourt, 1993). During the interval of 20,000 to 16,800 YBP, the Pacific Coastal Plain had a subalpine climate with lowland and montane plant species. Temperate trees began to migrate into the mountains 12,000 to 10,000 YBP as substantial glacial melting took place. Following this warm and wet period, summers became warmer and drier and plants experienced drought stress during the Hypsithermal Interval of 10,000 to 7,000 YBP. As a result, the forest canopy opened and herbaceous plants migrated northward. After the Hypsithermal Interval, the climate cooled to modern conditions.

From the present study, it cannot be determined where *I. bakeri* or *I. latibracteata* originated or to what extent their distributions once covered. Presently, *I. bakeri* is found in drier habitats at elevations ranging from 1000 m to 2500 m, growing in volcanic ash soil on mountain slopes and lava beds (Hickman, 1993). *Iliamna latibracteata* is uncommon in California and Oregon, but grows at lower elevations (500 m to 2000 m) in conifer forests near streams. During the Hypsithermal Interval, these populations were probably much larger and had a greater extension because of the opened forest canopy.

4.3.2 Western North America

Habitats of western North America during the Late Pleistocene were quite different from those of modern times. Lower unglaciated elevations had cool moist climates and the area was scattered with pluvial lakes (15,000 YBP) (Delcourt and Delcourt, 1993). Local pockets of areas of high precipitation led to decreasing seasonal climatic variation and an increase in riparian communities. Under such conditions, ranges of forest plants extended along their maximum low elevational and southern distributional ranges and woodland communities dominated. If *Iliamna* were present during this time, it would have had an extensive distribution throughout western North America.

As in the Pacific Northwest, a climatic warming, the Hypsithermal Interval, occurred (approximately 8,000 YBP) and resulted in a shift in vegetation (Delcourt and Delcourt, 1993). The low elevation woodlands were replaced by desert scrub vegetation. The forest communities persisted at intermediate elevations and expanded higher into the mountains as temperatures increased. Prairie plants and animals, such as Richardson's ground squirrel, migrated eastward during this time through grassland mountain passes that are now heavily forested (Pielou, 1992). Palynological evidence indicates that the prairie community extended north to Manitoba, Canada 7,000 YBP with fossilized *Sphaeralcea coccinea* pollen being found in northern Canada (Ritchie, 1983). After the climatic warming, the pluvial lakes did not return to the North American southwest, the desert scrub persisted (Delcourt and Delcourt, 1993).

With respect to hypothetical *Iliamna* distributions, some populations to the south may have become extinct during the warming of the Hypsithermal Interval. Other populations would have become isolated from the main species pool as the riparian system shrank. This isolation may have led to the ecotypes or species we see today in *I. grandiflora* and *I. crandallii.* Since the riparian communities did not return to their full extent after the Hypsithermal Interval, *Iliamna* may not have been able to recover its former continuous distribution.

4.3.3 Midwestern and Eastern North America

During the Late Quaternary glaciations, the Appalachian Region functioned as a refuge for plant species (Davis, 1983). During periods of glaciation, temperate species in eastern North America survived in small populations subject to extinction. Interglacial periods resulted in vegetational instability and shifting distributions of the flora and fauna. The rate of expansion of species during the more stable periods depended on the suitability of the climate, and the ability of a plant to disperse seeds and become established.

In the central Appalachians, the forest tundra gave way to a rich deciduous hardwood and conifer forest as the Wisconsin ice sheet retreated from Ohio and Indiana 13,000 YBP (Watts, 1979; Delcourt and Delcourt, 1993). Massive migrations of mesic forests from 12,000 to 9,000 YBP introduced *Castanea, Fagus, Juglans, Quercus,* and *Tilia* to the central Appalachians.

Castanea pollen dated from approximately 15,000 YBP found near Memphis, TN, is believed to indicate the starting point for a migrational pattern northeast along the Appalachians during the forest migration (Davis, 1983).

The dry, warm weather of the Hypsithermal Interval (9,000 to 6,000 YBP) led to further northward and higher elevational migration of taxa along the Appalachian Mountains (Watts, 1979). The water table decreased in the lowlands during this interval, causing a disequilibrium of plant species composition (Delcourt and Delcourt, 1993). As a result of the drying conditions in the Hypsithermal Interval, canopies opened and herbaceous taxa were able to migrate into and along the Appalachians as the hardwoods did. During this time, prairie habitats also reached their maximum eastern distribution, 120 km further east than their present boundary (Pielou, 1992)

By 5,000 YBP the modern climatic regime was established in the Appalachians and southeastern North America, with precipitation being available throughout the growing season (Delcourt and Delcourt, 1993). As the Hypsithermal Interval ended, there was a marked sudden loss of *Tsuga* populations throughout the Central Appalachians approximately 4,900 YBP, possibly due to disease or pathogens (Watts, 1979; Davis, 1981). Approximately 4,000 YBP the climate cooled and became more like the present day. Tree species common in modern times became dominant along the Appalachians as well. In the Midwest, the prairies were no longer a continuous band stretching from Indiana to the Dakotas, only fragments remained in Illinois, Indiana, and Ohio that were separated from the vast western prairies (Delcourt and Delcourt, 1981, 1993).

With this in mind, it is plausible that *I. remota* is a remnant of a once extensive *Iliamna* distribution from the Rocky Mountains to the East. This founding population could have migrated with the prairies into Illinois and Indiana 9,000 YBP and become separated when prairie habitat was reduced (4,000 YBP). During the Hypsithermal Interval, *I. remota* could have also migrated eastward into Virginia following the opening of the forest canopy. Further support for this migrational path comes from similar migration patterns of tree pollen seen in the fossil record (Davis, 1983). The present location for *I. corei* could be a further result of the eastwardly migration of *I. remota.* Upon inhabiting Peter's Mountain and the Blue Ridge, an archaic *I. remota* population may have led to the present day species *I. corei.* Once the climate cooled and habitats returned to their pre-Hypsithermal state, *I. corei* and *I. remota* of Virginia became separated from the ancestral populations of the west and from each other. From the ITS phylogeny produced, this hypothetical migration and foundation is plausible and suggests that *I. remota* has been in Virginia much longer than the suspected introduction in the early 1900s (Stewart and Porter, 1995).

4.4 Species Designation

Defining a species has been a long-standing discussion in biology. Among the definitions, the biological species concept (BSC) developed by the zoologist Ernst Mayr has been the most widely accepted (Judd et al., 1999). The BSC is simple, easily understood and defines a species as a reproductive community of population(s) that are isolated (Briggs and Walters, 1997; Judd et al., 1999). In plants, however, reproductive isolation cannot be tested unambiguously since interfertility varies widely among plants (Judd et al., 1999). The frequency of hybridization in plants further complicates designating species under the BSC. With frequent hybridization, the BSC would create species that are broadly inclusive of a wide array of closely related taxa, since they are not reproductively isolated. Furthermore, the BSC was developed with animal species in mind and has been almost abandoned in plant systematics (Judd et al., 1999).

Other species concepts include the evolutionary species concept and the diagnosability species concept (Judd et al.,1999). Simpson developed the evolutionary species concept (ESC) in 1961 based on the evolutionary history of organisms. The ESC defines a species as a lineage that has evolved separately from others with its own evolutionary role and tendencies (Briggs and Walters, 1997). Lack of clarity in the ESC lies in its discrepancy to define evolutionary lineages and the feasibility of labeling such in nature.

