FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTOCTORALES

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FACULTY OF GRADUATE AND POSDOCTORAL STUDIES

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GRADE / DEGREE

Faculty of Science – Department of Biology
FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Taxonomic Status of the Narrow Endemic *Astragalus Robbinsii* var. *Fernaldii* (Fernald's Milkvetch – Fabaceae): Molecules, Morphology, and Implication for Conservation
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Taxonomic status of the narrow endemic *Astragalus robbinsii* var. *fernaldii* (Fernald's Milkvetch - Fabaceae): molecules, morphology, and implications for conservation

by

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Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
University of Ottawa
In partial fulfilment of the requirements for the
degree of Master of Science in the

Ottawa Carleton Institute of Biology

Thèse soumise à
La Faculté des études supérieures et postdoctorales
Université d'Ottawa
En vue de l'obtnetion de la maîtrise ès science à

L'Institut de Biologie d'Ottawa-Carleton

Library and Archives Canada
Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
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Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-73750-7
Our file Notre référence
ISBN: 978-0-494-73750-7

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Abstract

Astragalus robbinsii var. *fernaldii* (Fernald's Milkvetch) is a federally listed taxon of "special concern" due for re-assessment by COSEWIC. Restricted to the Strait of Belle Isle region of Atlantic Canada, its taxonomy in respect to two co-occurring congeners, *Astragalus eucosmus* and *Astragalus robbinsii* var. *minor*, has been questioned due to the variable states of the varieties' diagnostic characters. To clarify phylogenetic relationships within this species complex we studied variation in chloroplast DNA, AFLPs and morphology.

Chloroplast DNA sequence data distinguished *A. r.* var. *minor* from *A. eucosmus* and most *A. r.* var. *fernaldii*. Three *A. r.* var. *fernaldii* populations possessed the *A. r.* var. *minor* haplotype, and AFLPs revealed no population structure, indicative of gene flow between taxa and populations. Morphometric analysis indicates that *A. r.* var. *fernaldii* is closer to *A. eucosmus* than *A. r.* var. *minor*. Based on these results taxonomic recognition of *A. r.* var. *fernaldii* is unwarranted, and we recommend that the taxon be merged into *A. eucosmus*.

Resumé

Astragalus robbinsii var. *fernaldii* (Fernald's Milkvetch), taxon «preoccupant» selon le gouvernement fédéral, doit être réévalué selon COSEPAC. Étant limité au détroit de Belle Isle, la variabilité dans les caractères diagnostiques met en doute sa taxonomie par rapport aux deux taxons sympatriques, *Astragalus eucosmus* and *Astragalus robbinsii* var. *minor*, du même genre. Afin de préciser la phylogénie de ce complexe taxonomique on a analysé l'ADN du choloroplaste, les PTFR et la morphologie.

Les séquences d'ADN ont distingué entre *A. r.* var. *minor* et les deux autres_taxons. Trois populations du premier ont la même haplotype que A. *eucosmus*, et l'analyse PTFR n'a démontré aucune structure génétique dans la population, signifiant le flux des gènes entre les taxons et les populations. L'analyse morphométrique a indiqué que *A. r.* var. *fernaldii* s'approche morphologiquement plus à A. *eucosmus* qu'à *A. r.* var. *minor.* D'après ces résultats, on recommande le transfère de *A. r.* var. *fernaldii* à *A. eucosmus.*

Statement of Contributions

The author thanks and acknowledges Claudia Hanel for collection assistance, and Jessica LeClerc-Blain and Rachel Vallender for their contribution to the sequencing effort outlined in chapter two. Thorough comments from Lynn Gillespie, John Maunder and David Carpenter have greatly improved early drafts of this work. The author would also like to thank Wayne Sawtell for translation of the abstract.

Acknowledgments

This thesis would not have been possible without the kindness, patience, and hard work of many people over the last two years, and the following have truly made this project an enjoyable experience and are addressed in no particular order.

Firstly, I would like to thank my supervisor, Lynn Gillespie, for all her help, encouragement, patience, and time, it has been much appreciated. From comments on this manuscript to guidance on all aspects of botany, she has been a fantastic mentor. This project has been incredibly enjoyable; I would like to thank her for the opportunity to work on it. Thanks also go to the members of my committee: Anne Bruneau, Naomi Cappuccino and Julian Starr have all provided advice, perspective, and much insight into the "*fernaldii* problem", many thanks to them!

Secondly, I would like to thank John Maunder; this whole project was his damn fault! It was his keen observation of the variability in Fernald's Milkvetch that started him questioning the taxonomic status of the variety, and paved the way for this thesis. Furthermore, John and his wife Susan made for excellent travelling companions in Blanc Sablon.

Thanks go to Claudia Hanel for carting me around the entire province of Newfoundland and Labrador. Both an expert botanist and sled-dog musher, without her I would have no samples, no field season, and no clothes! (It's a long story...).

Nathalie Djan-Chekar and C.C. Chinappa are the curators of the herbaria at the Provincial Museum of Newfoundland (NFM) and Labrador and the University of Calgary

(UAC) respectively, and welcomed me into their collections with open arms. Thanks to both of them for allowing me to poke around their cabinets.

Alex MacPhail, Veronic Pruneau, Daniela Gamboratta, Carmen Fletcher, Jeff Graham, Tasfia Ahmed Iqbal, Wayne Sawtell, Tamara Villaverde, Michel Paradis, Mitch Sprague and the rest of my labmates have kept me from going insane in the (windowless) DNA Lab. Without these folks I'm sure I could have gone weeks without seeing sunlight, you all make excellent coffee-break companions. I would like to thank Anna Ginter especially: for making sure that I ate, for helping me with sequencing and genotyping, and for giving me a sympathetic ear when I began shouting obscenities at the computer.

Thanks go to Jennifer Doubt and Micheline Beaulieu-Bouchard for the use of their excellent facilities at the National Herbarium of Canada (CAN), their incredible specimen-sorting speed, and the occasional drive home. Rosita Scherson and Martin Wojciechowski have both assisted with outgroup selection and PCR suggestions, and Janice's yoga classes mean I haven't packed on the thesis pounds.

My sampling trip to New Brunswick would not have been possible without the help of Don McAlpine, Stephen Clayden and Katelyn Vandenbroeck. Thanks to them for transportation, accommodation, advice, and the most enjoyable job I've never been paid for!

Jeff Saarela and Laurie Consaul have both provided insight into taxonomy, help with my molecular and morphological work, and thoughtful comments on my talks and papers, thanks to them both! I would like to thank Jessica LeClerc-Blain and Rachel Vallender for their assistance with sequencing, and their advice to a PCR novice.

Brianna Chouinard has been a good friend throughout my masters, and has helped me in more ways than I can count: running plates, driving me home, troubleshooting reactions, suggesting good books, and the list goes on! Brianna, thanks for everything!

I am extremely grateful to Roger Bull for teaching me just about everything I needed to know about DNA, for all his advice on surviving grad school, for having excellent musical taste, for rides home when I was stranded, for keeping us organized. Roger has been instrumental in the success of this thesis, and has cultivated one of the best working environments I've had the pleasure of being in, thanks!

Finally, I would like to thank David Carpenter for all his love, for patience when I was talking taxonomic gibberish, for making sure I ate and got out every once and a while, and for editing my thesis! All my love to you, sweetheart.

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Chapter 1: Introduction

The *fernaldii* problem

Astragalus robbinsii (Oakes) A. Gray var. *fernaldii* (Rydb.) Barneby, Fernald's Milkvetch, is a federally listed taxon of "special concern" endemic to the Strait of Belle Isle region of Newfoundland and Labrador and Québec. Provincially, this taxon is classified as "vulnerable" in Newfoundland and Labrador, and "menacée" in Québec (Morrisset, 1996; Hanel and Keeping, 2006). Currently due for re-assessment by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), this variety of Robbins' Milkvetch (*Astragalus robbinsii*) co-occurs with two congeneric taxa: *Astragalus eucosmus* B.L. Rob. (Elegant Milkvetch) and *Astragalus robbinsii* (Oakes) A. Gray var. *minor* (Hook.) Barneby (Robbins' Milkvetch). All three taxa are very closely related (Wojciechowski et al., 1993) and are thought to comprise a species complex, though the extent of reproductive isolation between the taxa has not been established (Maunder, 2006).

Morphologically, all three members of the *fernaldii-eucosmus-minor* species complex are outwardly similar. Fernald's milkvetch is differentiated from *A. eucosmus* and *A. r.* var. *minor* by three diagnostic characters: a stipe of intermediate length, the presence of white hairy fruit and calyces (Figs. 1-6), and white pubescence on the adaxial surface of the leaflets (Fig. 7) (Rydberg, 1928). However, these distinguishing traits are quite variable among the different populations presently deemed to belong to *Astragalus robbinsii* var. *fernaldii*, and are occasionally found in individuals of *A. eucosmus* and *A. robbinsii* var. *minor* (Maunder, 2006). For example, both calyx and pod pubescence appear to be correlated with the age of the individual plant; white pubescence has been observed on the immature pods of all three taxa, and has been observed to be gradually lost with age, leaving only sparse black pubescence by the time the pods begin to dehisce (Maunder, 2006).

Analysis of stipe lengths has also proven difficult due to a wide range of sampling dates on voucher specimens, representing stipes in varying degrees of development.

Due to the variability seen in the diagnostic characters, Maunder (2006) questioned the taxonomic status, distribution, and very existence of Fernald's Milkvetch. Here we endeavor to resolve the taxonomic identity and position of *A. r.* var. *fernaldii* using both morphological and molecular techniques.

Aside from the desire to confirm the status of Fernald's Milkvetch towards a greater understanding of the Canadian Flora, this taxonomic uncertainty is especially problematic as this entity is both a provincially and federally listed conservation target. There are both ethical and legal obligations to preserve endangered wildlife in Canada, but effective conservation requires a thorough taxonomic understanding of the target group to delineate populations of conservation concern. If Fernald's Milkvetch cannot be clearly separated from *Astragalus robbinsii* var. *minor* and *Astragalus eucosmus*, conservation managers will not be able to adequately identify and protect this narrowly endemic plant.

Natural history and biology of the *eucosmus-fernaldii-minor* complex

Astragalus L. is an extremely large genus that has radiated throughout the temperate regions of North and South America and Eurasia (Kazempour Osaloo et al., 2003).

Fig. 1: Inflorescence of *Astragalus robbinsii* var. *fernaldii*, pale calyx hairs evident. Photograph taken by Paul Sokoloff at Mont Bonenfant population, QC

Fig. 2: Inflorescence of *Astragalus robbinsii* var. *minor*, dark calyx hairs and pale corolla evident. Photograph taken by Paul Sokoloff at Pointe Amour population, NL.

Fig. 3: Inflorescence of *Astragalus eucosmus,* featuring mixed white and dark calyx hairs (paler than usual) and elongate raceme. Photograph taken by Paul Sokoloff at Indian River population, NL.

Fig. 4: Immature pods of *Astragalus robbinsii* var. *fernaldii*, pale calyx hairs and pale pod hairs evident. Photograph taken by Paul Sokoloff at Mont Bonenfant population.

Fig. 5: Pods of *Astragalus robbinsii* var. *minor*, mixed black and white pod hairs and elongate pod visible. Photograph taken by Paul Sokoloff at Battery Trail population.

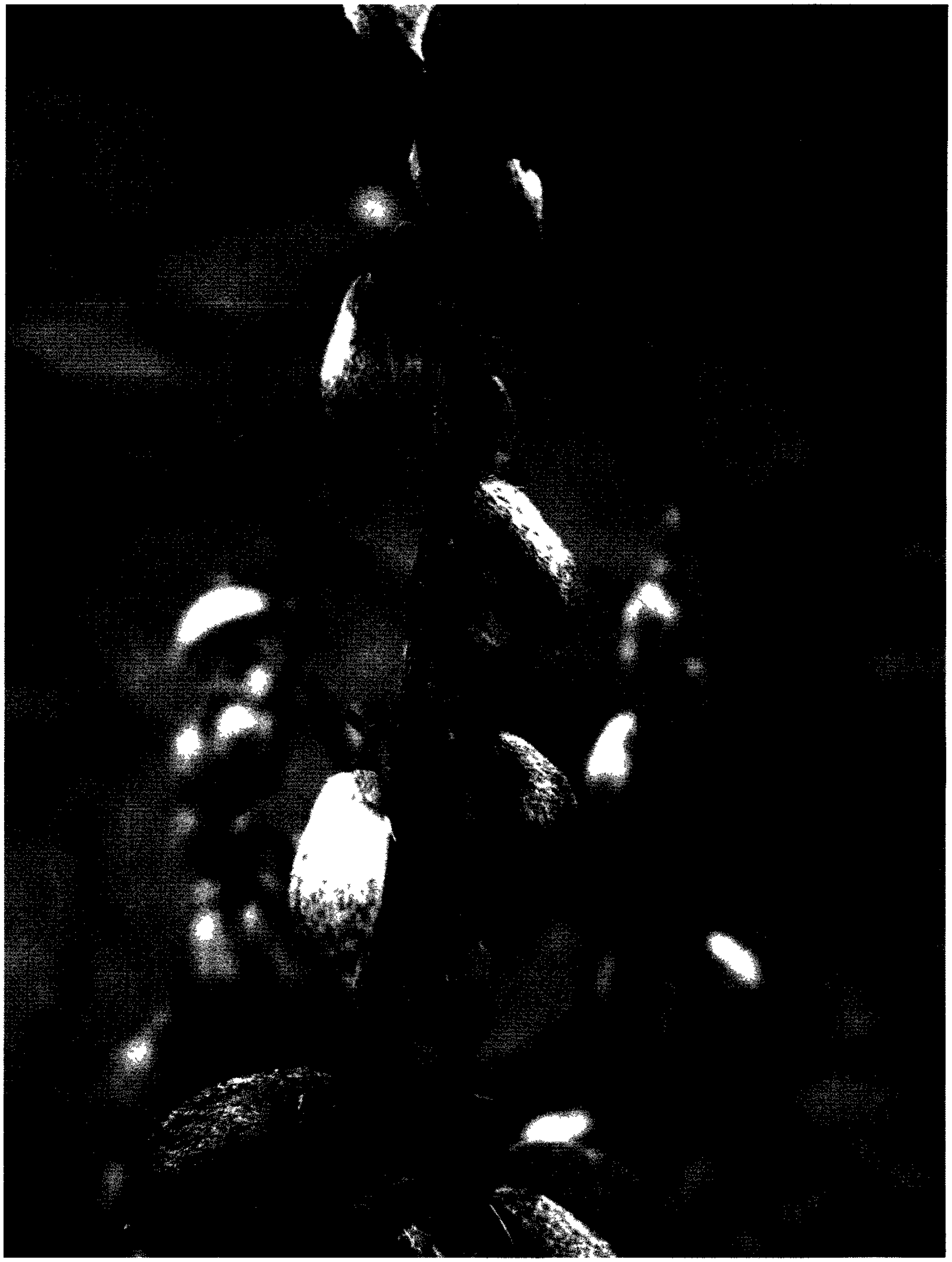

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Fig. 7: Comparison of adaxial leaf states in the *eucosmus-fernaldii-minor* species complex. Top panel shows dense white pubescence common *in A. r.* var. *fernaldii*, while bottom panel shows glabrous leaflets common in *A. eucosmus* and *A. r.* var. *minor*. Top photo taken by Paul Sokoloff at Mont Bonenfant. Bottom photo taken by Paul Sokoloff at Battery Trail, NL.

Nested within the legume subfamily Faboidae, an economically valuable and widespread herbaceous clade (Kass and Wink, 1997), *Astragalus* consists of over 2500 taxa which have diversified into a wide array of niche habitats, and they are generally regarded as highly adaptable habitat specialists (Barneby, 1964).

Members of this genus are split between two large clades that roughly correspond to their geographic distributions: the euploid Old World *Astragalus* of Eurasia, and the aneuploid New World taxa found in North and South America (Wojciechowski et al., 1999). These clades are generally not morphologically distinct from one another, but are separated on the basis of chromosome number and DNA phylogenies: species of aneuploid *Astragalus* form a monophyletic group nested within the euploid clade (Wojciechowski, 2005). Furthermore, they are not restricted to their namesake region, and a number of euploid *Astragalus* are found in North America, including both *A. eucosmus* and *A. robbinsii* (Barneby, 1964).

Astragalus robbinsii and *A. eucosmus* are the only North American representatives of sect. *Oroboidei* A. Gray., one of the rarer Old World sections (Spellenberg, 1974). Both are members of subgenus *Phaca* L. (their original generic designation), and are so morphologically similar that Barneby himself remarked that well formed fruits were a necessity for proper identification (Barneby, 1964) The only reliable diagnostic features separating the two taxa are stipe and pod length, as well as knowledge about the geographic distribution of each taxon. In *A. eucosmus,* the pods tend to be shorter (~10 mm), and the stipe extremely short, or missing altogether (0-0.5 mm), whereas the opposite is true in both cases for *A. robbinsii*, in particular var. *minor,* where pods range from 13-25 mm long, and the stipes average 1.5 mm (Rydberg, 1928).

Neither species is regarded as a weed, as having any commercial value, or as possessing any unique biological properties; hence little research has been done into the biology and ecology of either taxon, aside from the systematics and the conservation biology of two endangered varieties, Fernald's Milkvetch in Eastern Canada, and Jesup's Milkvetch in Vermont (Morisset, 1997; Brumback, 1989).

Astragalus eucosmus – Elegant Milkvetch

Originally described as *Phaca elegans* by Hooker in 1831, then synonymised with *Astragalus oroboides* var. *americanus* (a Eurasian species) by Gray in 1864, *Astragalus eucosmus* was named in its present form by Robinson in 1908 (Barneby, 1964; Robinson, 1908).