In the diagnosability species concept (DSC), Cracraft considered a species as the smallest diagnosable cluster of individual organisms that exhibits a pattern of ancestor and decendent relationships (Briggs and Walters, 1997). The character states used to define a cluster must be invariant and fixed (Judd et al., 1999). Like the BSC, this is an easily understood definition, yet it is flawed. If a series of populations differs by one small genetic characteristic, then it would be a separate species under this concept. Most taxonomists, however, would not recognize the series as separate species. Also, populations in the process of speciation would have some characters that are not fixed, and therefore would not be a species under the DSC.

Despite the numerous definitions of a species, there is little consensus as to what constitutes a species in plants (Judd et al., 1999). Species can be considered as artificial classification units used by systematists (Briggs and Walters, 1997). Taxa considered as species are hypotheses that are open to repeated testing as new data and methods become available (Judd et al., 1999). Most importantly, taxa designated as species should be easily recognizable by non-specialists of that group. Similar consideration should be used in designating subspecies (Weatherby, 1942). Often subspecies are named if they are the most important or distinguishable variants under a species.

In *Iliamna*, the eight designated species are easily recognized as separate species and should remain as such. The Rocky Mountain group does pose some difficulties, however. As has been suggested by Harrington (1964), *I.grandiflora* and *I. crandallii* vary only in sizes of parts and may be considered the same species. Rydberg (1904) also noticed a high degreee of similarity in the RMG when he first described *I. grandiflora* and *I. crandallii.* He noted that *I. grandiflora* and *I. rivularis* were close allies. He based his splitting of the two on the larger flowers, broader bractlets, longer 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. rivularis*, but differs in the long bractlets and the lanceolate sepals" (p. 564). The last member of the RMG, *I. longisepala*, has not been directly compared to *I. rivularis, I. grandiflora,* and *I. crandallii.* When Wiggins (1936) developed a taxonomic key for the genus, *I. longisepala* was most similar to *I. crandallii.* In the present study, it can be speculated that these three taxa are indeed not separate species, but since they are recognizable as such, their status as distinct species should be maintained and tested further.

As for *I. remota* and *I. corei*, the position of the Illinois *I. remota* as basal to the other two is interesting. This may imply that *I. corei* and *I. remota* from Virginia should be considered as subspecies of the Illinois *I. remota.* Or, the three may have diverged and become separate species. As with the Rocky Mountain group, *I. remota* and *I. corei* are easily recognized as distinct entities and should be considered as separate species until further evidence to the contrary is obtained.

Lastly, *I. bakeri* and *I. latibracteata* are clearly separate species in the molecular phylogeny produced here. The main concern regarding these two is their placement in the genus *Iliamna.* Since their sequences were more closely allied with *Sphaeralcea*, it may be that *I. bakeri* and *I. latibracteata* are the basal-most species in *Iliamna* or that they belong in a separate genus. In an analysis with the related genera *Malacothamnus* and *Phymosia*, the inclusion or exclusion of *I. bakeri* and *I. latibracteata* in *Iliamna* may be resolved.

4.5 Future Direction

In order to bring further resolution as to the species status of *I. corei* and *I. remota* and to understand the history of the disjunct populations of *I. remota* a finer scale analysis should be conducted. Inter-short sequence repeat (ISSR) genetic markers may provided the needed information. ISSRs are loci that are evenly dispersed throughout the genome and are inherited as dominant or codominant genetic markers in a Mendelian fashion (Wolfe and Liston, 1999). The use of ISSRs have revealed that these markers may be useful in assaying patterns of gene flow among species and populations.

A further expansion of this project would include *Phymosia* and *Malacothamnus* in the ITS data set. Since these two genera have been suggested to be the ancestor(s) to *Iliamna* their relationships to one another and to *Iliamna* could be seen with an analysis of the ITS region. Additional sequence data from either the alcohol dehydrogenase (adh) gene or the waxy gene could provide further resolution to the phylogenetic analysis. Phylogenies resulting from either of these genes could further support finding of this study. By including several genes and morphology in a phylogenetic analysis, a species tree could be inferred for *Iliamna*.