Astragalus eucosmus is a near circumpolar species, with a contiguous distribution across every Canadian province and territory and south through the Rocky Mountains into the western United States (Gray, 1864). It is morphologically similar to *Astragalus norvegicus* Weber, and may represent a descendant of this species through vicariance, differing primarily in the narrower leaflets (Jones, 1923; Love, 1954). This speciation event is thought to have stabilized before the last ice age, as seen in the relative morphological heterogeneity of this widespread species (Barneby, 1964)

This species possesses a tetraploid chromosome count of $2n = 32$, and unlike many other *Astragalus* it contains only trace amounts of organic nitrates, a secondary metabolite involved in herbivore defence (Ledingham and Fahselt, 1964, Williams and Parker, 1974). As with many other legumes, *Astragalus eucosmus* is an early colonizer of disturbed sites, and easily gains a foothold when natural or anthropogenic activities expose bare soil (Bishop and Chapin, 1989).

Astragalus eucosmus is an obligate calciphile, and inhabits a wide range of limestone-based habitats, from talus along inland riverbeds to limestone bluffs in coastal regions (Fernald, 1911). Though described by Barneby as "only moderately" variable throughout its range, the habit of *A. eucosmus* seems to be directly affected by both this variation in habitat type and latitude, encompassing a wide range of ecotypes (Barneby, 1964). Sheltered inland populations at lower latitudes possess relatively long, erect racemes and elongate leaves, while exposed northern populations, especially in coastal areas, display prostrate growth with shortened peduncles and leaves (Barneby, 1964).

In Newfoundland and Labrador, this phenotypic variation was used by Fernald (1926) and Rousseau (1933) to partition *A. eucosmus* into five infraspecific taxa: *A. eucosmus* var. *facinorum* Fern., a glabrous variety from Grand Falls-Windsor, *A. eucosmus* f. *albinus* Fern., a population with white inflorescences and calices from Ha-Ha Mountain, *A. eucosmus* var. *terrae-novae* Rouss. and *A. eucosmus* f. *villosus* Rouss., both with tomentose abaxial leaflet surfaces from the tip of Newfoundland's Great Northern Peninsula, and *A. eucosmus* f. *caespitosus* Rouss., a diminutive form from Bay of Island (Rousseau, 1933). Outside of Newfoundland and Labrador two additional forms exist: *A. eucosmus* f. *minor* Hook., a slight form from Baffin Island (Rousseau, 1933), and *A. eucosmus* f. *leucocarpus* Lepage, a white-fruited form from Alaska (Lepage, 1954). This variability, both within and between the various infraspecific taxa, suggests that *Astragalus eucosmus* possesses a greater degree of phenotypic plasticity than has been previously reported.

Astragalus robbinsii – Robbins' Milkvetch

Astragalus robbinsii is endemic to North America, and consists of five extant varieties; the type variety, *Astragalus robbinsii* var. *robbinsii* is believed to be extinct after the only known population was obliterated during the construction of a dam on the Winooski River in Vermont, USA (Pringle, 1897).

Four of the five remaining varieties of this species are spread throughout North America: *A. robbinsii* var. *alpiniformis* Rydb. in the Wallowa Mountains of Oregon, *A. robbinsii* var. *harringtonii* Rydb. along the southern Alaskan coast, *A. robbinsii* var. *occidentalis* Wats. in north-eastern Nevada, *A. robbinsii* var. *jesupii* Egglest. & Sheld. along the Connecticut River in Vermont, and *A. robbinsii* var. *fernaldii* Rydb. at Blanc Sablon, Québec (Barneby, 1964). These are all extremely narrowly endemic and represent highly derived forms adapted to restricted niche habitats; all but var. *harringtonii* are known from only one or two stations (USDA, 2009)

Astragalus robbinsii var. *minor* is the most prolific variety of Robbins' Milkvetch, and has a disjunct distribution in North America: the Rocky Mountains from Alaska to New Mexico, and Eastern Canada south through the Appalachian Mountains (Jones, 1923). Robbins' Milkvetch (var. *minor*) from these two regions differ in their stipe lengths. Eastern populations possess a stipe that ranges from 1 to 1.5 millimetres in length, whereas western representatives of this taxon have stipe lengths extending to 6.5 mm (Barneby, 1964).

Not only are the populations in these regions morphologically distinct, and potentially (as yet undescribed) distinct evolutionary entities, it is believed that the additional varieties of *Astragalus robbinsii,* i.e. var. *alpiniformis*, var. *harringtonii*, etc., are derived from *A. r.* var. *minor* through isolation and local selection pressures within these areas (Barneby, 1964).

Astragalus robbinsii var. *minor* – Robbins' Milkvetch

Astragalus robbinsii var. *minor* was originally described as *Phaca elegans* β *minor,* a variation of our modern day *A. eucosmus* by Hooker in 1831. Indeed the holotypes for *A. eucosmus* and *A. r.* var. *minor* are found on the same herbarium sheet (Jones, 1923).

The relatively wide distribution, and putative basal position of var. *minor,* in respect to its conspecific varieties, positions *A. r.* var. *minor* as the most important extant form of Robbins' Milkvetch in North America (Barneby, 1964).

Though originally thought to require calcareous soils like *A. eucosmus*, *A. r.* var. *minor* occupies a variety of limestone and granitic substrates throughout its range, and inhabits a wide range of habitat types, from shaded moist meadows to exposed cliff faces, highlighting the plasticity characteristic of *A. robbinsii* (Barneby, 1964). The habit of the variety is directly dependent on the ecology of the habitat; plants in sheltered regions at lower latitudes grow large and erect, while those in exposed northern populations are diffuse and prostrate, as with *A. eucosmus* (Barneby, 1964).

The ploidy level for *A. r.* var. *minor* has not been determined; however, ploidy for the now extinct *A. r. robbinsii* was $2n = 16$, determined through germination of stored seed (Ledingham, 1960), and *A. r. occidentalis* has been reported as $2n = 32$ from one count (Ledingham, 1960). Further work should ascertain the ploidy in *A. r.* var. *minor.*

Astragalus robbinsii var. *fernaldii* – Fernald's Milkvetch

Fernald's Milkvetch is the easternmost putative narrowly-endemic derivation of *Astragalus robbinsii* var. *minor*. Presently, it is known from only five adjacent sites in the Blanc Sablon area in Québec and adjacent Labrador: Cap Crow, Mont Parent, Mont Bonenfant, the Québec/Labrador border and Île-au-Bois (Hanel and Keeping, 2006). The range of this taxon was previously believed to extend down the Long Range Mountains in nearby Newfoundland (Maunder, 2006), and even as far away as New Brunswick (Hinds, 1986). However, over the past 50 years re-identification and redescription of these populations have left the taxon in its currently restricted state (Morriset, 1997; Maunder, 2006).

Like *A. eucosmus*, *A. r.* var. *fernaldii* is an obligate calciphile and a pioneer taxon, and seems to readily germinate in anthropogenically disturbed areas within the five known localities, with seedlings making up anywhere from 1 to 30% of each population (Morisset, 1997). However, it does not seem to utilize all potential habitat within the Blanc Sablon region; all five sites occur on exposed limestone bluffs within one kilometre of the ocean, and even as the same bluffs extend inland, the range of each population of Fernald's Milkvetch does not (Rousseau, 1933).

Personal observation by the author suggests that the intensity of both anthropogenic (ATV's, hiking, fire) and natural disturbance (ice scour, wind, etc.) decreases northwards from the shore, and subsequently, away from human settlement. This may not allow for the disruption and weathering of the heathy vegetation characteristic of the region that would allow new plants to further colonize the bluffs, though this hypothesis bears further testing (Muller, 1952). Moreover, seed dispersal within other members of the subgenus *Phaca* is limited to a few meters from the parent plant at best (there is no specialized dispersal mechanism) and *A. r.* var. *fernaldii* does not reproduce vegetatively (Kaye, 1999; Hanel and Keeping, 2006).

Fernald's Milkvetch is shade intolerant, further restricting its range to open lands, and appears to be a poor competitor in diverse climax communities (Morisset, 1997). It flowers throughout July and August, and generally sets seed in mid-August, lagging behind the conspecific taxa that are found in the milder parts of the province (Hanel and Keeping, 2006). This variety possesses a diploid chromosome count of 2n = 32, from a single count given verbally to Morriset for his 1997 report, the taxonomy of the voucher specimen was confirmed for this study. The lifespan of this perennial taxon is currently unknown, but was estimated by Morrisett (1997) to be in the order of decades.

Taxonomic History of Fernald's Milkvetch

Fernald's Milkvetch was first described by Per Axel Rydberg in 1928 from a type specimen currently held by the New York Botanical Garden (NY). Collected from Blanc Sablon, Québec in 1910 by M.L. Fernald himself, it was first classified as *Astragalus eucosmus* (Fernald, 1913). Rydberg felt that the presence of larger flowers, a longer,acute pod, white pubescence on the abaxial surface of the leaflets, and the development of a slightly longer yet still acutely angled stipe, merited the creation of a new species *Atelophragma fernaldii*, synonomous with our present day *A. r.* var. *fernaldii* (Rydberg, 1928). Rydberg also believed the colour of the calyx hairs to be important; indeed *A. r.* var. *fernaldii* often possesses a densely white pubescent calyx. However, this has been observed as variable between individuals within a population, and often changes with the age of the plant (Maunder, 2006). Rupert C. Barneby, in his atlas of North American *Astragalus*, felt that this character was, at best, fallible for the purposes of identification (Barneby, 1964).

Atelophragma fernaldii was transferred to the genus *Astragalus*, as *Astragalus fernaldii* by Lewis in 1932, who noted a perceived paler corolla than seen in its congeners. This was concurrent with the lumping of Rydberg's genera back to *Astragalus* (Rousseau, 1933). This was concurrent with the transfer of the remaining varieties of what is now

Robbins' Milkvetch to *Astragalus* as distinct species. These were not assembled as varieties of *Astragalus robbinsii* until Barneby's treatment in 1964.

Barneby's placement of var. *fernaldii* within *A. robbinsii* appears to be largely due to the length of the pod and the definite stipe. In *A. eucosmus*, he found pods to be short in comparison to *A. r.* var. *minor* (0.5 - 13 mm versus 8 - 25 mm), and the stipe in *A. eucosmus*, which is often less than a millimetre in length, was considered by Barneby to be vestigal (Barneby, 1964).

Barneby's classification of the *eucosmus-fernaldii-minor* species complex has become strained in recent years, primarily due to the high variability of these diagnostic characters within, and between, populations of all three taxa. Barneby's 1964 description of the variety allows for a large amount of variation within the defining characters (e.g., pod length of 10-15 mm, stipe length of 0.5-3 mm, leaf pubescence "above over the whole surface, or at least marginally"). It is still unknown whether this was simply due to the large amount of variation (plasticity) observed within the variety or incomplete sampling of the morphotypes. Consequently, many occurrences of *Astragalus* within Newfoundland, which may fit the typology of *A. eucosmus* or *A. r.* var. *minor,* have been chronically misidentified as *A. r.* var. *fernaldii,* if the collecting botanist referenced Barneby's work (Maunder, 2006).

Bernard Boivin of the National Herbarium of Canada re-examined the taxonomy of Fernald's Milkvetch for his Flora of the Prairie Provinces, possibly looking to correct the confusion inherent in the disagreement between Rydberg's and Barneby's classifications. Subsequently, he transferred var. *fernaldii* from *Astragalus robbinsii* to *Astragalus eucosmus*, as he felt that their range in the Strait of Belle Isle was essentially sympatric with that of *A. eucosmus* (Boivin, 1967). As such, many collections of Fernald's Milkvetch in Eastern Canadian herbaria bear the annotation *Astragalus eucosmus* var. *fernaldii* Boivin., and though this redescription has not been accepted by either the systematics or policy communities, many of these specimens have not been re-identified.

Compounding this taxonomic confusion has been the high number of misidentified *Astragalus* populations deemed to belong to var. *fernaldii*. In three separate instances, specimens from Newfoundland's Western shore, coastal New Brunswick and northern Ontario, all originally identified as var. *fernaldii*, were actually re-determined as *Astragalus alpinus,* a new-world *Astragalus* distantly related to the species complex (Morriset, 1997). Another example is the former population of *A. r.* var. *fernaldii* located at Cook's Harbour, near L'Anse aux Meadows in Newfoundland, that has been formally re-identified as a slightly hairy variation of *A. eucosmus*, known now as forma *villosus* as described above (Morriset, 1997). An additional collection of putative *A. r.* var. *fernaldii* from St. Paul's Inlet, Newfoundland, was re-identified as *A. r.* var. *minor* by Barneby (Maunder, 2006).

The most interesting, and in conservation terms, the most critical case of this sort of taxonomic mix-up, is a population of *Astragalus* at Barr'd Harbour Hill, on the Great Northern Peninsula of Newfoundland. This population, originally classified as *Astragalus blakei* (synonymous with *A. r.* var. *minor*), was reclassified by B. Boivin, as var. *fernaldii* in 1962. This was accepted until the rediscovery of the population in 2002, at which point John E. Maunder, then Curator of Natural History at the Provincial Museum of Newfoundland and Labrador, believed the newly rediscovered population more closely resembled *A. r.* var. *minor* than *A. r.* var. *fernaldii*. In this population, there is very little pubescence upon the leaflet tops, and the stipe lengths (1-3 mm) were found to be much longer than typical *A. r.* var. *fernaldii*. (Maunder, 2006). If this re-determination is supported, and the Barr'd Harbour Hill population is indeed *A. r.* var. *minor*, then the number of populations of var.

fernaldii present in Newfoundland and Labrador, and its geographic range in the province, would be drastically reduced, necessitating changes to current conservation priorities.

In itself, this potential misidentification of a population would just be a matter of an obscure taxonomic mix-up. The "taxon of special concern" designation of Fernald's Milkvetch makes the true identity of this population critical. During the variety's last reassessment by the government of Newfoundland and Labrador, the population at Barr'd Harbour Hill was included as *Astragalus robbinsii* var. *fernaldii*, the same classification Morriset gave to the population in his 1997 report to COSEWIC (Morriset, 1997; Hanel and Keeping, 2006). However, if this population is not Fernald's Milkvetch, then this taxon may not even occur in the province of Newfoundland and Labrador, as the exact location of the putative var. *fernaldii* population on the Québec/Labrador border is disputed (Maunder, 2006).

Each of the five remaining populations of Fernald's Milkvetch appears to vary in morphology: stipe and pod lengths at the Québec/Labrador border and Île-au-Bois populations are closer to *A. eucosmus* than *A. robbinsii*, as are the calyx and pod pubescence (Maunder, 2006). The population at Mont Parent seems intermediate between Île-au-Bois and "good" var. *fernaldii* (closest to the plants at the type locality at Cap Crow). At Mont Bonenfant and Cap Crow, where stipe lengths and pod pubescence are closest to the typical for the variety; there exists a heterogeneous mix of forms for all three diagnostic characters. Clearly, in reassessing the taxonomic status of *A. r.* var. *fernaldii*, each population must be carefully considered in context with the taxon as a whole.

Objectives

Clearly, the taxonomic history of Fernald's Milkvetch and the variability in its diagnostic characters makes the proper delineation of this variety difficult, more so considering that all previous systematic research has focused only on one or two characters (Maunder, 2006). Therefore, we performed a phylogenetic study on the *eucosmus-fernaldii-minor* complex to determine whether these taxa could be differentiated at the molecular level using chloroplast DNA sequences and amplified fragment length polymorphisms (AFLPs). As well, morphometric analysis of the species complex was used to document patterns of morphological variation in these taxa, and test the usefulness of the three diagnostic characters.

There are a several hypotheses that may account for the variation seen in *A. r.* var. *fernaldii* and the subsequent confusion surrounding the taxonomy of this narrowly endemic plant, and the three outlined below be tested in this study.

Astragalus robbinsii var. *fernaldii* may be a white-pubescent mutation of *A. eucosmus* restricted to calcareous soils in the Blanc Sablon region in Québec, as per Boivin's classification (Boivin, 1967). *Astragalus robbinsii* var. *fernaldii* was indeed thought by Rousseau (1993) and Barneby (1964) to be closer in appearance and morphology to *A. eucosmus* than *A. robbinsii*, and was theorized to have arisen from the former species by at least one author (Rousseau, 1933).

Astragalus robbinsii var. *fernaldii* could also be of hybrid origin, arising from a crossing between *A. eucosmus* and *A. r.* var. *minor*.

Finally, it is also possible that *A. r.* var. *fernaldii* is a variety that evolved from *A. r.* var. *minor* and morphologically and genetically distinct, and consistent with the current classification. In this case, a re-description of the variety would be necessary to eliminate the ambiguity present in Barneby's description.

Infraspecific taxa – species concepts and application

Effective conservation of Fernald's Milkvetch, if a valid entity of whatever lianeage described above, is clearly dependent upon the clear delineation of distinct populations of the variety. Unfortunately, the use of infraspecific taxa as a rank, and specifically, the distinction between varieties can be extremely subjective (Gianattasio and Spooner, 1994). While most governmental endangered species initiatives recognize infraspecific taxa as evolutionarily significant units worthy of protection, proper delineation of these ùnits requires two things: authors must make absolutely clear the species concept they are utilizing (Hamilton and Reichard, 1992), and the infraspecific taxon must be a biologically real entity, with a standardized criterion that sets it apart (McDade, 1995).