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Appendix A

Sequence Alignment

Complete Sequence Alignment for *Iliamna*. Bases 1-295 are the ITS-1 Region, 297-460, the 5.8s nrDNA, and 461-677, the ITS-2 Region.

```
                   1                                               50
Bakeri(MC)2B       TCGAAACCTGCCTAGCAGAACGACCCGTGAACGTGATATCAAACAACAAA
Bakeri(MC)1B       .....................A............................
Bakeri(MC)4        .....................A............................
Bakeri(MB)2        ..................................................
Bakeri(MB)3        ..................................................
Corei1             ....................T..................TG......TC.
Corei2             ....................T..................TG......TC.
Corei4             ....................T..................TG......TC.
Crandallii(F)4     ....................T..................TG......TC.
Crandallii(F)1B    ....................T..................TG......TC.
Crandallii(F)2B    ....................T..................TG......TC.
Crandallii(B)1     ....................T..................TG......TC.
Crandallii(B)2     ....................T..................TG......TC.
Crandallii(B)3     ....................T..................TG......TC.
Grandiflora1       ....................T..................TG......TC.
Grandiflora2       ....................T..................TG......TC.
Grandiflora3       ....................T..................TG......TC.
Grandiflora4       ....................T..................TG......TC.
Latabracteata1     ......T..............A............................
Latabracteata1B    ......T..............A............................
Latabracteata2     ......T..T...........A............................
Latabracteata2B    ......T.C................A........................
Longisepala(R)1    ....................T..................TG......TC.
Longisepala(R)2    ....................T..................TG......TC.
Longisepala(R)3    ....................T..................TG......TC.
Longisepala(S)1    ....................T..................TG......TC.
Longisepala(S)2    ....................T..................TG......TC.
Longisepala(S)3    ....................T..................TG......TC.
Remota(VA)1        ....................T..........T.......TG......TC.
Remota(VA)2        ....................T..................TG......TC.
Remota(VA)3        ..............T.....T..........T.......TG......TC.
Remota(IL)2        ....................T..................TG......TC.
Remota(IL)3        ....................T..................TG......TC.
Rivularis(CL)1     .........A..........T..................TG......TC.
Rivularis(CL)2     ....................T..................TG......TC.
Rivularis(CL)3     ..............C.....T..................TG......TC.
Rivularis(UT)1     ....................T..................TG......TC.
Rivularis(UT)2     ....................T..................TG......TC.
Rivularis(UT)3     ....................T..................TG......TC.
Sphaeralcea2       ...............................................TCC
Sphaeralcea3       .............................................G.TCC
```

```
                51                                             100
BakMC2B         ACGAGGGG-CGCGGATGCATCCTTGCCCCAAACCCCCTCGATGTCTTGGT
BakMC1B         ........G.........................................
BakMC4          ........G.........................................
BakMB2          ........GTA.......................................
BakMB3          ........GTA.......................................
Corei1          ........GT.....A....A..-...................C......
Corei2          ........GT.....A....A......................C......
Corei4          ........GT.....A....A......................C......
CrandFC4        ........GT.....A....A......................CT.....
CrandFC1B       ........GT.....A....A......................C......
CrandFC2B       ........GT.....A....A......................C......
CrandBP1        ........GT.....A....A......................C......
CrandBP2        ........GT.....A....A......................C......
CrandBP3        ........GT.....A....A...................G..C......
Grandif1        ........GT.....A....A.................T....C......
Grandif2        ........GT.....A....A.................T....C......
Grandif3        ........GT.....A....A.................T....C......
Grandif4        ........GT.....A....A.................T....C......
LatabrAg1       ........G.........................................
LatabrAg1B      ........G.........................................
LatabrAg2       ........G.........................................
LatabrAg2B      ........G.............C...........................
LongisRR1       ........GT.....A....A......................C......
LongisRR2       ........GT.....A....A......................C......
LongisRR3       ........GT.....A....A......................C......
LongisS1        ........GT.....A....A......................C......
LongisS2        ........GT.....A....A......................C......
LongisS3        ........GT.....A....A......................C......
RemotaVA1       ........GT.....C....A......................C......
RemotaVA2       ........GT.....A....A......................C......
RemotaVA3       ........GT.....C....A......................C......
RemotaIL2       ........GT.....A....A......................C......
RemotaIL3       ........GT.....A....A......................C......
RivularCL1      ........GT.....A....A......................C...T..
RivularCL2      ........GT.....A....A.................T....C......
RivularCL3      ........GT.....A....A......................C...T..
RivularUT1      ........GT.....A....A......................C......
RivularUT2      ........GT.....A....A................C.....C......
RivularUT3      ........GT.....A....A......................C......
Sphaera2        ........GT...................G..........G..C......
Sphaera3        ........GT..................TG..........G..C......
```

```
                  101                                             150
BakMC2B           GTGCTTTCCCTTGCACCATCCGCTCTTT-GGGCGGTTGAGATGCCTGGTC
BakMC1B           ......G.....................-......G.T............
BakMC4            A.....G.....................-.....................
BakMB2            ............................-......G..............
BakMB3            ............................-......G..............
Corei1            ...T..GG.T...TC...A.....A...-......A..............
Corei2            ...T..GG.T...TC...A.....A...G..CG..A..............
Corei4            ...T..GG.T...TC...A.....A...-......A..............
CrandFC4          ...T..GG.T...TC...A.....T...-......A.........C....
CrandFC1B         ...T..GG.T...TC...A.....A...T......A..............
CrandFC2B         ...T..GG.T...TC...A.....A...T......A..............
CrandBP1          ...T..GG.T...TC...A.....A...-......A..............
CrandBP2          ...T..GG.T...TC...A.....A...T......A..............
CrandBP3          ...T..GG.T...TC...A.....A...-......A..............
Grandif1          ...T..GG.T...TC...A.....T...-......A..............
Grandif2          ...T..GG.T...TC...A.....T...-......A..............
Grandif3          ...T..GG.T...TC...A.....T...-......A..............
Grandif4          ...T..GG.T...TC...A.....T...-......A..............
LatabrAg1         ......G...................C.-.T....G..............
LatabrAg1B        ......G...................C.-.T....G..............
LatabrAg2         ......G...................C.-.T....G..............
LatabrAg2B        ......G...A.................-.T....G..............
LongisRR1         ...T..GG.T...TC...A.....A...T......A..............
LongisRR2         ...T..GG.T...TC...A.....A...T......A..............
LongisRR3         ...T..GG.T...TC...A.....A...T......A..............
LongisS1          ...T..GG.T...TC...A.....A...T......A..............
LongisS2          ...T..GG.T...TC...A.....A...T......A..............
LongisS3          ...T..GG.T...TC...A.....A...T......A..............
RemotaVA1         ...T..GG.T...TC...A.....A...-......A..............
RemotaVA2         ...T..GG.T...TC...A.....A...-......A..............
RemotaVA3         ...T..GG.T...-C...A.....A...-......A..............
RemotaIL2         ...T..GG.T...TC...A.....A...-......A.............T
RemotaIL3         ...T..GG.T...TC...A.....A...-......A..............
RivularCL1        ...T..GG.T...TC...A.....A...-......A..............
RivularCL2        ...T..GG.T...TC...A.....T...-......A..............
RivularCL3        ...T..GG.T...TC...A.....A...-......A..............
RivularUT1        ...T..GG.T...TC...A.....A...T......A..............
RivularUT2        ...T..GG.T...TC...A.....A...T......A..............
RivularUT3        ...T..GG.T...TC...A.....A..CT......A..............
Sphaera2          ......G..T....C......A....C--.........T.T...AA....
Sphaera3          ......G..T....C......A....C--...........T...AA....
```

```
                151                                             200
BakMC2B         TTGTCACACTCCTAGGCAAAACGAACAAACCCCGGCGCGAATTGCGCCAA
BakMC1B         ............................C.....................
BakMC4          .G................................................
BakMB2          ............................C.....................
BakMB3          ............................C.....................
Corei1          .GTA........A.........C.....C........T............
Corei2          .ATA........A.........C.....C........T............
Corei4          .ATA........A.........C.....C........T............
CrandFC4        ..TA........A.........C.....C........T............
CrandFC1B       ..TA........A.....G...C.....C........T............
CrandFC2B       ..TA........A.........C.....C........T............
CrandBP1        ..TA........A.........C.....C........T............
CrandBP2        ..TA........A.........C.....C........T............
CrandBP3        ..TA........A.........C.....C........T............
Grandif1        ..TA........A.........C.....C........T............
Grandif2        ..TA........A.........C.....C........T............
Grandif3        ..TA........A.........C.....C........T............
Grandif4        ..TA........A.........C.....C........T............
LatabrAg1       ............A...............C........T............
LatabrAg1B      ............A..............TC........T............
LatabrAg2       ............A...............C........T............
LatabrAg2B      ............A..........G....C.....................
LongisRR1       ..TA........A.........C.....C........T............
LongisRR2       ..TA........A.........C...T.C........T............
LongisRR3       ..TA........A.........C.....C........T............
LongisS1        ..TA........A.........C.....C........T............
LongisS2        ..TA........A.........C.....C........T............
LongisS3        ..TA........A.........C.....C........T............
RemotaVA1       .ATA........A.........C...G.C.......AT............
RemotaVA2       .ATA........A...T.....C.....C........T..........T.
RemotaVA3       .ATA........A.........C...G.C.......AT............
RemotaIL2       .ATA........A.........C.....C........T............
RemotaIL3       .ATA........A.........C.....C........T............
RivularCL1      ..TA........A.........C.....C........T............
RivularCL2      ..TA........A.........C.....C........T............
RivularCL3      ..TA........A.........C.....C........T............
RivularUT1      ..TA.T......A.........C.....C........T............
RivularUT2      ..TA........A.........C.....C........T............
RivularUT3      ..TA........A.........C.....C........T............
Sphaera2        ............A...............C........T............
Sphaera3        ............A...............C........T............
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```
                201                                            250
BakMC2B         GGAAT--AATAAAATGAAAAGAGTGCACGTCTACTATTGCCGTCCCGTAC
BakMC1B         .....--..A.........................G.C............
BakMC4          .....--...........................................
BakMB2          .....--...........................................
BakMB3          .....--.............................A.............
Corei1          .....---.A.................T.......G....T.........
Corei2          .....AA..A...T.............T.......G..............
Corei4          .....-A..A...T............GT.......G..............
CrandFC4        .....--..A..........T..............G..............
CrandFC1B       .....--..A.......G.................G........T.....
CrandFC2B       .....-A..A.........................G..............
CrandBP1        .....-T..A.........................G..............
CrandBP2        .....-A..A.........................G..............
CrandBP3        .....--..A.........................G..............
Grandif1        ....---..A.........................G..............
Grandif2        .....--..A.........................G..............
Grandif3        .....--..A.................T.......G..............
Grandif4        .....--..A.........................G..............
LatabrAg1       .....-A..A...TC......................C..T.........
LatabrAg1B      .....-A..A...T.....................G.C..T.........
LatabrAg2       .....-A..A...T.....................G.C...AA.......
LatabrAg2B      .....-A..A...T...........T.T.....T.G.C............
LongisRR1       .....-A..A.........................G..............
LongisRR2       .....-A..A.........................G..............
LongisRR3       .....--..A.........................G..............
LongisS1        .....--..A.........................G..............
LongisS2        .....-A..A....................G....G..............
LongisS3        .....-A..A.........................G..............
RemotaVA1       .....AA..A...T.............T.......G..............
RemotaVA2       .....-A..A.................T.......G.....A........
RemotaVA3       .....AA..A...T.............T.......G..............
RemotaIL2       .....-A..A.................T.......G..............
RemotaIL3       .....-A..A.................T.......G........T.....
RivularCL1      .....--..A.....A...................G..............
RivularCL2      .....--..A.........................G..............
RivularCL3      .....--..A.....A...................G..............
RivularUT1      .....-A..A.........................G..............
RivularUT2      .....-A..A.........................G..............
RivularUT3      .....--..A.........................GCC............
Sphaera2        ...T.--TGA....................T.G..G.C............
Sphaera3        ...T.--TGA....................T.G..G.C............
```

```
                251                                              300
BakMC2B         GCGGTGTCTGTGTGGTAGAGACGTTGTTACTTTTGTCGTAAAAATACAAA
BakMC1B         .......................C.........................T
BakMC4          ..................................................
BakMB2          A.................................................
BakMB3          ..................................................
Corei1          ...............C..T..T.................G.....TT...
Corei2          ...............C..T..T.................G.....TT...
Corei4          ...............C..T..T.................T.....TT...
CrandFC4        ...............C..T..T.................G.....TT...
CrandFC1B       ...............C..T..T.................G.....TT...
CrandFC2B       ...............C..T..T.................G.....TT...
CrandBP1        ...............C..T..T.................G.....TT...
CrandBP2        ...............C..T..T.................G.....TT...
CrandBP3        ...............C..T..T.................G......T...
Grandif1        ...............C..T..T.................G.....TT...
Grandif2        ...............C..T..T.................G.....TT...
Grandif3        ...............C..T..T.................G.....TT...
Grandif4        ...............C..T..T.................G.....TT...
LatabrAg1       .......A..........T.G..C...............G..........
LatabrAg1B      .......A........G.T.G..C...............G..........
LatabrAg2       .......A..........T.G..C...............G..........
LatabrAg2B      ....C..........C..T.G..C...............G..........
LongisRR1       ...............C..T..T.G...............G.....TT...
LongisRR2       ...............C..T..T.................G.....TT...
LongisRR3       ...............C..T..T.................G.....TT...
LongisS1        ...............C..T..T.................G.....TT...
LongisS2        ...............C..T..T.................G.....TT...
LongisS3        ...............C..T..T.................G.....TT...
RemotaVA1       ...............C..T..T.................G......T...
RemotaVA2       ...............C..T..T.................G......T...
RemotaVA3       ...............C..T..T.................G......T...
RemotaIL2       ...............C..T..T.................G.....TT...
RemotaIL3       ...............C..T..T.................G.....TT...
RivularCL1      ...............C..T..T...........-.....G.....TT...
RivularCL2      ...............C..T..T.................G.....TT...
RivularCL3      ...............C..T..T...........-.....G.....TT...
RivularUT1      ...............C..T..T.................G.....TT...
RivularUT2      ...............C..T..T.................G.....TT...
RivularUT3      ...............C..T..T..C..............G.....TT...
Sphaera2        .........T..C..C..T..............C.....G.....-....
Sphaera3        .........T..C..C..T....................G..........
```

```
               301                                              350
BakMC2B        ACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAG
BakMC1B        .......................T..........................
BakMC4         ...............................................C..
BakMB2         ..................................................
BakMB3         ..................................................
Corei1         .T................................................
Corei2         .T................................................
Corei4         .T.......................................G........
CrandFC4       .....................................G............
CrandFC1B      ..................................................
CrandFC2B      ..................................................
CrandBP1       ..................................................
CrandBP2       ..............................A...................
CrandBP3       ..................................................
Grandif1       ..................................................
Grandif2       ..................................................
Grandif3       ..................................................
Grandif4       ..................................................
LatabrAg1      ..................................................
LatabrAg1B     ..................................................
LatabrAg2      ..................................................
LatabrAg2B     .......................T..........................
LongisRR1      ..................................................
LongisRR2      ..................................................
LongisRR3      ..................................................
LongisS1       ..................................................
LongisS2       ..................................................
LongisS3       ..................................................
RemotaVA1      .T................................................
RemotaVA2      .T................................................
RemotaVA3      .T................................................
RemotaIL2      .T................................................
RemotaIL3      .T................................................
RivularCL1     ..................................................
RivularCL2     ..............................T...................
RivularCL3     ..................................................
RivularUT1     ..................................................
RivularUT2     ......................A...........................
RivularUT3     ..................................................
Sphaera2       ..................................................
Sphaera3       ..................................................
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                351                                            400
BakMC2B         CGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTC
BakMC1B         ..................................................
BakMC4          ..................................................
BakMB2          ..................................................
BakMB3          ..................................................
Corei1          ..................................................
Corei2          .....A............................................
Corei4          ..................................................
CrandFC4        ..................................................
CrandFC1B       ..................................................
CrandFC2B       ..................................................
CrandBP1        ..................................................
CrandBP2        ..................................................
CrandBP3        ..................................................
Grandif1        ..................................................
Grandif2        .....C............................................
Grandif3        ..................................................
Grandif4        ..................................................
LatabrAg1       ..................................................
LatabrAg1B      ..................................................
LatabrAg2       ..................................................
LatabrAg2B      ..................................................
LongisRR1       ..................................................
LongisRR2       ..........................T.......................
LongisRR3       ..................................................
LongisS1        ..................................................
LongisS2        ..................................................
LongisS3        ..................................................
RemotaVA1       ..................................................
RemotaVA2       ..................................................
RemotaVA3       ..................................................
RemotaIL2       ..................................................
RemotaIL3       ..................................................
RivularCL1      .......................A..........T...............
RivularCL2      ..................................................
RivularCL3      .......................A..........T...............
RivularUT1      ..................................................
RivularUT2      ..................................................
RivularUT3      ..................................................
Sphaera2        ..................................................
Sphaera3        ..................................................
```