In their 2006 review on the inclusion of infraspecific taxa in the United States Endangered Species Act, Haig et al. recommend two important principles for including these taxa as valid entities: a) that some minimal form of the biological species concept is used, and b) there must be a geographic component to the separation of infraspecific taxa. The first criteria may be problematic to adopt in this case – Haig et al. (2006) focused on all taxa in their review, and reproductive isolation breaks down much more readily in plants than animals (Ornduff, 1968). However, the geographic range of Fernald's Milkvetch must be taken into account when deciding its taxonomic status, as it is sympatric with *A. robbinsii*, and nearly sympatric with *A. eucosmus* (Boivin, 1967). Under COSEWIC guidelines, geographic and genetic distinctiveness are essential criteria for listing entities below the species level (COSEWIC, 2007).

For this study, we will consider both morphological and genetic differences in assigning the taxonomic position of Fernald's Milkvetch. Specifically, we will adapt the system used by Pelser and Houchin (2004). In their analysis, morphological differences stood in for genetic differences in two species of *Senecio*. If in a multivariate analysis of the morphology the taxa were separated into two non-overlapping clusters, then it would be assumed that reproductive isolation was sufficient to maintain morphological distinctions and the taxa would be considered as distinct species. However, if the taxa overlapped significantly in their morphology and their range was sympatric, then they would be considered conspecific at the varietal level (Pelser and Houchin, 2004). For this study, I will consider both the morphology and molecular data in a similar manner; partial overlap is likely indicative of a close taxonomic association, and complete overlap may imply the taxa are not truly separate entities.

Chapter 2: Materials and Methods

Collection

Expeditions were made to Newfoundland and Labrador and Québec in July 2008 and to New Brunswick in June 2009 to collect *Astragalus eucosmus*, *A. robbinsii* var. *minor* and *A. robbinsii* var. *fernaldii* throughout their range in the Atlantic Provinces. Leaf material was collected in silica gel from 10-20 plants per population (see Appendix 1) to preserve DNA for later extraction (Chase and Hills, 1991). Together with samples collected for the pilot study, and specimens provided by participating botanists in Newfoundland, nearly 500 genetics samples have been collected from across the Québec-Newfoundland range of these species (Appendix 2). Herbarium specimens from Harvard University (HUH), the New York Botanical Gardens (NYBG), the University of British Columbia (UBC), Université Laval (QFA) and the University of Calgary (UAC) (see Appendix 3) were sampled for morphology and DNA to extend our sampling range across the continent (see Appendix 4 for geographic distribution of all plants sampled).

For each genetic sample collected from a plant in the field by the author, a macroscopic photograph, including the flowers, leaves, and fruit (if present), was taken, so that specific morphological characters could later be tied back to any genetic variation found between the populations sampled. Where populations of each taxon in the species complex were morphologically homogenous, one or two voucher specimens were also collected. However, if populations were morphologically heterogeneous, sufficient specimens were collected to represent the diversity of forms present at each site. As Fernald's Milkvetch is designated as a taxon "special concern", only a single branch was collected from each plant, as specified in the appropriate permits, so as not to remove an entire individual from a population. These voucher specimens have been deposited at the National Herbarium of Canada (CAN) at the Canadian Museum of Nature in Gatineau, QC.

DNA marker screening

Previous large scale studies in which both *A. eucosmus* and *A. r.* var. *minor* were included have found no differences in sequences within the commonly used phylogenetic markers, the nuclear ribosomal internal transcribed spacer (ITS) and the chloroplast (cp) DNA *trn*L intron, highlighting the taxonomic closeness of species and the dearth of molecular knowledge on these two taxa (Wojciechowski et al., 1999).

Initial screening of chloroplast and nuclear DNA for sufficient variation to detect the variety examined 13 markers, including: the ITS and external transcribed spacer (ETS) regions (nrDNA), *ndhF, matK, atpI-atpH, trnL-trnF* and *trnL* intron*, rpl32-trnL, trnD-rpoB, trnG* intron, and *trnH-psbA* (cpDNA intergenic spacers and introns). None of these regions contained enough useful variation for phylogenetic reconstruction. Only three cpDNA regions showed sufficient variation to be included in the present study: *ycf6-trnC*, *trnC-rpoB*, and *trnM-trnS.* Of these loci ycf6-*trnC* was once a proposed barcoding locus (Kress et al, 2006), *trnC-rpoB* was proven useful in low level phylogenetic work on the Polygonaceae (Ohsako and Ohnishi, 2000), and *trnM-trnS* has proven especially useful in systematics work on South American *Astragalus* (Scherson et al., 2008).

Outgroup selection

Earlier work on the taxonomy of *Astragalus robbinsii* has largely assumed that each of its narrowly endemic varieties arose through isolation of allopatric populations of *A. r.*

var. *minor*, resulting in an independent evolutionary trajectory and a new taxon (Barneby, 1964). If Fernald's Milkvetch arose from *A. r.* var. *minor* (or even from *A. eucosmus*), it is then imperative to understand the variation within this species complex on a continental scale. Accordingly, we have sampled all of the remaining extant varieties of *A. robbinsii* (aside from the endangered *A. r.* var. *jesupi*).

We have also sampled six additional outgroups to determine variability in the cpDNA regions chosen at mid and high phylogenetic levels. *Astragalus bodinii* Sheldon and *Astragalus alpinus* L. are both distantly related to *A. eucosmus* and *A. robbinsii* (*A. bodinii* is a representative of the New World aneuploid clade, *A. alpinus* is an Old World euploid species) (Wojciechowski et al., 1999). They only other *Astragalus* within the province of Newfoundland and Labrador (Maunder, 2006). *Astragalus williamsii* Rydb. and *Astragalus australis* (L.) Lam. are North American alpine species found within subgenus *Phaca* that form a sister group to *A. eucosmus* and *A. robbinsii* (Wojciechowski et al., 1999). *Astragalus corrugatus* Bertol. and *Astragalus siliquosus* Boiss. are euploid old world species endemic to Iran, and previous phylogenetic work indicates a close alliance to *A. eucosmus* (Wojciechowski et al., 1999).

DNA extraction

Genomic DNA was extracted from both silica gel-preserved and herbarium specimen leaf tissue samples using protocols adapted from Alexander et al. (2007). Herbarium specimens were only sampled if they were collected within the last thirty years and appeared to be well preserved (i.e. intact, green, etc.), to avoid degraded cpDNA.

Approximately 20 mg of leaf tissue from each sample was ground for 45 seconds using a BioSpec Mini Beadbeater-96 (BioSpec, Oklahoma). The powdered tissue was suspended in a mixture consisting of 320 μl homogenization buffer (0.1 m NaCl, 0.2 m Sucrose, 0.01 m EDTA, 0.03 m Tris-HCl pH 8.0), 80 μl lysis buffer (0.25 m EDTA, 2.5% SDS, 0.5 m Tris-HCl pH 9.2), and 4 μl RNase A. The samples were then incubated at 65°C for 30 minutes. Potassium acetate (130 μl) was added after incubation, and each sample was placed in a -20°C freezer for five minutes, then spun in an Eppendorf 5424 centrifuge at 15000 rpm for ten minutes. The resulting supernatant was transferred to a 1.5 μl tube containing 600 μl of plant binding buffer (2M guanidine hydrochloride, 95% EtOH), and incubated at room temperature (~20°C) for five minutes. Each sample was then transferred to a silica membrane spin column (Epoch Biolabs, Texas), and spun at 14000 rpm for one minute to collect the DNA. The DNA bound to the silica gel columns was washed to remove residual salts with 1 ml of 70% EtOH (14000 rpm, 1 minute), and eluted by incubation in 150 μl of warm THE Buffer (10 mM Tris-HCl pH 8.0, 0.5 mM EDTA) for 5 minutes and by spinning the columns at 14000 rpm for 1 minute. Extraction success was verified by running 4 μl from each sample on a 1.25 % agarose gel.

PCR and cpDNA sequencing

The primers used to amplify and sequence the *ycf6-trnC-rpoB* cpDNA region (Table 1) were taken from Shaw et al. (2004).

Table 1: Primers used in chloroplast DNA sequencing.

Primer	Sequence
ycf6R	GCCCAGCRAGACTTACTATATCCAT
trnC^{GCA}F	CCAGTCRAATCYGGGTG
trnC^{GCA}R	CACCCRGATTYGAACTGGGG
rpoB	CKACAAAAYCCYTCRAATTG

Each spacer region, *ycf6-trnC* and *trnC-rpoB*, was amplified and sequenced separately. For the *ycf6-trnC* intergenic spacer, each 15 µl PCR reaction volume contained 9.27 µl nuclease-free ddH_2O, 1.5 µl 10X reaction Buffer (Sigma Aldrich, Ontario), 2.5 µl $MgCl_2$ (25 mM – Sigma Aldrich), 0.3 µl dNTPs (10 mM), 0.375 µl of each primer (10 µm – Alpha DNA, Montreal), 0.12 µl JumpTaq DNA Polymerase (Sigma Aldrich) and 1.5 µl 1:10 concentration genomic DNA. The reactions for the *trnC-rpoB* intergenic spacer were identical, except that one µl of the ddH_2O was replaced with BSA (20 µg per ml) and full concentration DNA was used to increase the specificity of the product.

The amplification of both regions used the same protocol: 94°C for 3 minutes, followed by 35 full cycles of a) 94°C for 1 minute b) 45°C for 1 minute c) 72°C for 1 minute 20 seconds with ramping set to 50%, and a final extension step at 72°C for 5 minutes.

All reactions were carried out on an Eppendorf Mastercycler epgradient-S thermal cycler. Reaction success was verified by running PCR products out on a 1.25% agarose gel (Fig. 8).

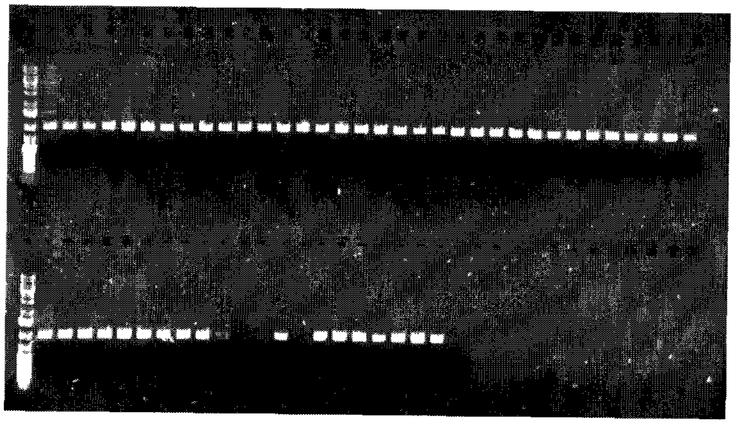


Fig. 8: *ycf6-trnC* amplification products from *A. eucosmus*, *A. r.* var. *minor* and *A. r.* var. *fernaldii* on 1.25% agarose gel. First lane in both rows contains a 1 kb DNA ladder.

PCR products were purified by adding 0.75 μl of Exonuclease I and Shrimp Alkaline Phosphatase (MJS Biolynx, Ontario) to successful reactions, and by incubating at 37°C for 30 minutes, followed by 90°C for 10 minutes on an Eppendorf Mastercycler epgradient-S thermal cycler.

Purified PCR products were sequenced on a 3130xl Genetic Analyser using BigDye v3.1 chemistry (Applied Biosystems, California). The 10 μl sequencing reactions contained 7.1 μl ddH_2O, 1.5 μl 5X ABI buffer, 0.5 μl primer, 0.4 μl BigDye Terminator v3.1 Ready Reaction Mix, and 0.5 μl purified PCR product.

Sequence Data Analysis

Sequence traces were assembled and base-calls were checked visually in Sequencher v.4.9 (GeneCodes, Michigan). Completed sequences were then aligned manually in BioEdit v.7 (Ibis Biosciences, California), and concatenated using Concatenator 1.0 (Pina-Martins and Paulo, 2008). Congruence of the two datasets was tested using an incongruence length difference (ILD) test (100 replicates with 100 trees saved at each step). At this point sequences that were missing one of the two regions (no data) were excluded. Indels were coded as a fifth state, and complex indels were coded as per Simmons and Ochterena's "simple indel coding" (2000). A maximum parsimony analysis was performed in PAUP 4.0 (Swofford, 2000), and a strict consensus of 1600000 trees was generated. Bootstrap support for the consensus tree was obtained by running 100 bootstrap replicates with 1000 trees each, with multiple trees (MULTREES) saved at each step (Swofford, 2000). A Bayesian analysis of the dataset was performed in MrBayes 3.1 run for 10000000 generations, with trees sampled every 100 generations and 2500 trees discarded as the initial burn-in (Huelsenbeck et al., 2001). This analysis was carried out using a using a GTR+G model of sequence evolution, determined using Mr.ModelTest 2.2 (Nylander, 2002).

AFLPs

To supplement the sequencing effort, we used AFLPs to genotype populations of the *eucosmus-fernaldii-minor* complex (including *A. eucosmus* and *A. r.* var. *minor*) throughout its range in Newfoundland and Labrador and Québec. This technique often detects genetic variation at the infraspecific level as it detects mutations within the entire genome of an organism, rather than the isolated spacer regions used in sequencing (Karudapuram and Larson, 2005). Highly reproducible and fast-evolving AFLP genotypes should allow us to infer an accurate phylogeny, and determine population structure within this narrowly endemic variety (Meudt and Clarke, 2007).

The protocol for generating AFLP fingerprints was adapted from procedures developed by the Canadian Museum of Nature molecular systematics laboratory (Checkland and Bull, 2008). Only samples preserved in silica gel were used to minimize use of degraded DNA (Lambertini et al., 2008). Initially 4 μl of genomic DNA was digested in a 20 μl reaction volume with 11.2 μl nuclease-free ddH_2O, 2 μl 10X NEBuffer 2 (New England Biosystems, Massachusetts), 0.2 μl 100X BSA (NEB, Massachusetts), 0.5 μl EcoRI (10 units) and 1 μl MseI (10 units) (NEB, Massachusetts). Digested DNA samples were ligated with EcoRI and MseI adaptor preparations according to the procedure in Checkland and Bull (2008). After ligation, each sample was diluted with 160 μl of 10 mM Tris-HCl (pH 8.5).

Pre-selective amplifications were carried out on an Eppendorf Mastercycler epgradient-S thermal cycler. Each 40 μl reaction contained 25 μl nuclease-free ddH_2O, 1 μl deionized formamide, 5 μl 10X reaction buffer (Fermentas, Maryland), 3 μl $MgCl_2$ (25mM –

Sigma Aldrich), 1 µl dNTPs (10mM), 0.75 µl of each primer (Table 2) diluted to 20 µm, 0.5 µl Taq DNA Polymerase (NEB), and a 10 µl sample dilution from the ligation step. The pre-selective amplification used the following PCR profile: 72°C for 15 minutes followed by 20 full cycles of a) 94°C for 50 seconds, b) 56°C for 1 minute, and c) 72°C for 2 minutes. For both the pre-selective and selective amplifications, reaction success was checked on a 1.25% agarose gel. Successful reactions were diluted with 180 µl of 10 mM Tris-HCl (pH 8.5) and carried forward to the selective amplification step.

Table 2: Primers used in AFLP pre-selective amplifications

Primer	Sequence
EcoRI+A	GACTGCGTACCATTCA
MseI+C	GATGAGTCCTGAGTAAC

Selective amplifications were carried out on an Eppendorf Mastercycler epgradient-S thermal cycler. The 20 µl reactions contained 12 µl nuclease-free ddH$_2$O, 0.5 µl deionized formamide, 2.5 µl 10X reaction buffer (Fermentas, Maryland), 1.5 µl MgCl$_2$ (25mM – Sigma Aldrich), 0.75 µl dNTPs (10mM), 0.25 µl EcoRI selective primer, fluorescently labeled with FAM or HEX (Table 3, 20 µm), 1.25 µl MseI selective primer (20 µm), 0.25 µl Taq DNA Polymerase (NEB) and 5 µl of the diluted pre-selective amplification. The Touchdown PCR profile used for the selective amplification was: 8 full cycles of a) 94°C for 50 seconds, b) 50°C for 1 minute, and c) 72°C for 2 minutes, followed by 30 full cycles of a) 94°C for 50 seconds, b) 45°C for 1 minute, and c) 72°C for 2 minutes, wioth a final extension step of 72°C for 10 minutes.

Table 3: Primer combinations and fluorescent labels used to generate AFLP profiles in selective amplifications

Primer Pair	Sequence
p1 (Fam)	EcoRI-AAC + MseI-CAA
p3 (Fam)	EcoRI-ACC + MseI-CAA
p7 (Fam)	EcoRI-ACC + MseI-CAC
p11 (Fam)	EcoRI-ACC + MseI-CAG
p13 (Fam)	EcoRI-AAC + MseI-CAT
p19 (Fam)	EcoRI-ACC + MseI-CTA
p12 (Hex)	EcoRI-AAG + MseI-CAG
p20 (Hex)	EcoRI-AAG + MseI-CTA

Selective PCR products were diluted by a factor of 1:28 with ddH_2O. FAM and HEX-labeled amplicons were also mixed at this point to allow for mixing during the ABI run. One µl of the resulting dilutions were mixed with 11.2 µl deionized formamide and 0.8 µl ROX size standard (Geneflo-625, CHIMERx, Wisconsin). Samples were then denatured at 95°C for 3 minutes, placed on ice for 2 minutes and spun at 3700 rpm for one minute after which samples were run on a 3130xl Genetic Analyzer (Applied Biosystems, California). Eight samples from each ABI run were repeated to ensure AFLP profiles were replicable.

AFLP Scoring and Data Analysis

Variability within an initial screening set of 32 primer combinations was assessed visually. Primer pairs that showed little variation or repeated failed reactions were eliminated, leaving the eight that were used in the final analysis (Table 3). Peak sizing was checked and bin positions were assigned using GeneMarker v1.75 (SoftGenetics, Pennsylvania). For each primer pair, the AFLP profiles (up to 100), were screened for variable and repeatable alleles with AFLPScore v1.4 (Whitlock et al., 2008). Allele calling thresholds were determined through calculating error rates based on repeated AFLP fingerprints, and the program was used to eliminate alleles that were not repeated, or where mean peak heights were low enough to be due to background noise (Whitlock et al., 2008). Retained alleles were visually checked to ensure accurate scoring. After scoring all eight primer combinations, 106 alleles were retained for further analysis. A principal components analysis on the data matrix was carried out in PAST 4.0 (Hammer et al., 2001). A neighbour-joining tree of the AFLP data was constructed in PAUP 4.0 (Swofford, 2000). We attempted to generate sequence and AFLP data for each morphological specimen tested, see Appendix 4 for genotype, sequencing, and morphology data obtained.

Mantel Test – Isolation by distance

To test for isolation by distance in *Astragalus robbinsii* / *Astragalus eucosmus* a Mantel test for correlation between the genetic and geographic distance of populations was performed. AFLP allele data was converted into pairwise F_{ST}s by population using Arlequin 3.11 (Excoffier et al., 2005). A corresponding geographic distance matrix was created using the Geographic Distance Matrix Calculator (Ersts, 2010). The Mantel test between these two data matrices was performed in PAST 4.0 (Hammer et al., 2001). Only Atlantic Canadian populations were included in this analysis.

Morphology

Twenty two quantitative and qualitative morphological characters characters taken from Rydberg's (1928) and Barnebys (1964) description of the variety were measured on 217 herbarium specimens (Appendix 3) for the morphological analysis (Table 4).

Quantitative characters were measured with a ruler and calipers in millimetres, while qualitative character states were visually scored.

Leaf, leaflet, pod, seed, beak, calyx, teeth and flower quantitative characters were recorded as averages from two to three separate measurements on each specimen. Stipule length was measured for the stipule closest to the base of the plant. Peduncle and raceme lengths were recorded for the most elongate stems on each specimen, flowering and fruiting peduncles and racemes were recorded separately.

Table 4: Morphological characters measured

Quantitative	**Qualitative**
Leaf Length	Bottom Leaf State (0-5) (1)
Leaflet Length	Top Leaf State (0-5) (1)
Peduncle Length	Calyx Pubescence (0-5) (2), (3)
Raceme Axis Length	Pod Pubescence (0-5) (2), (3)
Calyx Length	Petal Colour (0-3) (4)
Teeth Length	
Banner Petal Length	
Stipe Length	
Stipe Taper	
Pod Length	
Pod Depth	
Pod Width	
Beak Length	
Ovule Number	
Seed Diameter	
Stipule Length	
Flower Length	

(1) 0 glabrous, 1 white pubescence at margins, 2 sparse white pubescence, 3 intermediate white pubescence, 4 dense white pubescence

(2) 0 glabrous, 1 sparse white pubescence, 2 intermediate white pubescence, 3 dense white pubescence

(3) 0 glabrous, 1 sparse black pubescence, 2 intermediate black pubescence, 3 dense black pubescence

(4) 0 strictly white, 1 white flower, purple at center of banner petal, 2 pale purple flower, 3 dark purple flower

Prior to analysis, pod depth, ovule number and seed diameter were excluded as too few measurements were obtained for these three characters. Stipe taper was also excluded due to ambiguous scoring. A principal components analysis on the complete dataset was carried out using PAST to examine separation between the groups in multivariate space (Hammer et al., 2001). Missing data in the morphology PCA was corrected for using column average substitution (Hammer et al., 2001).

Interspecific variation for each quantitative variable between *Astragalus eucosmus*, *A. robbinsii* var. *minor*, and *A. robbinsii* var. *fernaldii* was explored using box and whisker plots, and analyzed using a Kruskal–Wallis one-way ANOVA (as the assumption of normalcy was violated), followed by post-hoc Mann-Whitney U pairwise comparisons to establish levels of significance. Variation within each qualitative variable was plotted as a histogram.

Chapter 3: Results

Chloroplast DNA sequencing

Parsimony and Bayesian analysis of 125 *ycf6-trnC-rpoB* sequences shows two major groupings within the *eucosmus-fernaldii-minor* species complex, separating *A. eucosmus* from the majority of *A. r.* var. *minor*. The topologies of the parsimony strict consensus tree and Bayesian phylogram were identical; therefore the Bayesian phylogram is presented in Fig. 9 with bootstrap values indicated where appropriate. An ILD test of the two spacer region datasets indicated no significant incongruency in the data (P = 0.01).

Four specimens of *A. r.* var. *minor* (three from Pointe Amour and one from Alberta) were found allied with *A. eucosmus,* however, they possess elongated stipes (1.3-1.6 mm) and black calyx pubescence as is typical for var. *minor*; they seem to differ from the remainder of their populations in cpDNA haplotype only, rather than morphology (Fig. 9).

Astragalus robbinsii var. *fernaldii* is split between the *A. eucosmus* or *A. r.* var. *minor* haplotypes, but all populations except Mont Bonenfant are found to possess at least one individual with the *A. eucosmus* genotype. Only a few members of the Québec/Labrador Border population are allied to *A. r.* var. *minor* in the analysis and the complete alliance of Mont Bonenfant with *A. r.* var. *minor* is not supported in the bootstrap analysis (Fig. 9). All individuals from Cap Crow are found within the *A. eucosmus* polytomy, save for one individual, although this separation is not supported by bootstrap analysis.

One small clade of *A. eucosmus* samples from Alberta (bootstrap value of 62%) and five samples of *A. r.* var. *fernaldii* are found allied to the *A. r.* var. *minor* lineage in a very poorly supported clade (bootstrap value of 51%). Within this clade, a bootstrap-unsupported grouping of two *A. eucosmus* from Alberta, one *A. eucosmus* from Cape Mauve, and one *A.*

r. var. *fernaldii* from Cap Crow emerge with a posterior probability score of 0.92. With such low bootstrap values, these two clusters may be more likely a part of the *A. eucosmus* polytomy, significantly different from the *A. r.* var. *minor* lineage (bootstrap value of 89%), but not from *A. eucosmus.*

One specimen from Alberta and one from Pointe Amour (*A. r.* var. *minor*) are found in a clade in the *A. eucosmus* polytomy with a bootstrap value of 51%. Two samples of *A. r.* var. *minor* from Alberta are found in this polytomy with longer branch lengths. An additional clade (bootstrap value of 57%) emerging from the *A. eucosmus* polytomy included both populations of *A. eucosmus* sampled from New Brunswick (NB) and a sample of *A. australis* from the Canadian arctic (Fig. 9). *Astragalus williamsii* also emerges from the *A. eucosmus* polytomy, but as with *A. australis* in the NB clade, emerges on a longer branch, indicative of differences in haplotype.

The one sample of *A. r. occidentalis* included in this analysis is found within *the A. eucosmus* polytomy, with a branch length similar *A. eucosmus*. Samples from the remaining varieties of *Astragalus robbinsii* failed to amplify, likely due to degradation during storage as a herbarium specimen, and are omitted here as a result.

AFLP genotyping

Both neighbour-joining (Fig. 10) and principal components analysis (Fig. 11) of the AFLP allele data revealed little genetic differentiation within the *eucosmus-fernaldii-minor* species complex throughout its range in Newfoundland and Labrador and Québec. All three taxa occupied the same area of multivariate space as indicated within the convex hulls, and many of the AFLP genotypes generated were shared, as indicated by the substantial overlap of individuals in the PCA plot (Fig. 11). In both analyses New Brunswick populations of

Astragalus eucosmus were supported as distinct groups. Outgroups were excluded from the principal coordinates analysis.

Though two samples from Cook's Harbour (*A. eucosmus*) appear to be divergent in the PCA, and the neighbour-joining tree (P0109 and P0110), both the neighbour joining tree and PCA indicate that the majority of genotypes from this locale were the same as the rest of the species complex in Newfoundland and Labrador. Samples from the Barr'd Harbour Hill population of *A. r.* var. *minor* also do not deviate from other provincial genotypes in the species complex.

Examination of both the raw and processed AFLP chromatograms revealed no additive alleles in *A. r.* var. *fernaldii*, i.e. no species-specific markers from *A. r.* var. *minor* or *A. eucosmus* were found together in Fernald's Milkvetch (Gobert et al., 2007). No species or variety specific alleles were seen in any member of the species complex.

Mantel test (isolation-by-distance)

Mantel's test for correlation between population Fst values and geographic distance was not significant (R = 0.041, p = 0.1042) indicating that despite differentiation of the New Brunswick populations, there is no overall isolation-by-distance pattern within the *eucosmus-fernaldii-minor* complex within Atlantic Canada (Manel et al., 2003).

Error Rates Analysis

When repeated AFLP profiles were generated for each marker, only two samples were in disagreement for all 94 alleles. Therefore, the allele-calling error rate in the AFLP dataset was estimated to be as low at 2.12%.

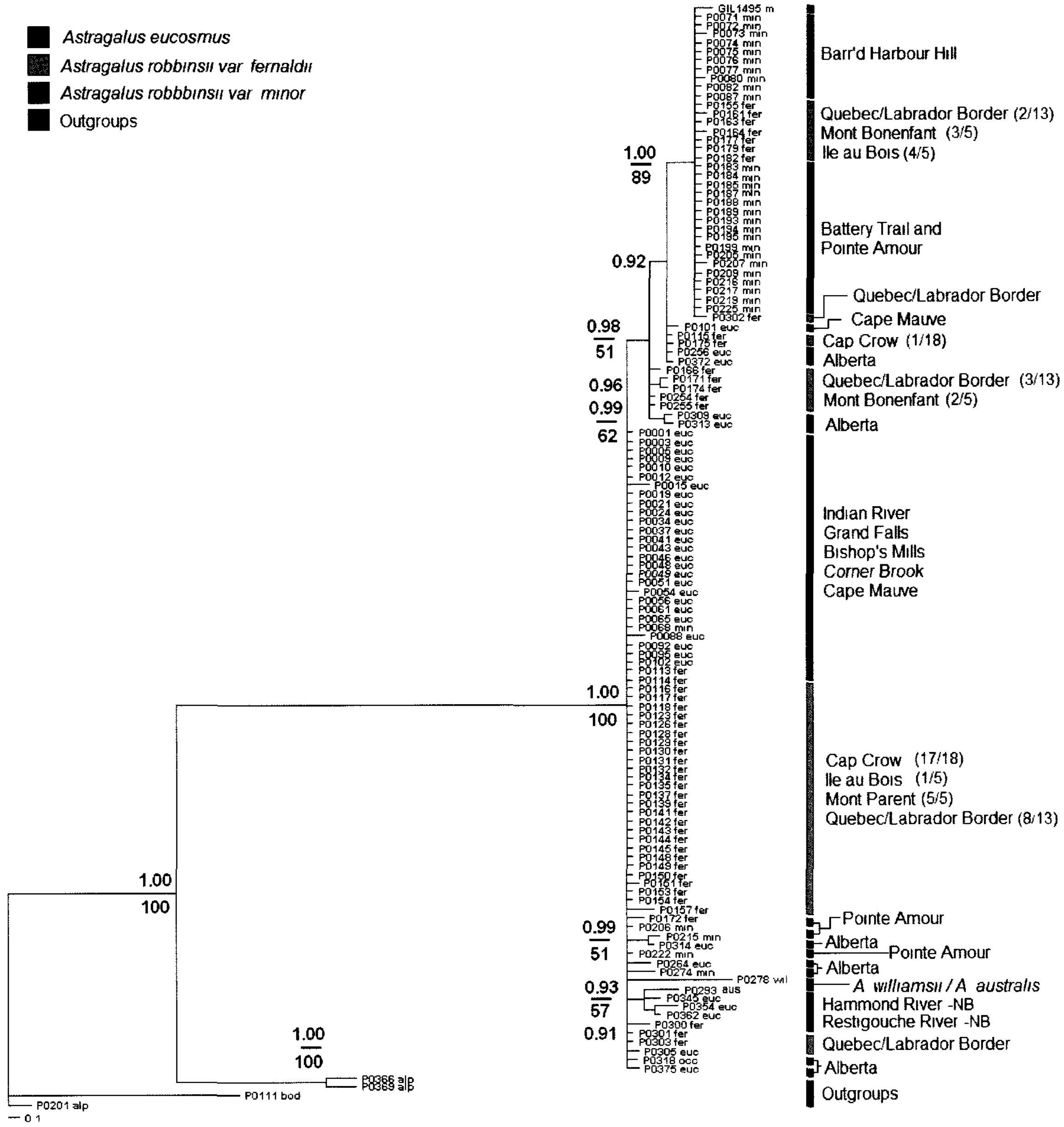


Fig. 9. Bayesian phylogram of 125 combined *ycf6-trnC-rpoB* chloroplast DNA sequences. Indels included in this analysis are coded as a fifth state. Taxa are indicated by the colour of the bar at branch tips. Populations included in this analysis are indicated by their position on tree. Posterior probability scores are indicated above the branching node. Bootstrap values for the identical strict consensus tree are indicated below each branching node.

Morphology – multivariate analysis

During analysis of the morphology dataset four quantitative variables were omitted due to insufficient data or difficulty to score, including stipe taper, pod depth, ovule number and seed diameter, leaving 19 combined quantitative and qualitative variables.

A principal components analysis of all 19 characters (Table 4), including Alberta and New Brunswick specimens belonging to the *eucosmus-fernaldii-minor* species complex revealed substantial overlap between all three taxa, with the majority of individuals sampled clustering in close proximity (Fig. 12). Even an analysis of the two putatively valid taxa within the region, *A. eucosmus* and *A. r minor,* showed a large shared area (Fig. 13). Drawing convex hulls around the extent of variation in each taxon in our first analysis shows that the morphological variation of *A. r.* var. *fernaldii* is almost completely enclosed within the variation seen in *A. eucosmus*.

These analyses account for relatively little of the variation seen in the data: principal components 1 and 2 account for just 15% and 10% of the variability in the species complex PCA alone.

When the same principal components analysis was performed while separately delimiting NL and QC samples, all other North American populations (including New Brunswick), and the individual *A. r.* var. *fernaldii* populations at Cap Crow, Barr'd Harbour Hill and Cook's Harbour, we see that in Newfoundland and Labrador *A. eucosmus* and *A. r.* var. *minor* are more or less variable with respect to their mainland conspecifics respectively (Fig. 14). Specimens of *A. r.* var. *fernaldii* at Cap Crow possesses much more morphological variation than the remaining four populations, which cluster together even closer to *Astragalus eucosmus*.

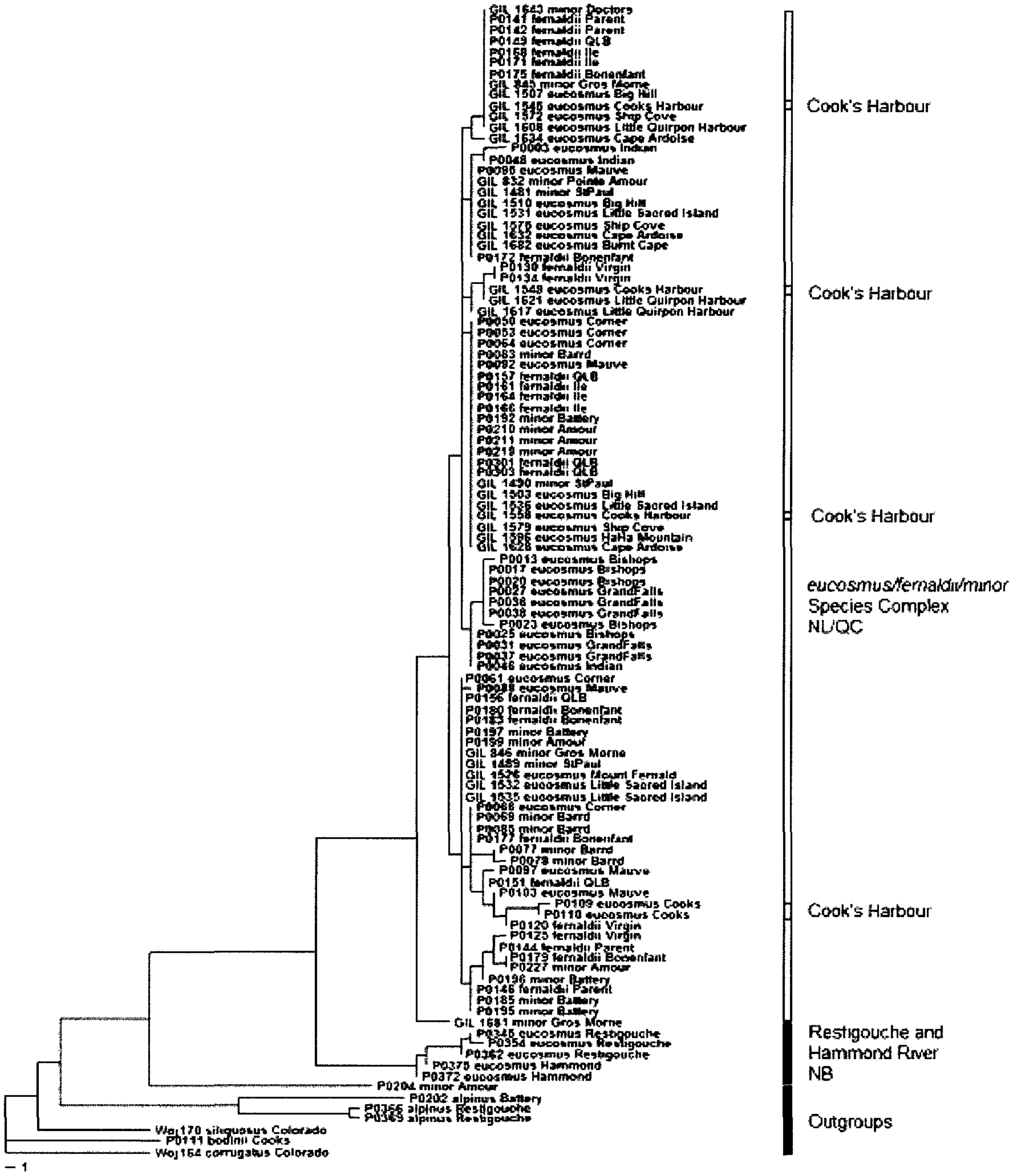


Fig. 10: Neighbour-joining dendrogram of AFLP dataset of 94 loci for 106 individuals from the *eucosmus-fernaldii-minor* complex in Atlantic Canada. Individuals of A. eucosmus from Cook's Harbour are indicated.