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               401                                              450
BakMC2B        TTTGAACGCAAGTTGCGCCCCAAGCCATTAGGCTGAGGGCACGTCTGCCT
BakMC1B        .................................C................
BakMC4         .................................C................
BakMB2         .................................C................
BakMB3         .................................C................
Corei1         .................................C..............A.
Corei2         .................................C..............A.
Corei4         ........T........................C..............A.
CrandFC4       .................................C..............A.
CrandFC1B      .................................C..............A.
CrandFC2B      .................................C..............A.
CrandBP1       .................................C..............A.
CrandBP2       .................................C..............A.
CrandBP3       .................................C..............A.
Grandif1       .................................C..............A.
Grandif2       .................................C..............A.
Grandif3       .................................C..............A.
Grandif4       .................................C..............A.
LatabrAg1      .................................C................
LatabrAg1B     .................................C................
LatabrAg2      .................................CA...............
LatabrAg2B     .................................C................
LongisRR1      .................................C..............A.
LongisRR2      .................................C..............A.
LongisRR3      .................................C..............A.
LongisS1       .................................C..............A.
LongisS2       .................................C..............A.
LongisS3       .................................C..............A.
RemotaVA1      .................................C..............A.
RemotaVA2      ............................C....C..............A.
RemotaVA3      .................................C..............A.
RemotaIL2      .................................C..............A.
RemotaIL3      .................................C..............A.
RivularCL1     .................................C..............A.
RivularCL2     .................................C..............A.
RivularCL3     .................................C..............A.
RivularUT1     .................................C..............A.
RivularUT2     .................................C..............A.
RivularUT3     .................................C..............A.
Sphaera2       .................................C................
Sphaera3       .................................C................
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               451                                              500
BakMC2B        GGGTGTCACGCATCGTCGCCCCC-TTTAAACCCTAAGCCCTCGGGCTATG
BakMC1B        ................T......-A.........................
BakMC4         .......................-.......A..................
BakMB2         .......................-.......A..................
BakMB3         .......................-.......A..................
Corei1         .....C.......T........--A.C....A..G......T......C.
Corei2         .A...........T....G...--A.C....A..G......T...A..C.
Corei4         .............T........--A.C....A..G......T...A..C.
CrandFC4       .............T........--A.C....A..G.............C.
CrandFC1B      .............T........--A.C....A..G.............C.
CrandFC2B      .............T........--A.C....A..G.............C.
CrandBP1       .............T........--A.C....A..G............GC.
CrandBP2       .............T........--A.C....A..G.............C.
CrandBP3       .............T........--..C....A..G.............C.
Grandif1       .............T........--A.C....A..G.............C.
Grandif2       .............T........--A.C....A..G.............C.
Grandif3       .............T........--A.C....A..G.............C.
Grandif4       .............T........--A.C....A..G.............C.
LatabrAg1      .....C.................-A.......................C.
LatabrAg1B     ................T...T..-A.........................
LatabrAg2      .......................-A.......................C.
LatabrAg2B     .......................-A.......................C.
LongisRR1      .............T........--A.C....A..G.............C.
LongisRR2      .............T........--A.C....A..G.............C.
LongisRR3      .............T........--A.C....A..G.............C.
LongisS1       .............T........--A.C....A..G.............C.
LongisS2       .............T........--A.C....A..G.............C.
LongisS3       .............TT.......--A.C....A..G.............C.
RemotaVA1      .............T........--A.C....A..G......T...A..C.
RemotaVA2      .............T........--A.C....A..G......T......C.
RemotaVA3      .............T........--A.C....A..G......T...A..C.
RemotaIL2      .............T........--A.C....AT.G......T......C.
RemotaIL3      .............T........--A.C....A..G......T.....CC.
RivularCL1     .............T........--A.C....A..G.............C.
RivularCL2     .............T........--A.C....A..G.............C.
RivularCL3     .............T........--A.C....A..G.............C.
RivularUT1     .............T........--A.C....A..G.............C.
RivularUT2     .......G.....T........--A.C....A..G......A......C.
RivularUT3     .............T........--..C....A..G.............C.
Sphaera2       .......................-A.C.....A.T..T..........C.
Sphaera3       .......................-A.C.....A.T..T..........C.
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                501                                              550
BakMC2B         GTTGAATTGTGGGCGGAAACTGGCCTCCCGTGCGCTCACCGCTCGTGGTT
BakMC1B         .............................................C....
BakMC4          ..................................................
BakMB2          ..................................................
BakMB3          ..................................................
Corei1          ....T.A............A............T..........T.C....
Corei2          ....T.A............A............T..........T.C....
Corei4          ....T.A............A............T..........T.C....
CrandFC4        ....T.A............T............T..........T.C....
CrandFC1B       ....T.A............T............T..........T.C....
CrandFC2B       ....T.A.C..........T..T.........T..........T.C....
CrandBP1        ....T.A............T............T..........T.C....
CrandBP2        ....T.A............T..T.........T..........T.C....
CrandBP3        ....T.A............T............T..........T.C....
Grandif1        ....T.A............T............T..........T.C....
Grandif2        ....T.A........T...T............T..........T.C....
Grandif3        ....T.A.....CG.-...T............T..........T.C....
Grandif4        ....T.A........T...T............T..........T.C....
LatabrAg1       ......C......................................C....
LatabrAg1B      .............................................C....
LatabrAg2       ......C......................................C....
LatabrAg2B      ......C..................A...................C....
LongisRR1       A...T.A............T............T..........T.C....
LongisRR2       ....T.A............T............T..........T.C....
LongisRR3       ....T.A............T............T..........T.C....
LongisS1        ....T.A............T............T..........T.C....
LongisS2        A...T.A............T............T..........T.C....
LongisS3        ....T.A............T............T..........T.C....
RemotaVA1       ....T.A............A............T..........T.C....
RemotaVA2       ....T.A............A..A.........T..........T.C....
RemotaVA3       ....T.A............A............T..........T.C....
RemotaIL2       ....T.A............A............T..........T.C....
RemotaIL3       ....T.A............A............T......T...T.C....
RivularCL1      ....T.A............T............T..........T.C....
RivularCL2      ....T.A............T............T..........T......
RivularCL3      ....T.A............T............T..........T.C....
RivularUT1      ....T.A.C..........T..T.........T..........T.C....
RivularUT2      ....T.A.....-......T............T..........T.C....
RivularUT3      ....T.A............T............T..........T.C....
Sphaera2        ......C............T.........................C....
Sphaera3        ...................T.........................C....
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```
                551                                              600
BakMC2B         GGCCTAAAATTGAGTCCTCGGCGATGAAGTGCCGCGACAATCGGTGGGAA
BakMC1B         ..................................................
BakMC4          ..........................G.......................
BakMB2          ..................................................
BakMB3          ..................................................
Corei1          ...................TATA...........................
Corei2          ...................TATA...........................
Corei4          ....CG.............TATA.........T.................
CrandFC4        .....................TA...........................
CrandFC1B       .....................TA...........................
CrandFC2B       .....................TA...........................
CrandBP1        .....................TA...........................
CrandBP2        .....................TA..........A......C.........
CrandBP3        .....................TA....-......................
Grandif1        .....................TA...........................
Grandif2        .....................TA...........................
Grandif3        .....................TA...........................
Grandif4        .....................TA...........................
LatabrAg1       ....................A.............................
LatabrAg1B      ..................................................
LatabrAg2       ....................................C.............
LatabrAg2B      ....................A.............................
LongisRR1       .....................TA...........................
LongisRR2       .....................TA...........................
LongisRR3       .....................TA...........................
LongisS1        .....................TA...........................
LongisS2        .....................TA...........................
LongisS3        .....................TA...........................
RemotaVA1       ...................TATA...........................
RemotaVA2       ...................TATA...........................
RemotaVA3       ...................TATA...........................
RemotaIL2       ...................T.TA...........................
RemotaIL3       ...................T.TA...........................
RivularCL1      .....................AA.............CG......A.....
RivularCL2      ......................A...........................
RivularCL3      ......................A..............G............
RivularUT1      .....................TA....G......................
RivularUT2      .....................TA...........................
RivularUT3      .....................TA...........................
Sphaera2        ..................................................
Sphaera3        ............................T.....................
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               601                                            650
BakMC2B        TGCTTTTT--GCTGCCTCGTTCGGAGTCGTGTGCGCTCGTTGATTAGGAC
BakMC1B        ........--..............T.........................
BakMC4         ........--...........................T............
BakMB2         ........--........................................
BakMB3         ........--........................................
Corei1         ...A...AA-.................T......A......T........
Corei2         ...A...AA-.................T......A......T........
Corei4         ...A...AA-.............T...T......A......T.......T
CrandFC4       .......AA-.............A...T......A...............
CrandFC1B      .......AA-.................T......A...............
CrandFC2B      .......AA-.................T......A...............
CrandBP1       .......AA-.................T......A...............
CrandBP2       .......AAT.A............T..T......A...............
CrandBP3       .......AA-.................T......A........C......
Grandif1       .......AA-.T...............T......A...............
Grandif2       .......AA-.................T......A...............
Grandif3       .......AA-.................T......A...............
Grandif4       .......AA-.................T......A...............
LatabrAg1      ........--...........................T............
LatabrAg1B     ........--.......................T...............A
LatabrAg2      ........--........................................
LatabrAg2B     ........--.......................T................
LongisRR1      .......AA-...........A.A...T......A...............
LongisRR2      .......AA-.................T......A...............
LongisRR3      .......AA-.................T......A...............
LongisS1       .......AA-.................T......A...............
LongisS2       .......AA-.................T......A...............
LongisS3       .......AA-.................T......A.......G.......
RemotaVA1      ...A...AA-.................T......A......T........
RemotaVA2      ...A...AA-.................T......A......T.......T
RemotaVA3      ...A...AA-...............A.T......A......T........
RemotaIL2      ...A...AA-.................T......A......T........
RemotaIL3      ...A...AA-.................T......A......T........
RivularCL1     .......AA-......A..........T......A...............
RivularCL2     .......AA-.................T......A...............
RivularCL3     .......AA-......A..........T......A...............
RivularUT1     .......AA-.................T......A...............
RivularUT2     .......AA-.................T......A...............
RivularUT3     .......AA-.................T......A...............
Sphaera2       .......A--..C...................A.................
Sphaera3       .......A--......................A.......C.........
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                651                          680
BakMC2B         CCTCTGACCCTTTTTGGCATCGCAATGTCG
BakMC1B         .........................C..A.
BakMC4          .........................C....
BakMB2          .........................C....
BakMB3          .........................C....
Corei1          .TGT.........C....T...........
Corei2          .TGT.........C....T...........
Corei4          .TGT.........C....T...........
CrandFC4        TTG..........C....T...........
CrandFC1B       .TG..........C....T...........
CrandFC2B       .TG..........C....T...........
CrandBP1        .TG..........C....T...........
CrandBP2        ATG..........C...AT...........
CrandBP3        .TG..........C....T...........
Grandif1        .TG..........C....T...........
Grandif2        TTG..........C....T...........
Grandif3        .TG..........C....T...........
Grandif4        TTG..........C....T...........
LatabrAg1       ....................T...CCA...
LatabrAg1B      ....................T....C....
LatabrAg2       ....................T.........
LatabrAg2B      ....................T....C....
LongisRR1       .TG..........C....T...........
LongisRR2       .TG..........C....T........G..
LongisRR3       .TG..........C....T...........
LongisS1        .TG..........C....T...........
LongisS2        .TG..........C....T...........
LongisS3        .TG..........C....T...........
RemotaVA1       .TGT.........C....T..-........
RemotaVA2       .TGT.........C....T...........
RemotaVA3       .TGT.........C....T...........
RemotaIL2       .TGT.........C....T...........
RemotaIL3       .TGT.........C....T...........
RivularCL1      .TG..........C....T.G........A
RivularCL2      .TG..........C....T...........
RivularCL3      .TG..........A....T..........A
RivularUT1      .TG..........C....T...........
RivularUT2      .TG..........C....T...........
RivularUT3      .TG..........C....T..........-
Sphaera2        .........................C....
Sphaera3        ....................T....C....
```

Appendix B

Pairwise Differences of Sequences

Similarity between all pairs of sequences minus gaps entered in the alignment for insertion deletion events.

		1	2	3	4	5	6	7	8