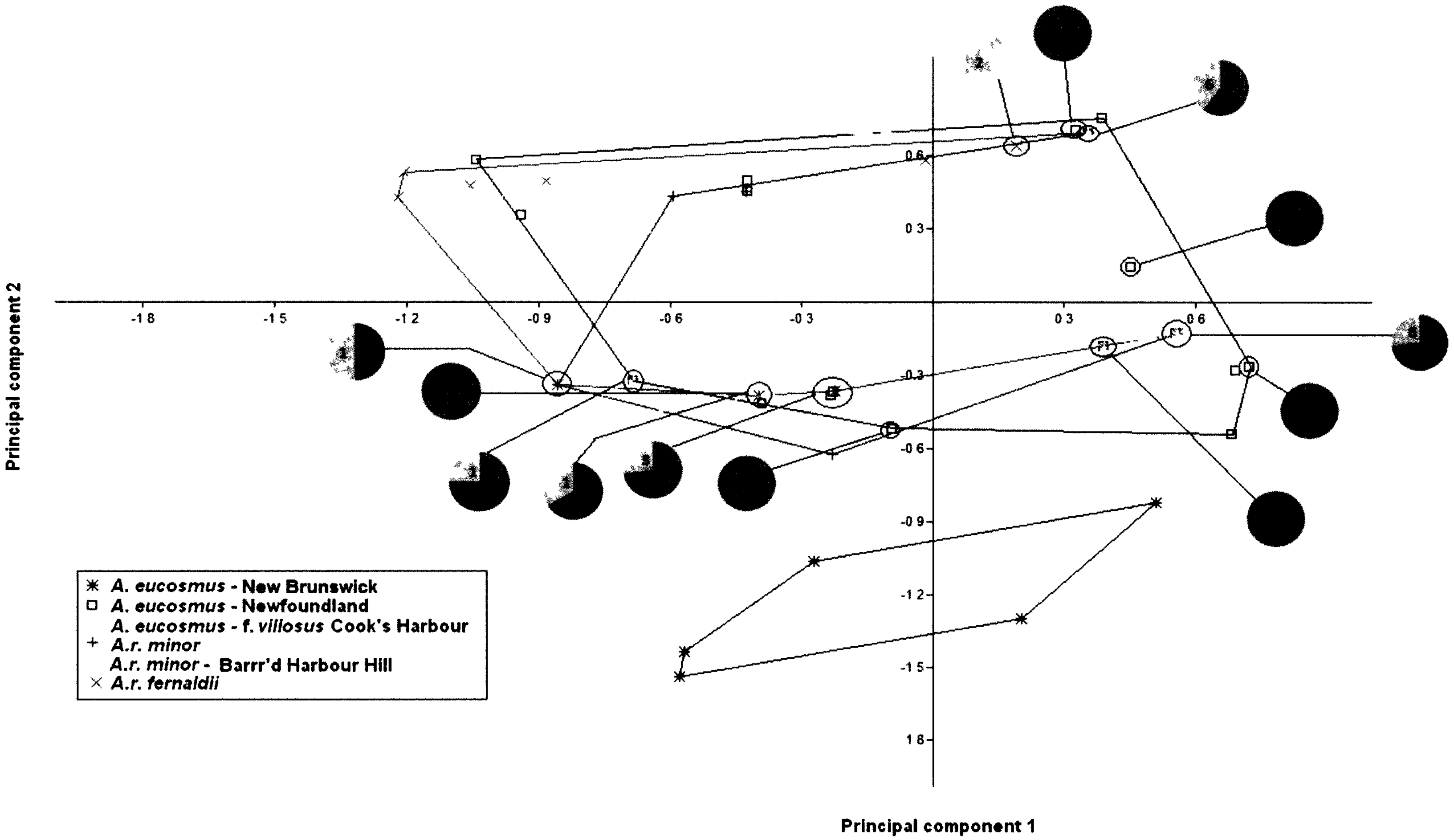


Fig. 11: Principal coordinates analysis of AFLP dataset consisting of 94 loci for 106 individuals of the *eucosmus-fernaldii-minor* complex within Atlantic Canada. Where genotypes overlap a pie chart indicates number of alleles and proportion of taxa present, as well as the number of individuals. The first and second principal component explains 22% and 19% of the variation in the dataset respectively, and convex hulls have been drawn around taxa and specified populations.

The previously re-determined populations of *A. r.* var. *fernaldii* at Cook's Harbour (*A. eucosmus* var. *terrae novae* f. *villosus* – see above), and Barr'd Harbour Hill (*A. r.* var. *minor*) cluster together with *A. eucosmus* and *A. r.* var. *minor* respectively, supporting their reassignments.

Morphology – character analysis

In addition to multivariate analysis of the complete morphology dataset, each measured character was examined for variation at the level of the *eucosmus-fernaldii-minor* species complex across North America and between the five extant populations of *A. r.* var. *fernaldii*. Of the 20 quantitative characters measured four were not significantly different among members of the species complex, including: leaflet length, fruiting peduncle and raceme length, stipule length, and pod width (Fig. 15, p values are presented in Appendix 6). Banner petal length, flower length, beak length, and calyx length were all significantly different among taxa, with *A. r.* var. *fernaldii* as an intermediate between *A. r.* var. *minor* and *A. eucosmus*.

Flowering peduncle length was lowest, significantly, in *A. r.* var. *fernaldii*. In the critical diagnostic character stipe length, as well as leaf length, pod length, calyx teeth length and flowering raceme length, *A. eucosmus* and *A. r.* var. *fernaldii* were indistinguishable from each other, but both were significantly different from *A. r.* var. *minor* (Fig. 15).

At the population level in *A. r.* var. *fernaldii*, average measures for the quantitative variables were overall very similar, for the most part.

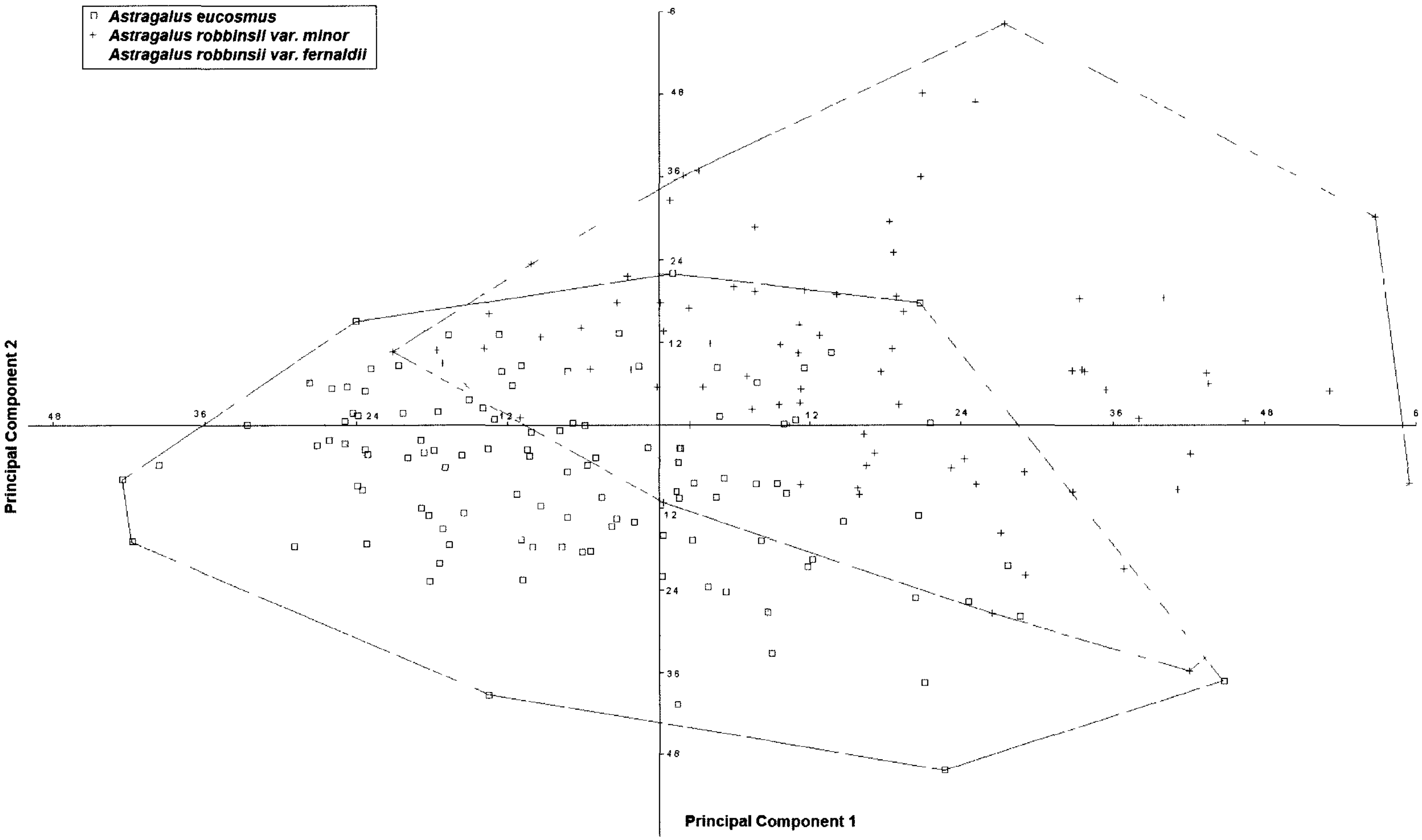


Fig. 12: Principal coordinates analysis of morphology dataset consisting of 20 morphological characters measured in 247 individuals of the *eucosmus-fernaldii-minor* species complex across North America. The first and second principal components explain 15% and 10% of the variation in the dataset respectively. Convex hulls are drawn around the extent of each taxon.

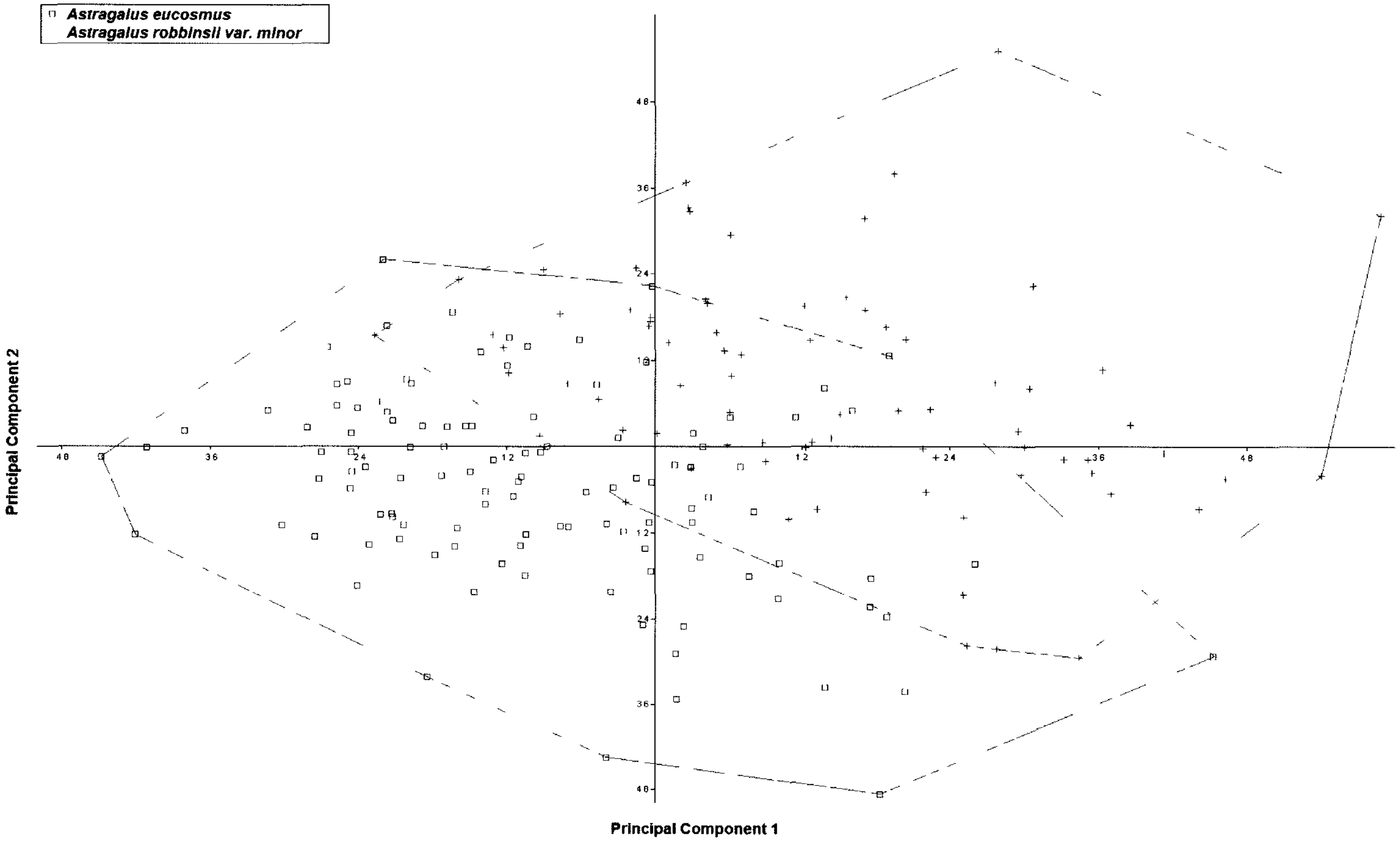


Fig. 13: Principal coordinates analysis of morphology dataset consisting of 20 morphological characters measured in 194 individuals of the *Astragalus eucosmus and Astragalus robbinsii* var. *minor* across North America. The first and second principal components explain 16% and 11% of the variation in the dataset respectively. Convex hulls are drawn around species.

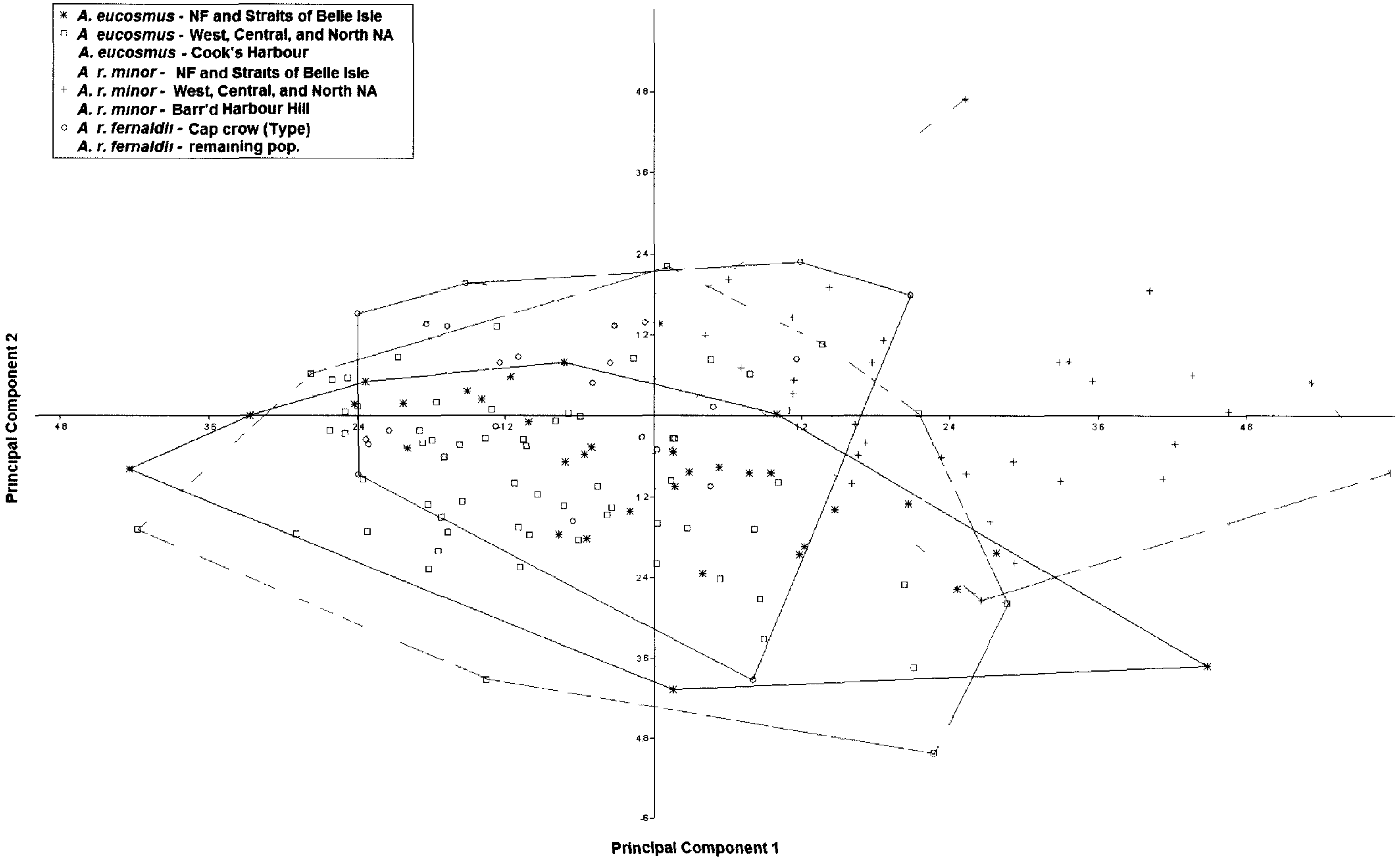


Fig. 14: Principal coordinates analysis of morphology dataset consisting of 20 morphological characters measured in 247 individuals of the *eucosmus-fernaldii-minor* species complex across North America. The first and second principal components explain 15% and 10% of the variation in the dataset respectively. Convex hulls are drawn around taxa, geographic regions, and specified populations of interest.