1	BakMC2B	-							
2	BakMC1B	0.02673	-						
3	BakMC4	0.01486	0.02967	-					
4	BakMB2	0.01188	0.02671	0.01780	-				
5	BakMB3	0.01188	0.02671	0.01780	0.00297	-			
6	Corei1	0.09854	0.10293	0.10145	0.09697	0.09697	-		
7	Corei2	0.10571	0.11008	0.11007	0.10414	0.10413	0.01492	-	
8	Corei4	0.11016	0.11455	0.11453	0.10858	0.10858	0.02084	0.02076	-
9	CrandFC4	0.09077	0.09517	0.09664	0.08920	0.08920	0.02827	0.03565	0.04007
10	CrandFC1B	0.08640	0.09081	0.09228	0.08483	0.08484	0.02231	0.03119	0.03561
11	CrandFC2B	0.08492	0.08934	0.09080	0.08335	0.08336	0.02080	0.02961	0.03407
12	CrandBP1	0.08342	0.08784	0.08930	0.08185	0.08186	0.01933	0.02816	0.03407
13	CrandBP2	0.09398	0.09543	0.09988	0.09242	0.09244	0.02980	0.03848	0.04294
14	CrandBP3	0.08197	0.08936	0.08786	0.08042	0.08042	0.02384	0.03129	0.03717
15	Grandif1	0.08499	0.08939	0.09086	0.08342	0.08342	0.02238	0.02977	0.03572
16	Grandif2	0.08779	0.09220	0.09366	0.08623	0.08623	0.02529	0.03119	0.03858
17	Grandif3	0.08785	0.09225	0.09373	0.08629	0.08629	0.02232	0.02974	0.03565
18	Grandif4	0.08631	0.09072	0.09218	0.08475	0.08475	0.02381	0.03119	0.03709
19	LatabrAg1	0.04603	0.04007	0.04599	0.04599	0.04599	0.09991	0.10987	0.11584
20	LatabrAg1B	0.04455	0.03264	0.04747	0.04450	0.04450	0.10586	0.11282	0.11731
21	LatabrAg2	0.04308	0.03711	0.04897	0.04600	0.04600	0.10290	0.10692	0.11287
22	LatabrAg2B	0.05197	0.04303	0.05786	0.05192	0.05192	0.10585	0.10984	0.11583
23	LongisRR1	0.08793	0.09086	0.09381	0.08636	0.08637	0.02380	0.03256	0.03554
24	LongisRR2	0.08642	0.09083	0.09230	0.08485	0.08486	0.02230	0.03109	0.03556
25	LongisRR3	0.08193	0.08636	0.08782	0.08037	0.08038	0.01785	0.02672	0.03115
26	LongisS1	0.08193	0.08636	0.08782	0.08037	0.08038	0.01785	0.02672	0.03115
27	LongisS2	0.08494	0.08936	0.09082	0.08337	0.08338	0.02080	0.02961	0.03406
28	LongisS3	0.08493	0.08934	0.09080	0.08335	0.08336	0.02080	0.02962	0.03407
29	RemotaVA1	0.10136	0.10575	0.10575	0.09979	0.09979	0.01495	0.01482	0.02079
30	RemotaVA2	0.10274	0.10714	0.10713	0.10117	0.10117	0.01492	0.02075	0.02370

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31 RemotaVA3   0.10300  0.10741  0.10738  0.10141  0.10141  0.01794  0.01778  0.02378
32 RemotaIL2   0.09677  0.10117  0.10116  0.09521  0.09521  0.00893  0.01482  0.02074
33 RemotaIL3   0.09825  0.10265  0.10264  0.09669  0.09669  0.01042  0.01631  0.02222
34 RivularCL1  0.09845  0.10283  0.10430  0.09686  0.09686  0.03583  0.04313  0.04908
35 RivularCL2  0.08185  0.08923  0.08773  0.08030  0.08030  0.02529  0.03266  0.03858
36 RivularCL3  0.09247  0.09686  0.09833  0.09089  0.09089  0.03283  0.04015  0.04610
37 RivularUT1  0.08789  0.09230  0.09377  0.08632  0.08633  0.02377  0.03258  0.03704
38 RivularUT2  0.08795  0.09237  0.09384  0.08639  0.08640  0.02231  0.03112  0.03556
39 RivularUT3  0.08640  0.09083  0.09231  0.08485  0.08337  0.02528  0.03415  0.03859
40 Sphaera2    0.07013  0.07006  0.07602  0.07008  0.07008  0.09907  0.10778  0.11375
41 Sphaera3    0.07144  0.07136  0.07729  0.07136  0.07136  0.10329  0.11194  0.11788


                     9       10       11       12       13       14       15       16
 9 CandFC4           -
10 CrandFC1B   0.01483        -
11 CrandFC2B   0.01335  0.00742        -
12 CrandBP1    0.01186  0.00593  0.00593        -
13 CrandBP2    0.02080  0.01633  0.01183  0.01481        -
14 CrandBP3    0.01635  0.01042  0.00894  0.00744  0.01793        -
15 Grandif1    0.01190  0.00891  0.00742  0.00593  0.01488  0.01041        -
16 Grandif2    0.01187  0.01187  0.01038  0.00890  0.01783  0.01337  0.00595        -
17 Grandif3    0.01485  0.01189  0.01038  0.00891  0.01930  0.01339  0.00595  0.00743
18 Grandif4    0.01039  0.01038  0.00889  0.00741  0.01634  0.01189  0.00447  0.00148
19 LatabrAg1   0.10111  0.09677  0.09514  0.09504  0.10418  0.09532  0.09532  0.09813
20 LatabrAg1B  0.10108  0.09672  0.09511  0.09503  0.10413  0.09527  0.09533  0.09810
21 LatabrAg2   0.09517  0.09082  0.08919  0.08910  0.09822  0.08938  0.08937  0.09219
22 LatabrAg2B  0.10406  0.09975  0.09812  0.09800  0.10718  0.09826  0.09829  0.10108
23 LongisRR1   0.01336  0.01040  0.00888  0.00888  0.01775  0.01195  0.01039  0.01336
24 LongisRR2   0.01484  0.00890  0.00740  0.00741  0.01627  0.01043  0.00892  0.01187
25 LongisRR3   0.01038  0.00444  0.00296  0.00148  0.01188  0.00596  0.00444  0.00742
26 LongisS1    0.01038  0.00444  0.00296  0.00148  0.01188  0.00596  0.00444  0.00742
27 LongisS2    0.01335  0.00743  0.00592  0.00592  0.01479  0.00895  0.00742  0.01038
28 LongisS3    0.01335  0.00742  0.00592  0.00593  0.01479  0.00894  0.00742  0.01038
29 RemotaVA1   0.03571  0.02980  0.02821  0.02823  0.03709  0.02837  0.02984  0.03273
30 RemotaVA2   0.03564  0.02972  0.02668  0.02815  0.03557  0.02830  0.02978  0.03267
31 RemotaVA3   0.03872  0.03277  0.03118  0.03120  0.04007  0.03135  0.03285  0.03574
32 RemotaIL2   0.02671  0.02079  0.01927  0.01926  0.02815  0.02232  0.02085  0.02374
33 RemotaIL3   0.02820  0.01930  0.02075  0.01926  0.02963  0.02380  0.02233  0.02522
34 RivularCL1  0.02826  0.02231  0.02081  0.01933  0.02976  0.02387  0.02234  0.02529
35 RivularCL2  0.01484  0.01187  0.01038  0.00890  0.01783  0.01337  0.00595  0.00890
36 RivularCL3  0.02528  0.01933  0.01782  0.01635  0.02677  0.02087  0.01936  0.02232
37 RivularUT1  0.01632  0.01037  0.00296  0.00889  0.01478  0.01041  0.01040  0.01335
38 RivularUT2  0.01633  0.01041  0.00888  0.00888  0.01776  0.01194  0.01038  0.01335
39 RivularUT3  0.01780  0.01187  0.01036  0.00893  0.01931  0.01041  0.01187  0.01483
40 Sphaera2    0.08974  0.08681  0.08539  0.08382  0.09447  0.08384  0.08542  0.08826
41 Sphaera3    0.09395  0.09103  0.08960  0.08805  0.09867  0.08659  0.08965  0.09246


                    17       18       19       20       21       22       23       24
17 Grandif3          -
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 18 Grandif4    0.00594        -
 19 LatabrAg1   0.09820  0.09665        -
 20 LatabrAg1B  0.09818  0.09662  0.02222        -
 21 LatabrAg2   0.09226  0.09071  0.02074  0.02222        -
 22 LatabrAg2B  0.09819  0.09961  0.03556  0.03704  0.03556        -
 23 LongisRR1   0.01337  0.01187  0.09663  0.09660  0.09068  0.09962        -
 24 LongisRR2   0.01188  0.01038  0.09663  0.09660  0.09068  0.09962  0.01036        -
 25 LongisRR3   0.00742  0.00593  0.09230  0.09226  0.08636  0.09528  0.00595  0.00444
 26 LongisS1    0.00742  0.00593  0.09230  0.09226  0.08636  0.09528  0.00595  0.00444
 27 LongisS2    0.01039  0.00889  0.09515  0.09512  0.08920  0.09813  0.00592  0.00740
 28 LongisS3    0.01039  0.00889  0.09514  0.09511  0.08919  0.09812  0.00888  0.00740
 29 RemotaVA1   0.02979  0.03125  0.10555  0.10854  0.10260  0.10555  0.03116  0.02822
 30 RemotaVA2   0.02971  0.03119  0.10992  0.11139  0.10398  0.10991  0.03111  0.02965
 31 RemotaVA3   0.03278  0.03426  0.10723  0.11017  0.10425  0.10722  0.03412  0.03120
 32 RemotaIL2   0.02079  0.02226  0.10695  0.10693  0.10101  0.10694  0.02222  0.02075
 33 RemotaIL3   0.02227  0.02374  0.10843  0.10841  0.10249  0.10842  0.02370  0.02223
 34 RivularCL1  0.02531  0.02381  0.10726  0.10727  0.09684  0.11023  0.02378  0.02231
 35 RivularCL2  0.00891  0.00742  0.09517  0.09514  0.08923  0.09813  0.01336  0.01187
 36 RivularCL3  0.02232  0.02083  0.10278  0.10279  0.09683  0.10575  0.02079  0.01932
 37 RivularUT1  0.01336  0.01186  0.09810  0.09807  0.09215  0.10108  0.01183  0.01036
 38 RivularUT2  0.01188  0.01186  0.09816  0.09814  0.09221  0.10115  0.01184  0.01036
 39 RivularUT3  0.01484  0.01334  0.09679  0.09675  0.09084  0.09977  0.01336  0.01185
 40 Sphaera2    0.08833  0.08678  0.07160  0.07309  0.06864  0.07754  0.08838  0.08691
 41 Sphaera3    0.09254  0.09098  0.07288  0.07142  0.06992  0.07883  0.09260  0.09111

                     25       26       27       28       29       30       31       32
 25 LongisRR3         -
 26 LongisS1    0.00000        -
 27 LongisS2    0.00297  0.00297        -
 28 LongisS3    0.00296  0.00296  0.00592        -
 29 RemotaVA1   0.02531  0.02531  0.02821  0.02822        -
 30 RemotaVA2   0.02526  0.02526  0.02815  0.02816  0.01779        -
 31 RemotaVA3   0.02829  0.02829  0.03116  0.03119  0.00296  0.02077        -