Stipule and pod lengths are quite variable, and flowering raceme length at Cap Crow seemed higher than usual. Flower, banner petal, and stipe length average values for Île-au-Bois and Mont Parent are lower than the other populations, being much closer to *A. eucosmus* for these variables (Fig. 16). In particular, stipe and pod length at Cap Crow and Mont Bonenfant were longer than the remaining three populations (Fig. 16).

The seven qualitative figures measured were compared using histograms, both by taxa at the species complex level (Fig. 17) and by population in the case of *A. r.* var. *fernaldii* (Fig. 18). Pubescence on the bottom of the leaflets was almost exclusively dense white hairy in *A. r.* var. *fernaldii*, while in *A. eucosmus* and *A. r.* var. *minor* it varied from glabrous to dense white hairy. Top leaf pubescence in *A. r.* var. *fernaldii* encompassed a variety of states, but was almost entirely glabrous in the other two taxa. Petal colour in A. *eucosmus* and *A. r.* var. *fernaldii* was most often dark purple, in *A. r.* var. *minor* it ranged from creamy white to dark purple. Both black and white pod and calyx pubescence shared a similar pattern of character states, with the pods and calyces *A. r.* var. *minor* possessing a moderately high density of black hairs with little to no white pubescence. Both *A. eucosmus* and *A. r.* var. *fernaldii* possessed a similar mix of intermediate to dense white and black pubescence, although *A. r.* var. *fernaldii* tends towards white pubescence.

At the population level, all locales except for Île-au-Bois are similar with to the measured qualitative variables. The Île-au-Bois population possesses calyx and adaxial hair profiles similar to that of *A. eucosmus*.

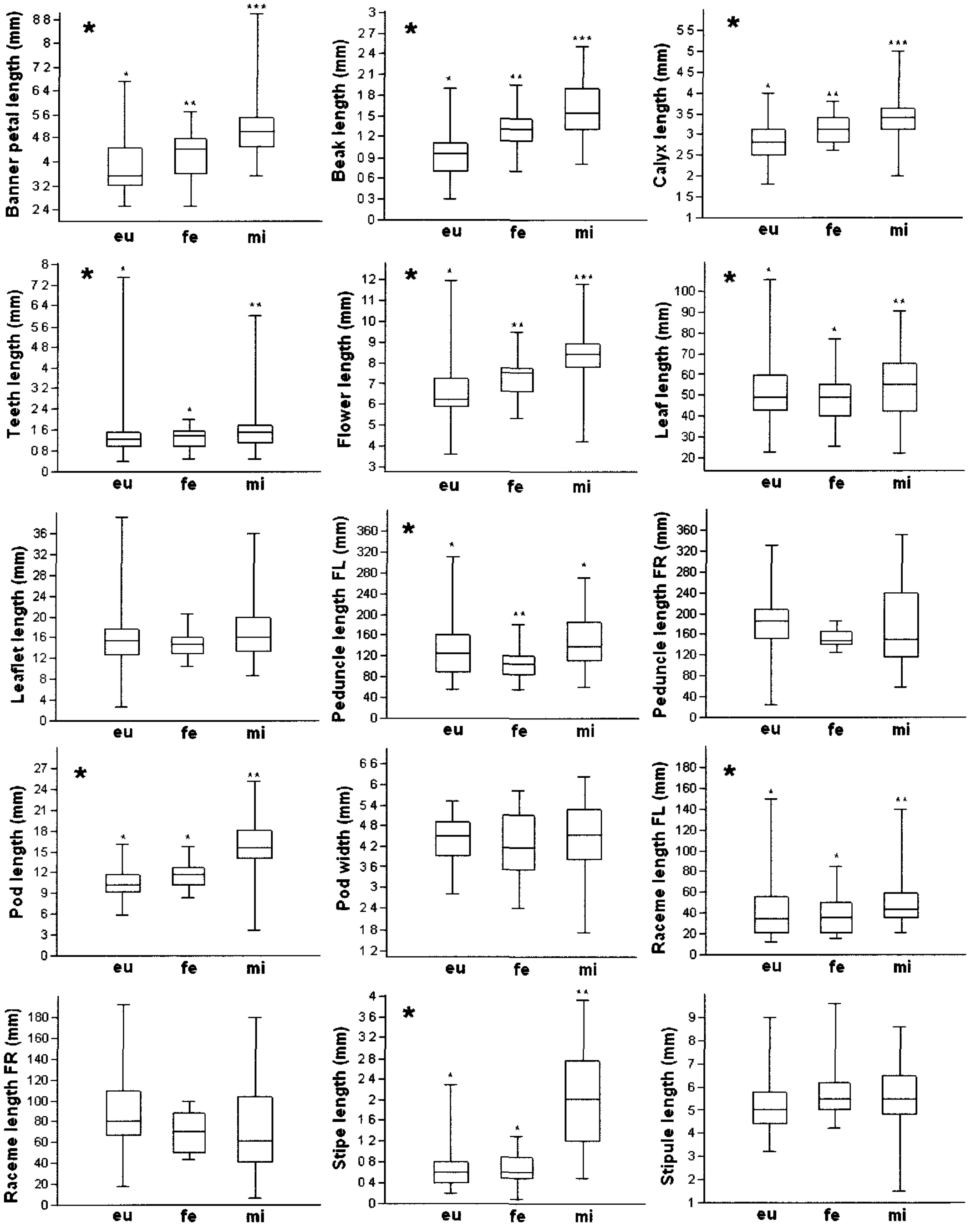


Fig. 15: Box plots showing interspecific differences between *A. eucosmus*, *A. r.* var. *minor* and *A. r.* var. *fernaldii* for fifteen quantitative variables. Variable measured shown to left of each plot, asterisks in the left of each box denote level of statistical significance based on a Kruskall-Wallis ANOVA, blue indicates p>0.05, red indicates p>0.001. Asterisks above each taxon indicate levels of significance based upon a post-hoc Mann-Whitney U test, bars sharing the same number of asterisks are not significantly different.

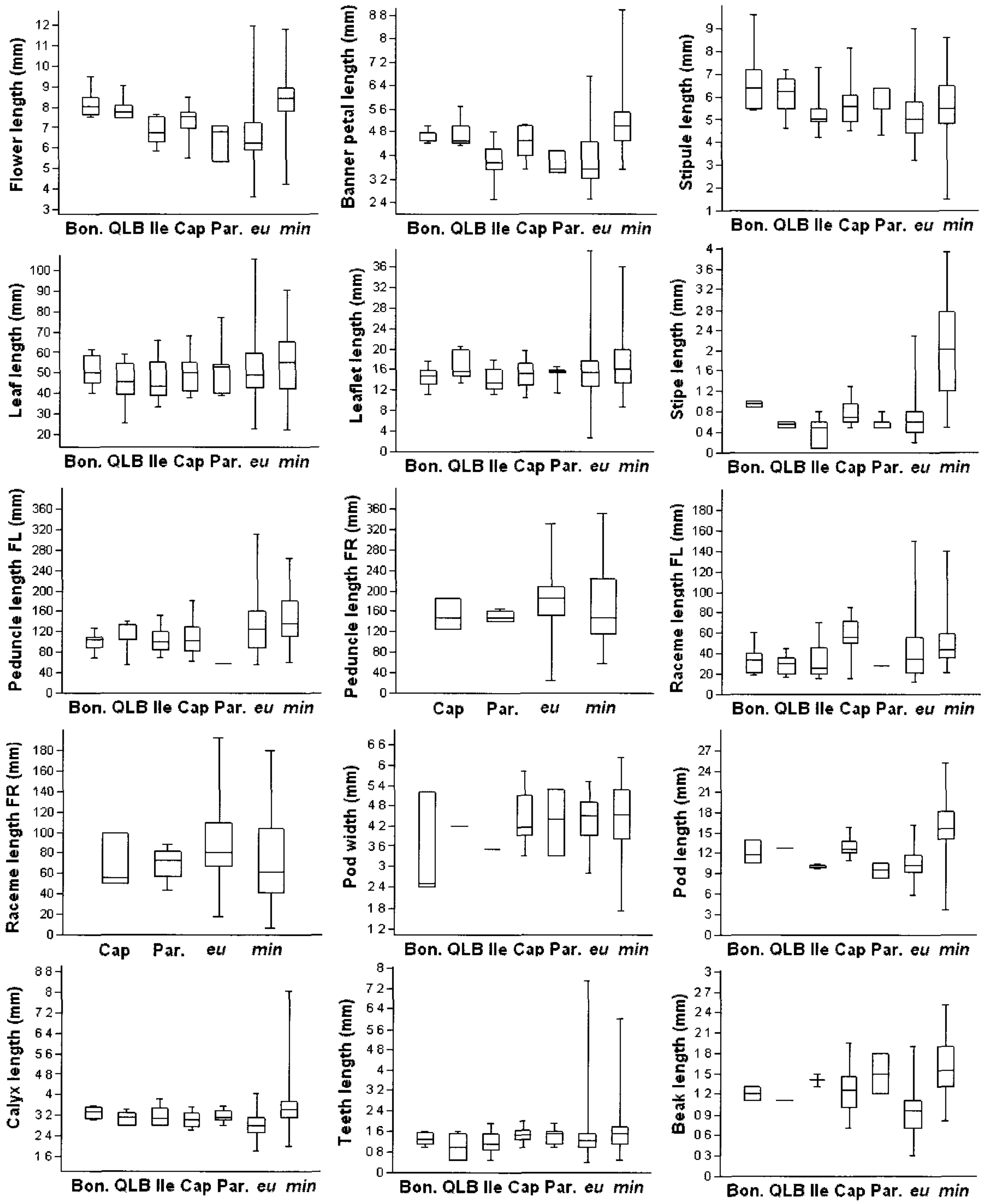
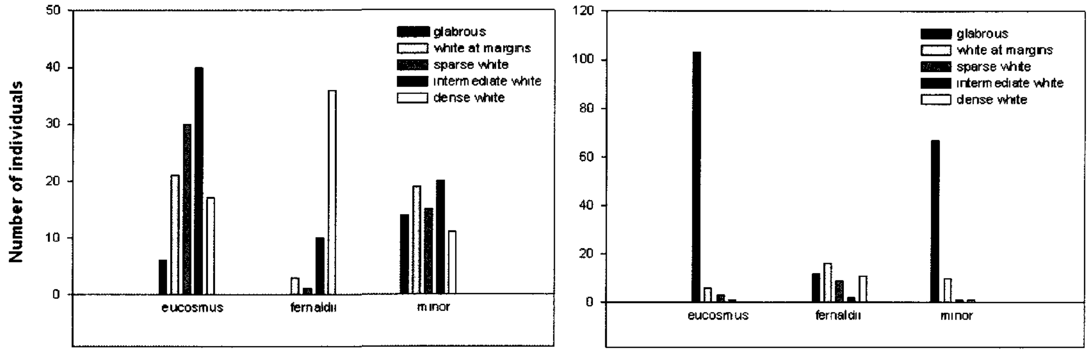
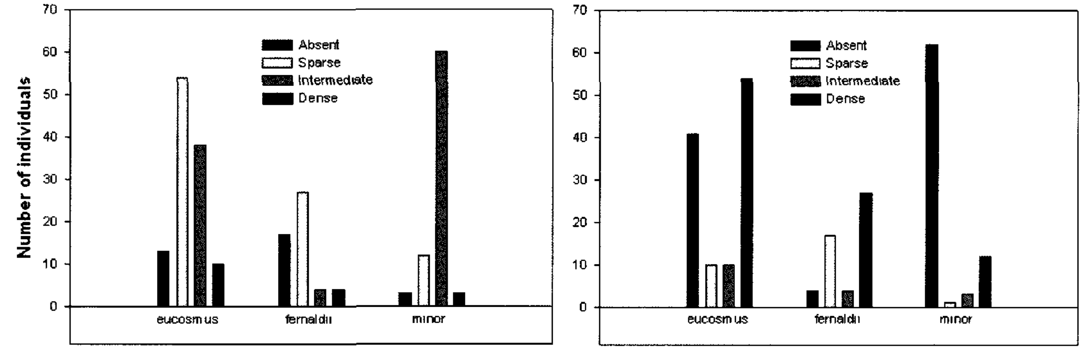


Fig. 16: Box plots showing population differences for fifteen quantitative variables in *A. r.* var. *fernaldii*. Variable measured shown to left of each plot, average values for *A. eucosmus* and *A. r.* var. *minor* are presented for comparison. Due to low numbers of individual counts, statistical analysis of this data was omitted. Due to sampling error, fruiting peduncle length data is unavailable for Mont Bonenfant, Île-au-Bois, and Mont Parent.



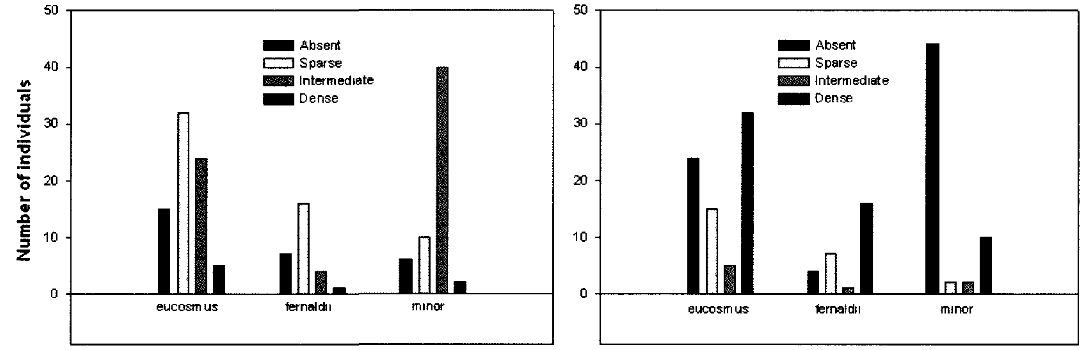
Abaxial leaflet pubescence

Adaxial leaflet pubescence



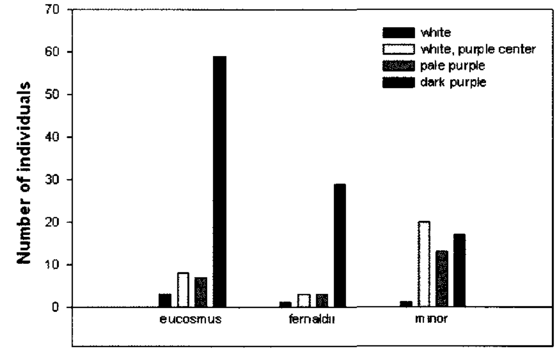
Calyx hair density (black)

Calyx hair density (white)



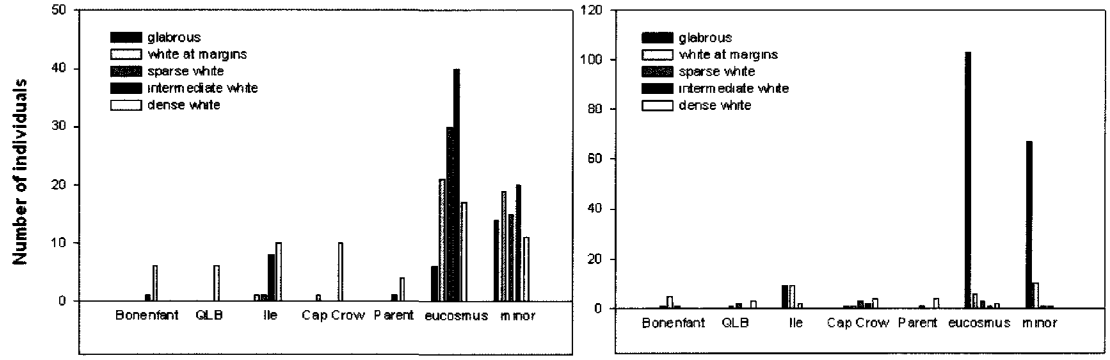
Pod hair density (black)

Pod hair density (white)



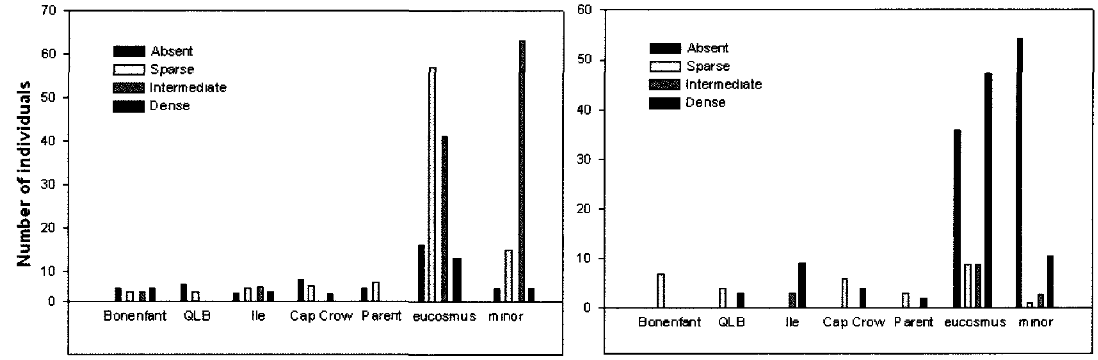
Corolla colour

Fig. 17: Histograms showing interspecific frequency differences between *A. eucosmus*, *A. r.* var. *minor* and *A. r.* var. *fernaldii* for seven quantitative variables. Variable measured shown below each histogram.