 32 RemotaIL2   0.01633  0.01633  0.01926  0.01927  0.01485  0.01481  0.01782        -
 33 RemotaIL3   0.01781  0.01781  0.02074  0.02075  0.01634  0.01630  0.01931  0.00741
 34 RivularCL1  0.01785  0.01785  0.02080  0.02081  0.04320  0.04315  0.04619  0.03422
 35 RivularCL2  0.00742  0.00742  0.01038  0.01038  0.03273  0.03267  0.03574  0.02374
 36 RivularCL3  0.01487  0.01487  0.01781  0.01782  0.04021  0.04017  0.04173  0.03124
 37 RivularUT1  0.00592  0.00592  0.00888  0.00888  0.03120  0.02965  0.03417  0.02223
 38 RivularUT2  0.00594  0.00594  0.00888  0.00888  0.02970  0.02963  0.03266  0.02074
 39 RivularUT3  0.00741  0.00741  0.01038  0.01037  0.03273  0.03267  0.03572  0.02374
 40 Sphaera2    0.08234  0.08234  0.08391  0.08539  0.10494  0.10627  0.10658  0.09735
 41 Sphaera3    0.08657  0.08657  0.08813  0.08961  0.10765  0.10896  0.10926  0.10151


                     33       34       35       36       37       38       39       40
 33 RemotaIL3         -
 34 RivularCL1  0.03570        -
 35 RivularCL2  0.02522  0.02380        -
 36 RivularCL3  0.03272  0.01040  0.01934        -
 37 RivularUT1  0.02371  0.02378  0.01335  0.02080        -
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38 RivularUT2  0.02222  0.02379  0.01335  0.02079  0.01184        -
39 RivularUT3  0.02522  0.02386  0.01483  0.02086  0.01333  0.01335        -
40 Sphaera2    0.10032  0.09737  0.08528  0.09140  0.08837  0.08844  0.08523        -
41 Sphaera3    0.10448  0.10159  0.08949  0.09711  0.09258  0.09266  0.08948  0.01338


                    41
41 Sphaera3          -
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Appendix C

Taxonomy of *Iliamna*

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

Stems erect, pubescence stellate, 0.6 m to 2 m, emerging from woody rootstalk. Leaves palmately 3- to 7-lobed, sparse to dense pubescence, cuneate, truncate, or cordate at the 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. Eight species in North America.

1 Leaves shallowly 3-lobed; base tapered to truncate; petioles stout, 1-6 cm; petals 3 cm or larger.
I. bakeri
1 Leaves 5- to 7-lobed; cordate to truncate at base (2)
2 Sepals ovate; seeds puberulent(3)
3 Sepals as wide as long, or nearly so; plants sparingly branched (4)
4 Bracts linear, half as long as sepals.
I. rivularis
4 Bracts lanceolate to elliptic, 2/3 as long or longer than sepals (5)
5 Lobes of leaves broadest at base; seeds sparsely puberulent.
I. grandiflora
5 Lobes of leaves broadest at middle; leaf sinuses acute; leaves pale beneath.
I. latibracteata
3 Sepals longer than wide; plants profusely branched (6)
6 Leaf sinuses shallow; leaf teeth rounded to mucronate.
I. remota
6 Leaf sinuses deep; leaf teeth cupsidate.
I. corei
2 Sepals narrowly lanceolate; seeds glabrous (7)
7 Petals white to pale pink; sepals quarter length of petals.

I. crandallii

7 Petals deep rose-purple; petioles 1/2 length of leaf blades; bracts longer than sepals.

I. longisepala

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

Sphaeralcea bakeri Jepson. *A Manual of the Flowering Plants of California* p. 635, 1925.

Stems puberulent, 0.3-0.7 m. 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. Leaf margin mucronate to crenate. Inflorescence of axilary clusters of flowers. Calyx 9-12 mm long, 1-2 mm wide, puberulent. Pedicels 5 mm. Bracts 5-8 mm. 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.

Iliamna remota var. corei Sherff. *Rhodora* 48: 96, 1946.

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

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

Sphaeralcea crandallii Rydberg. *Bulletin of the Torrey Botanical Club* 31: 564, 1904.

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

Stems sparingly stellate-pubescent, 0.5 m. Leaves deeply 5- to 7-lobed, 2-5 cm long, 5-10 cm wide, sparingly stellate. Leaf base cordate. Petioles 1-3 cm. Leaf margin coarsely serrated. Inflorescence a raceme. Calyx 10 mm long, 5 mm wide, sparingly to finely puberulent. Pedicels up to 1-3 cm. 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 grandiflora (Rydberg) Wiggins. *Contributions from the Dudley Herbarium* 1: 223, 1936.

Sphaeralcea grandiflora Rydberg. *Bulletin of the Torrey Botanical Club* 31: 564, 1904.

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.

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

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

Stems sparingly stellate pubescent, 1-2 m. 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. 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.

Sphaeralcea longisepala Torrey in Wilkes. *United States Exploring Expedition* 17: 255, 1874.

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

Stems sparingly stellate-pubescent, 1.2-2 m. 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 leaves. Leaf margin coarsely serrated. Inflorescence of few-flowered axilary clusters. Calyx 15-20 mm long, <5 mm wide, sparsely pubescent. Pedicels 2.5 cm. Bracts 8-10 mm long. Flowers pink to lavendar, 2-4 cm in diameter. Seeds 3 per carpel, pubescence unknown. Streambanks and mountain slopes in Chelan, Douglas, and Kittitas Co., WA.

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

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. Leaves shallowly 5- to 7-lobed, 15-20 cm long and about as wide, pubescence dense. Leaf base cordate. Petioles 5-10 cm. Leaf margin mucronate to rounded. Inflorescence of axilary clusters. Calyx 10-15 mm long, 5 mm wide, hirsute. Pedicels 1-3 cm. 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 along 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.

Malva rivularis Douglas ex Hooker. *Flora boreali-americana* 2: 107. 1838.

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. 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. Leaf margin coarsely serrated. Inflorescence racemose to paniculate. Calyx 5-8 mm long, 3-5 mm wide, hirsute. Pedicels 1.5-4 cm. 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 SD, west to WA and south to CO.

Appendix D

Vita

Tracey A. Bodo Slotta

Biology Department, Virginia Tech, Blacksburg, VA 24061

Education

1997-Present	Virginia Tech, Blacksburg, VA. Master of Science in Biology (June 2000 expected).
1995-1990	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.

Honors

April 2000	Awarded Travel Fund Program Grant, Graduate Student Assembly, Virginia Tech
January 2000	Awarded Graduate Research Development Program Grant, Graduate Student Assembly, Virginia Tech
May 1999	Awarded Virginia Academy of Science Small Research Project Grant
January 1998	Awarded Graduate Research Development Program Grant, Graduate Student Assembly, Virginia Tech

Affiliations

2000	Member of the American Society of Plant Taxonomists
1999-Present	Member of Sigma Xi, American Institute of Biological Sciences, and Phi Sigma
1998-Present	Member of the Botanical Society of America and the Society of Systematic

	Biologists
1997-Present	Member of Virginia Academy of Science

Professional Experience

2000	Vice-President of Programs, Graduate Student Assembly, Virginia Tech
1999-2000	Chair, Graduate Research Development Program Grant, Graduate Student Assembly, Virginia Tech
1998-1999	Delegate for the Biology Department to the Graduate Student Assembly, Virginia Tech
1997-Present	Graduate Teaching Assistant, Biology Department, Virginia Tech. Taught introductory biology labs during fall semesters and Plant Taxonomy labs during spring semesters.
Summer 1996	Undergraduate Assistant, Biology Department, Bowling Green State University. Collected seeds for Lupinus perennis from an oak savanna habitat, planted lupine seedlings in a reintroduction project.
1993-1995	Lab Assistant. Biology Department, University of Dayton. Organized laboratory supplies. Collected, prepared and analyzed roots for mycorrhizal fungi project in a wetland prairie.

Presentations

August 2000	Seminar to be presented at the Botanical Society of America, Portland, OR
May 2000	Seminar to be presented at the Virginia Academy of Science, Radford, VA
March 2000	Poster presented at the American Institute of Biological Sciences, Washington, D.C.
March 2000	Poster presented at the Graduate Research Symposium, Virginia Tech