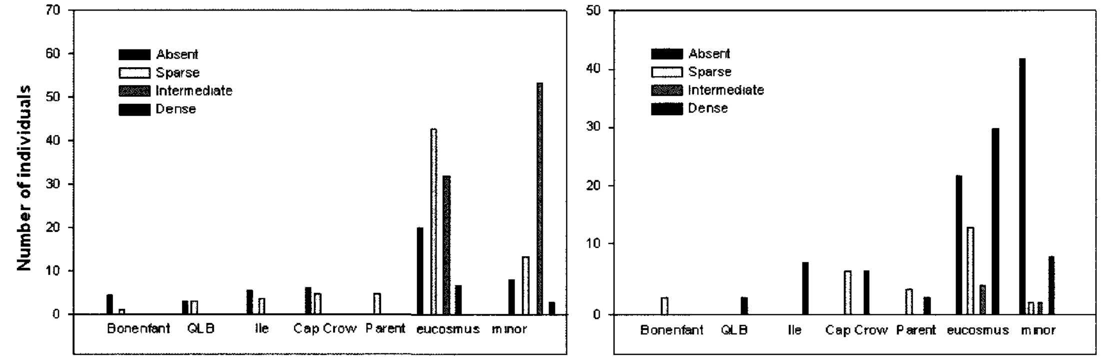
Abaxial leaflet pubescence

Adaxial leaflet pubescence



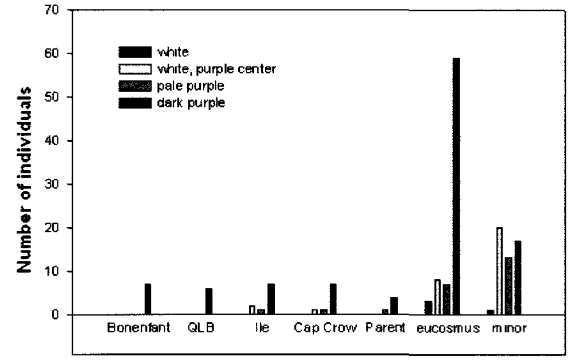
Calyx hair density (black)

Calyx hair density (white)



Pod hair density (black)

Pod hair density (white)



Corolla color

Fig. 18: Histograms showing population frequency differences in *A. r.* var. *fernaldii* for seven quantitative variables. Variable measured shown below each histogram, frequencies for *A. eucosmus* and *A. r.* var. *minor* are presented for comparison.

All five populations displayed sparse black pod hairs and a proportion of individuals without black pod hairs, and black calyx hairs are found in various densities in all populations. Cap Crow, the population closest to typical *A. r.* var. *fernaldii*, had the highest number of individuals with dense white adaxial pubescence, and high proportions of adaxial white pubescence was seen at Mont Parent and Île-au-Bois.

Chapter 4: Discussion

cpDNA sequencing

There were relatively few phylogenetic characters seen in each cpDNA dataset, mostly large indels coded as a fifth state to retain maximum phylogenetic signal (Simmons and Ochoterena, 2000). However, through concatenation of the *ycf6-trnC* and *trnC-rpoB* datasets phylogenetic resolution was increased using the total-evidence approach: where multiple weak phylogenies possess increased support in the combined tree (Olmstead and Sweere, 1994). The ILD test for incongruence (P = 0.01) supported concatenation of the dataset to increase phylogenetic accuracy (Cunningham, 1997).

In both the parsimony and Bayesian analyses of this combined dataset, *A. r.* var. *minor* and *A. eucosmus* are well resolved from each other, with the majority of *A. r.* var. *fernaldii* samples are not found in the *A. r.* var. *minor* lineage (Fig. 9). Both the Bayesian and strict consensus trees had the same topology, increasing the likelihood that the tree is accurate, as two fundamentally different analyses have returned the same result (Alfaro et al., 2003). While parsimony bootstrap values and Bayesian posterior probability scores are not directly comparable, there is high support in both these values for the *A. r.* var. *minor* clade, distinguishing this group as a distinct lineage (Simmons et al., 2004).

While the majority of *A. r.* var. *fernaldii* specimens were found to be allied with *A. eucosmus*, three of five sampled Mont Bonenfant (white hairy) specimens, and most Île-au-Bois specimens (*eucosmus*-like), as well as two specimens from the Québec Labrador border, share the same haplotype as *A. r.* var. *minor*. All of the samples taken from Cap Crow, the most "typical" locality for *A. r.* var. *fernaldii*, were allied with *A. eucosmus*. As such, the chloroplast DNA data indicates a closer relationship between *A. eucosmus* and *A. r.* var. *fernaldii* than with *A. r.* var. *minor*, consistent with Rousseau's (1933) hypothesis regarding the origin of the taxon. However, that some of the sampled populations display the *minor* haplotype indicates that gene flow is likely occurring between at least three of the *A. r.* var. *fernaldii* populations and the nearby populations of *A. r.* var. *minor* at Battery Trail and Point Amour (Appendix 2). Introgression may be occurring through long distance dispersal, or hybridization and back-crossing of these closely related taxa (Liston and Kadereit, 1994).

As the *A. r.* var. *fernaldii* populations are relatively homogenous in terms of morphology and AFLP genotype (nuclear DNA and morphology are in agreement), differences in cpDNA haplotype may indicate chloroplast capture and gene flow between three *A. r.* var. *fernaldii* populations and *the A. r.* var. *minor* localities (Soltis and Kuzoff, 1995). This is supported by the fact that the westernmost populations of *A. r.* var. *fernaldii* in the Blanc Sablon region, the ones with the highest proportion of *A. r.* var. *minor* genotypes, are the closest (geographically) to the above mentioned *A. r.* var. *minor* populations, at a distance of only 16 km (Morrisett, 1997). In fact, among known populations, this is the only region in Atlantic Canada where two different members of the relatively rare *eucosmus-fernaldii-minor* species complex occur in such close proximity (Appendix 2).

Gene flow in the reverse direction may also be true for the population of *A. r.* var. *minor* at Pointe Amour, where the *A. eucosmus*/*A. r.* var. *fernaldii* haplotype is found in three sampled individuals (Fig. 9), also indicative of a localized chloroplast capture (Kron et al., 1993). The hypothesis of cpDNA transfer between the populations is additionally supported by the fact that the eight putatively introgressed var. *fernaldii* and three var. *minor* are morphologically "good" representatives of their taxa (they do not appear to be intermediates or hybrid forms). This may indicate that the nuclear genomes of these plant populations are relatively homogenous among taxa at sampled loci (supported by AFLP data), and that only the cpDNA haplotypes have been transferred, most likely through introgression (Soltis and Kuzoff, 1995). There are distinct morphological differences, however, indicative of nuclear DNA differentiation not detected by our neutral markers.

The populations of *A. eucosmus* sampled from New Brunswick form a weakly supported clade in the combined tree perhaps indicating a phylogeographic effect on genotype, as supported by our AFLP results (Fig. 10). Both *A. eucosmus* and *A. r.* var. *minor* sampled from Alberta, however, are found within both the *A. eucosmus* and *A. r.* var. *minor* clades. While nuclear DNA was not sampled for western Canadian populations, additional screening and sequencing these samples for a nuclear marker, such as ITS, may indicate introgression of cpDNA genotypes within these populations as well, if the nuclear genotypes are homogenous and the cpDNA haplotypes are mixed.

While *A. alpinus* and *A. bodinii*, both aneuploid New World taxa, were easily resolved using these cpDNA spacer regions, *Astragalus williamsii, Astragalus australis*, and *Astragalus robbinsii* var. *occidentalis* were not. The former two species are both euploid *Astragalus*, and have been found to form a sister clade to *A. eucosmus* and *A. robbinsii* (Wojciechowski et al., 1999).

Here, *A. r. occidentalis, A. williamsii* and *A. australis* are found allied to *A. eucosmus*, either as part of the large polytomy of Newfoundland samples, or allied with the cluster of New Brunswick *A. eucosmus* populations. When compared to the phylogeny of

North American *Astragalus* published in Wojciechowski et al. (1999), which shows *A. eucosmus* and *A. r.* var. *minor* as sister groups based on ITS data, the *A. r.* var. *minor* clade indicates that the predominant *A. eucosmus* haplotype is potentially an ancestral state which gave rise to new haplotypes in *A. r.* var. *minor.* This is supported in that *A. australis* and A. *williamsii*, our sister taxa, possess haplotypes allied with *A. eucosmus.*

AFLPs

Both principal components and neighbor-joining analysis of the AFLP allele data show little variation within the *eucosmus-fernaldii-minor* species complex (Figures 3 and 4). *Astragalus alpinus*, *A. corrugatus* and *A. siliquosus*, our "close" and "distant" outgroup taxa, are separated from this species complex in the neighbour-joining analysis, at the very least confirming the utility of AFLPs to differentiate members of this genus (Knaus et al., 2005). This data suggests that there may not be a high degree of interspecific variation within this species complex at the regional level, seen in the minimal variation present in NL/QC samples, when compared to other *Astragalus*.

Previous population genetic studies have succeeded in delineating varieties of *Astragalus lentiginousus* (Knaus et al., 2005), determining F_{ST}s in geographically isolated populations of *Astragalus cremnophylax* var. *cremnophylax* (Travis et al., 1996), and reconstructing the genetic history of *Astragalus bibullatus* (Baskauf and Burke, 2009), where it was possible to detect infraspecific variation even from relatively close populations. Here, there is no evidence of differentiation between *A. eucosmus* and *A. r.* var. *fernaldii*, or evidence of population level differentiation within Newfoundland and Labrador and adjacent Québec. Indeed there does not seem to be any differentiation between members of the species complex at all, aside from the separation of the two New Brunswick populations of *A. eucosmus*, which represent the most geographically distant populations sampled for AFLPs in this study.

While *A. eucosmus* and *A. r.* var. *fernaldii* possess 11 and 6 haplotypes respectively (Fig. 11), *A. r.* var. *minor* possesses only 4. This is likely due to limited sampling of *A. r.* var. *minor* populations (only six, when compared to 14 populations of *A. eucosmus*). Additional sampling would likely reveal additional variation within *A. r.* var. *minor.*

The lack of discernable structure in genotypes between populations of *A. r.* var. *fernaldii* could be due to their proximity: all populations occur within a five km strip of coast within the Blanc Sablon region (Appendix 2). At such a small scale it is likely that gene flow is occurring between these populations. Cross-pollination is likely occurring as many *Astragalus* are insect pollinated (Alexander et al, 2004).

Even on a regional scale, disjunct populations may still experience high levels of pollen-mediated gene flow, perhaps leading to high outcrossing between all members of the species complex (*A. eucosmus, A. r.* var. *minor*, and *A. r.* var. *fernaldii*) even in the more remote localities (Ellstrand, 1992). The high degree of overlap between all three taxa in the *eucosmus-fernaldii-minor* complex indicates that this gene flow is likely taking place between all three taxa, and does not support complete reproductive isolation between our two putative species: *A. r.* var. *minor* and *A. eucosmus* (Ellis et al., 2009).

The Mantel test conducted on the AFLP allele data matrix and a matrix of their geographic distance was not significant, indicating that there is no isolation-by-distance dynamic present in Atlantic Canadian populations of *A. eucosmus, A. r.* var. *minor* and *A. r.* var. *fernaldii* (Schaal, 1972). As mentioned above, inclusion of samples from *A. eucosmus* and *A. r.* var. *minor* populations across North America would likely indicate a phylogeographic effect through the introduction of region-specific alleles commonly seen in isolated populations (Neel, 2008), such as those already seen in New Brunswick populations of *A. eucosmus*.

While our AFLP study was confined to samples from Atlantic Canada, addition of AFLP profiles from *A. eucosmus* and *A. r.* var. *minor* taken from throughout their North American range may allow for the detection of phylogeographic effects, as was seen in the delination of the New Brunswick populations. This would also allow for the assessment of outcrossing and the potential for gene flow between the species at a continental scale (Breinholt et al., 2009).

Two plant samples (*A. eucosmus,* potentially f. *villosus*) sampled from Cook's Harbour, Newfoundland cluster together in the PCA, forming a cluster distinct from the rest of the species complex. In the neighbor joining tree and morphological analyses this population is not significantly different from the remaining populations of *A. eucosmus*.

The lack of genetic resolution in Newfoundland and Labrador and Québec *Astragali* observed in this study contrasts with ideas proposed by Scherson et al. (2008), and Wojciechowski et al. (1993), who both assert that New World aneuploid *Astragalus* are less genetically variable than their euploid counterparts, including *A. eucosmus* and *A. robbinsii*. However, no other genetic study to date has examined euploid *Astragalus* at the species level. Additional genetic work on subgenus *Phaca*, the group containing *A. eucosmus* and *A. robbinsii*, may reveal less variation than previously thought (Scherson et al., 2008).

Conservative allele choice may also be responsible for the AFLP profile similarity within the *eucosmus-fernaldii-minor* complex. Although eight AFLP markers were used, only the 106 alleles that were repeatable and easily quantifiable (i.e., distinct from background noise) were included; previous AFLP studies have often used hundreds of alleles

(Chung et al., 2004; Baskauf and Burke, 2009). Screening of additional markers could increase the number of variable alleles (Walker and Metcalf, 2008), but, this could also introduce confounding, difficult to score alleles into the dataset that could potentially reduce the confidence in our results (Whitlock et al., 2007).

Though many species of *Astragalus*, unlike *A. eucosmus* or *A. r.* var. *minor*, are highly endemic, representing a wide array of derived morphologies, molecular studies have previously discovered relatively poor resolution, or even lack of resolution at the species level (Schlee et al., 2010). In these cases, Schlee et al. (2010) have concluded that not all narrow endemics or even distinct morphotypes can be considered endemic, relict populations, lest phenotypic variation be mistaken for taxonomic distinction. Based upon our AFLP data, we cannot conclude that *A. r.* var. *fernaldii*, despite its subtly distinct morphology, represents a genetically distinct, independently evolving entity (Haig et al., 2006). The lack of genetic distinction seen between the putative taxa within this complex indicates that the infraspecific recognition may be unwarranted (Ellis et al., 2009).

Morphology

Morphometric analysis of the *eucosmus-fernaldii-minor* species complex elucidates more variation than did AFLP and cpDNA analyses. Principal coordinates analysis of both quantitative and qualitative characters show a partial, but not complete separation of *A. eucosmus* and *A. r.* var. *minor* (Fig. 13), as well as an almost complete overlap between *A. r.* var. *fernaldii* and *A. eucosmus* (Fig. 12). This is in agreement with Barneby's statement that *A. eucosmus* and *A. r.* var. *minor* are so morphologically similar that "fruits at least half formed" are necessary for correct identification (Barneby, 1964). PCA analysis of the morphology data from all three taxa only explains 15% and 10% of the variation within the first two principal components, indicative of the phylogenetic closeness of these three taxa (Maunder, 2006).

Principal coordinate one possesses moderately negative loadings for size variables (i.e., leaf length, pod width, see Appendix 6), and moderate positive loadings on qualitative variables; it only allows for moderate separation of the species along the x-axis (Henderson and Ferreira, 2002). Conversely, principal coordinate two consists of high positive loadings for vegetative characters and moderate to high negative loadings for pod and floral characters, but again, only allowing for slight separation of the taxa. The almost complete overlap of *A. r.* var. *fernaldii* within *A. eucosmus* along both axes indicates a closer relationship with *A. eucosmus* than *A. r.* var. *minor.* Though *A. eucosmus* and *A. r.* var. *minor* do overlap in the morphometric PCA (and the AFLP analyses), their continental range is not completely sympatric, and our sampling for both taxa is incomplete at a continental scale – this study focus on the complex as it occurs in Newfoundland and Labrador and Quebec. This indicates a very close relationship, but not a conspecific one once we take into account the geographic component of our infraspecific species concept (Pelser and Houchin, 2004).

By delineating populations of *A. eucosmus* and *A. robbinsii* by location (NF and Strait of Belle Isle compared to the rest of North America) within the principal coordinates analysis, we see that there is no differentiation between populations of *A. eucosmus* and *A. r.* var. *minor* in Newfoundland and Labrador and Québec when compared to their western and central North American conspecifics (Fig. 14). These Newfoundland and Strait of Belle Isle populations overlap to a greater extent than their mainland counterparts, indicating a potential higher degree of gene flow (Neel, 2008).

The Cap Crow population of *A. r.* var. *fernaldii* possesses a much greater amount of morphological variation than the four remaining populations, which cluster together on the *A. eucosmus* side of the analysis. As Cap Crow is thought to be most typical for this variety, and potentially the origin of the type specimen, this could explain why Rydberg's type specimen was distinctive enough for taxonomic delineation, hence leading to the subsequent taxonomic confusion.

The population of *A. eucosmus* var. *terrae novae* f. *villosus* at Cook's Harbour, while unique in the AFLP analysis, falls within the variation encompasssing *A. eucosmus* in Newfoundland and Labrador. Likewise, the contested population of *A. r.* var. *minor* at Barr'd Harbour Hill is found within *A. r.* var. *minor* in the PCA, further confirming its re-identification (Maunder, 2006).

Examination of individual quantitative characters of *A. r.* var. *fernaldii* reveals that average beak, calyx, banner petal, and flower lengths are all intermediate between those of *A. eucosmus* and *A. r.* var. *minor* (Fig. 15). This is consistent with the original treatment of the taxon, which also describes it as being intermediate within this species complex (Rydberg, 1928). By population, the *A. r.* var. *fernaldii* plants at Mont Bonenfant and Cap Crow possess beaks, calices, banner petals and flowers all longer than the remaining populations, but still consistent with *A. eucosmus*. Pod lengths for *A. r.* var. *fernaldii* were slightly longer, but not significantly different from *A. eucosmus*. Additionally, all of these measures are concordant with the variation seen in *A. eucosmus*, and stipe and pod length fall well short of the average measures seen in *A. r.* var. *minor* (Fig. 16). In light of the relatively low levels of sampling seen at each of these populations, this variability may be an artifact in the data.

Most other vegetative characters, however, including leaf and leaflet length, and stipule length show little to no differentiation between all three taxa, or by population, providing additional evidence that these taxa are phylogenetically close and difficult to separate (as per Barneby, 1964).

Peduncle and raceme lengths were similar for both flowering and fruiting states in each taxon, and were not significantly different; however, *A. r.* var. *fernaldii* possessed shorter peduncles than the other two taxa on average when flowering. This may be a phenotypically plastic adaptive trait, as Barneby noted that populations of *A. eucosmus* found in proximity to exposed shorelines are often shorter and/or more prostrate than inland populations (Barneby, 1964). In both *A. eucosmus* and *A. r.* var. *fernaldii*, the peduncle and raceme elongate markedly, while no such elongation is observed in *A. r.* var. *minor*, and may indicate that the former are more closely related (Fig. 15). This elongation contrasts sharply with a recent revision of North American *Astragalus*, which indicates that the raceme in Fernald's Milkvetch "hardly elongates" (Welsh, 2007).

In the qualitative variables scored, *A. r.* var. *fernaldii* as a whole, and in each of the five populations, is more similar to *A. eucosmus* than *A. r.* var. *minor* in both black and white calyx and pod hair densities and in corolla colour. Adaxial leaf surfaces in *A. r.* var. *fernaldii* encompass a range of states, compared to the mostly glabrous occurences in *A. eucosmus* and *A. r.* var. *minor*.

Nonetheless, adaxial pubescence was observed in both the Cook's Harbour population of *A. eucosmus* and *A. r.* var. *minor* at Pointe Amour, and ten samples of A. *r. fernaldii* were observed to be adaxially glabrous.

On stipe length and adaxial pubescence

The original diagnostic characters for *Atelophragma fernaldii*, and the now synonymous *A. r.* var. *fernaldii* are a stipe of intermediate length and white adaxial pubescence on the leaflets (Barneby, 1964). Stipe length, the only unambiguous, quantitative diagnostic character for *A. r.* var. *fernaldii* (Maunder, 2006), and the reason that the variety was placed within *A. robbinsii* in the first place, was found to be the same, on average, to that measured in *A. eucosmus*, and significantly different from the average value for *A. r.* var. *minor*. Specimens from Cap Crow, Mont Bonenfant, and one specimen from the Québec/Labrador Border have longer pods and stipes than the remaining populations of *A. r.* var. *fernaldii* (Fig. 16); however, even here these lengths are consistent with *A. eucosmus*, thus eliminating the usefulness of this diagnostic character for identification (Morisset, 1997).

As stipe length in *A. r.* var. *fernaldii* is not statistically different from *A. eucosmus*, the only diagnostic character retained in Fernald's Milkvetch is white adaxial pubescence, a trait found in *A. eucosmus* as well. Pubescence was disregarded by Barneby during his treatment of the species complex as it was considered too highly variable (Barneby, 1964).

Additionally, hair characters have been found to be phenotypically plastic (Zarre, 2003), and the presence of hairs is plesiomorphic (Taeb et al., 2007) in *Astragalus*, thus increasing the ambiguity when scoring. Taxa described by only one or two ambiguous characters are often treated as suspect until confirmation of genetic distinction. For example, when Perrie and Brownsey found similar patterns in New Zealand *Asplenium*, they argued for the discontinued recognition of a taxon (Perrie and Brownsey, 2005).

Morphologically, the entire Astragalean clade within the Fabaceae is regarded as highly diverse, but Sanderson and Wojciechowski (1996) failed to find a specific morphological innovation or ecological factor that may be responsible for its radiation. They concluded that some fundamental trait within the lineage, likely genetic or biochemical, was responsible for this expansion, and that all of *Astragalus* had an extremely plastic morphology. Most importantly, they found that the number of species described by these data was entirely dependent upon personal taxonomic practice, and hindered by the genus' predilection for parallel evolution.

Rydberg, who was known as a "splitter" within the systematic community (one who extensively divides taxa based on minute differences), may have interpreted this high within-species variability as useful systematic data, as per his personal taxonomic practice. Frequently, genera and species circumscribed by his methods were based on variable and “trivial” diagnostic characters (Walsh et al., 2007). In fact, nearly all of the genera he initially split from *Astragalus* have since been returned to the genus (Barneby, 1964). With the majority of morphological data showing a closer relationship between *A. r.* var. *fernaldii* and *A. eucosmus* than with *A. r.* var. *minor*, consistent with Rousseau (1933) and Boivin (1967), it seems likely that Rydberg's original description of *Atelophragma fernaldii* (from Fernald’s identification of *A. eucosmus*) failed to consider the plasticity inherent in Atlantic Canadian *Astragalus* (Maunder, 2006). This was continued in Barneby’s treatment, as a result of the type specimen possessing a longer than average stipe.

Putting it all together – the taxonomic fate of *A. r.* var. *fernaldii*

On the basis of AFLP, cpDNA, and morphometric data, the continued taxonomic recognition of *Astragalus robbinsii* var. *fernaldii* is not warranted.

AFLPs failed to detect phylogenetically informative inter or infra-specific variation in the *eucosmus-fernaldii-minor* complex in the Strait of Belle Isle Region, and both chloroplast DNA sequences and morphometric analysis indicate a closer relationship between *A. eucosmus* and the populations currently classified as *A. r.* var. *fernaldii* than to *A. r.* var. *minor*.

As per our infraspecific species concept from Pelser and Houchin (2004), the complete overlap of *A. r.* var. *fernaldii* with *A. eucosmus* in the morphometric analysis, and to a lesser extent the cpDNA and AFLP data, indicates a conspecific relationship. This data supports our hypothesis that *A. r.* var. *fernaldii* is more closely related to *A. eucosmus* than *A. r.* var. *minor*, and does not represent a derived form of the latter species.

Astragalus eucosmus is already considered to be a highly phenotypically plastic species, consisting of multiple varieties and forms endemic to Atlantic Canada, and as a whole is spread throughout North America (Barneby, 1964). The phenotypic traits that have previously been used to distinguish *A. r.* var. *fernaldii* from *A. eucosmus* may not be correlated with genetic differentiation, but rather may be a result of climatological or edaphic conditions, and are hence indicative of plasticity within a larger species rather than delineation as a threatened entity (Haig et al., 2006).

In light of the sympatric range of *A. eucosmus*, and similarities in habitat type (obligate calciphile), genetics and morphology, our data support the complete transfer of *A. r.* var. *fernaldii* to *A. eucosmus* (for a similar study and conclusion in *Cerastium* L. see Gustafson et al., 2003). Boivin's proposed *A. eucosmus* var. *fernaldii* is not appropriate as the primary defining character, stipe length, is not significantly different from that seen in *A. eucosmus*. Additionally, the difficulty in circumscribing these five populations due to the inconsistent appearance of each taxon makes describing natural infraspecific taxa of any rank (subspecies, variety, or form) impossible.

Conservation and implications for policymakers

In their article on the listing of subspecies under endangered species guidelines, O'Brien and Mayr (1991) noted that for endangered subspecies, or any distinct taxonomic grouping, genetic and morphological data are frequently contradictory. To clarify which morphologically distinct populations are deserving of protection, they published three guidelines for biologists and conservationists: (1) that a population display phylogenetically concordant phenotypic traits, (2) that they are geographically distinct, and (3) that they have a unique evolutionary history. The five Blanc Sablon area populations of *A. eucosmus* (formerly *A. r.* var. *fernaldii*) fail to meet these criteria. If we omit pubescence as a useful character for the above mentioned reasons, then the most important character is stipe length. In the five populations of *A. eucosmus/A. r.* var. *fernaldii,* stipe length is the same as in *A. eucosmus*, and does not support taxonomic separation. On a broad geographic scale, the range of the Blanc Sablon populations is sympatric with both *A. r.* var. *minor* and *A. eucosmus*. Finally, while we were unable to differentiate the evolutionary history of the Blanc Sablon populations, there is no evidence that it would be different from local congeners, or other *Astragalus* in the Strait of Belle Isle region (Morriset, 1997). Under the following guidelines, the Blanc Sablon populations of *A. eucosmus* (the five previous *A. r.* var. *fernaldii* populations) would likely not be a conservation priority, as these populations currently cannot be confirmed to be a distinct evolutionary entity (Chung et al., 2004).

Although we are recommending the rejection of *A. r.* var. *fernaldii* as a valid taxon and the subsequent transfer of the five remaining Blanc Sablon populations to *A. eucosmus*, we are not endorsing the wholesale neglect of these populations. *Astragalus eucosmus* is still a provincially uncommon species in Newfoundland and Labrador (Maunder, 2006), and certainly these populations represent a distinctly hairy morphotype of this circumpolar species. While these populations may no longer be federally or provincially warded, monitoring of these populations to prevent extirpation from anthropogenic activities and illegal quarrying is encouraged (Morriset, 1997).

Additionally, the residents of the community of Blanc Sablon should be made aware of this proposed taxonomic revision. Previous conservation efforts have made inroads in communicating the need to conserve this rare plant through the construction of an interpretive walking trail and the education of local youth. Notification of the taxonomic revision to local stakeholders would help show that science is indeed at work in their community. We should stress that the habitat that these populations of *A. eucosmus* inhabit in the Blanc Sablon area still vulnerable to human influence (notably from illegal quarrying), and it is still one of the only known stations for another endemic taxon, *Draba glabella* Pursh. var. *pycnosperma* Fernald & C.H. Knowlt. (Al-Shebaz et al., 2009). Conservation of the five Blanc Sablon populations, regardless of their status as a rare variety, represents the opportunity to maintain a wild area significant to the natural heritage of the region within this increasingly developed North Shore community (Morriset, 1997).

Future research

Future work should attempt to confirm that *A. r.* var. *fernaldii* is a phenotypically plastic form of *A. eucosmus* through reciprocal transplants throughout its range in Atlantic Canada, and the use of common garden/glasshouse experiments to determine the plasticity of the genus (Gustafson et al., 2003).

As mentioned before, AFLP sampling should be expanded to include populations of *A. eucosmus* and *A. r.* var. *minor* from across North America, to determine if in actuality there is a phylogeographic effect on genotype within this species complex. Additionally, inclusion of the remaining varieties of *A. robbinsii* in the genetic analysis, possibly as newly collected material, may provide insight into patterns of genetic diversity and varietal delineation in this compound species.

Finally, additional basic research into the habitat requirements, reproduction biology, and ecology of *A. eucosmus* and *A. robbinsii*, two of the most widely spread species of North American *Astragalus*, would greatly benefit our understanding of the causes of endemism and the biogeography within this extremely diverse clade.

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Appendix 1: Voucher data for *Astragalus* specimens collected for study, Paul Sokoloff et al., 2008-2009.

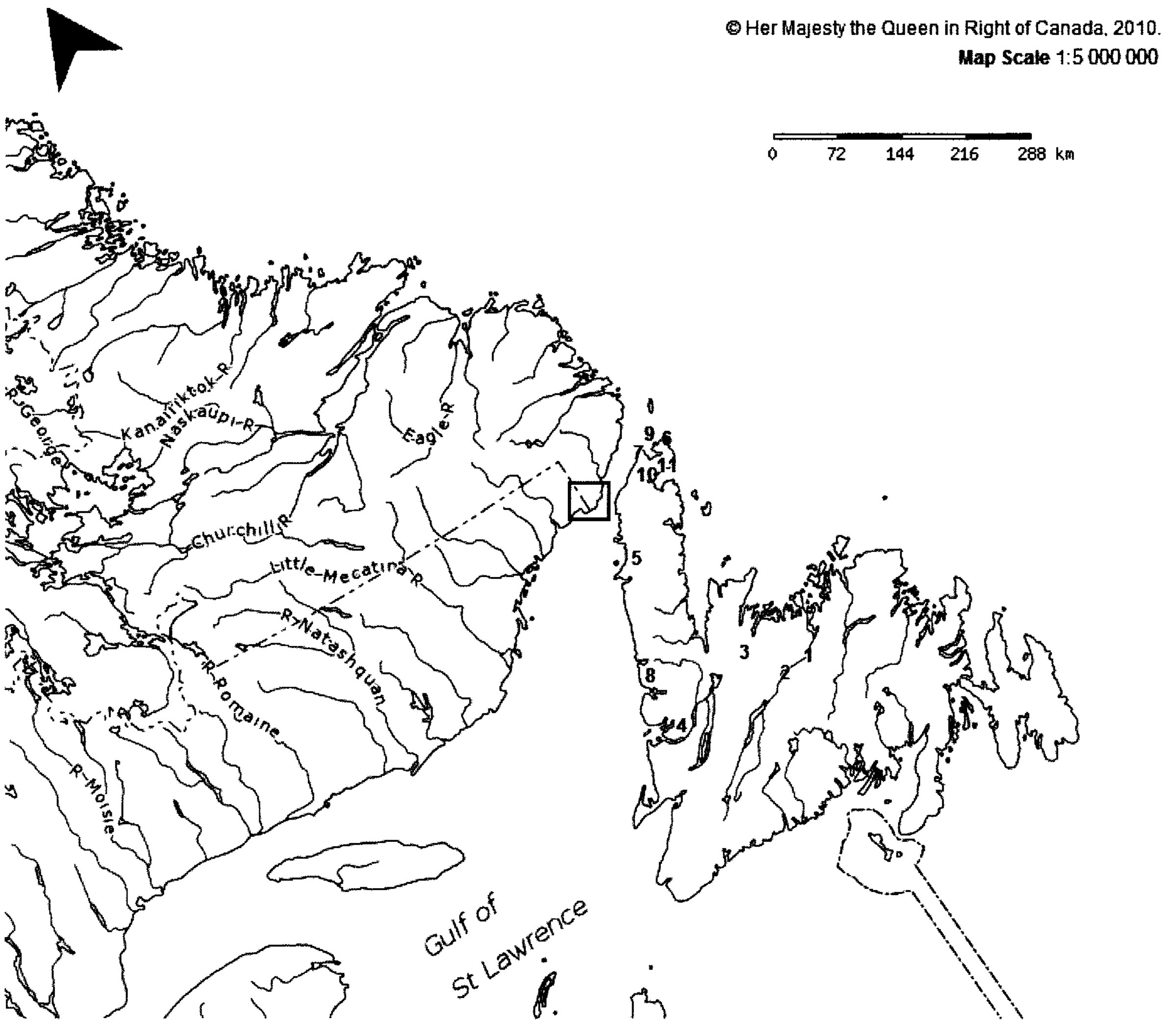
Taxon	**Collection # (DNA#)**	**CAN #**	**Prov.**	**Site Name**	**Lat**	**Long**	**Elev. (m)**
Astragalus eucosmus	Cape Mauve 1	593743	NL	Cape Mauve	51°35`56.62"N	-55°28`13.83"W	54m
Astragalus eucosmus	Sokoloff 1	593742	NL	Indian River	49°29`48.72"N	-56°10`41.12"W	58m
Astragalus eucosmus	Sokoloff 2	593740	NL	Bishop's Falls	49°29`48.72"N	-56°10`41.12"W	8m
Astragalus eucosmus	Sokoloff 3	593738	NL	Grand Falls	48°55`25.95"N	-55°39`11.47"W	36m
Astragalus eucosmus	Sokoloff 4	593741	NL	Grand Falls, Trail	48°55`30.61"N	-55°39`21.52"W	36m
Astragalus eucosmus	Sokoloff 5	593739	NL	Indian River,	49°29`45.11"N	-56°10`48.04"W	58m
Astragalus eucosmus	Sokoloff 5.1	593746	NL	Indian River	49°29`45.11"N	-56°10`48.04"W	58m
Astragalus eucosmus	Sokoloff 5.2	593745	NL	Indian River	49°29`45.11"N	-56°10`48.04"W	58m
Astragalus eucosmus	Sokoloff 6	593737	NL	Corner Brook	48°56`58.55"N	-57°52`27.1"W	10m
Astragalus eucosmus	Sokoloff 6.1	593705	NL	Corner Brook	48°56`58.55"N	-57°52`27.1"W	10m
Astragalus robbinsii var. *minor*	Sokoloff 7	593744	NL	Barr'd Hill	50°49`53.58"N	-57°1`19.69"W	364m
Astragalus eucosmus	Sokoloff 8	593708	NL	Cook's Harbour	51°36`30.08"N	-55°53`5.13"W	0m
Astragalus bodinii	Sokoloff 9 (P0111)	593747	NL	Cook's Harbour	51°36`30.08"N	-55°53`5.13"W	0m
Astragalus robbinsii var. *fernaldii*	Sokoloff 10 (P0115)	593779	QC	Cap Crow	51°24`55.69"N	-57°11`56.67"W	29m
Astragalus robbinsii var. *fernaldii*	Sokoloff 11 (P0126)	593780	QC	Cap Crow	51°24`55.29"N	-57°11`59.98"W	29m
Astragalus robbinsii var. *fernaldii*	Sokoloff 12 (P0131)	593777	QC	Cap Crow	51°25`6.7"N	-57°11`51.02"W	29m
Astragalus robbinsii var. *fernaldii*	Sokoloff 12.5 (P0132)	593710	QC	Cap Crow	51°25`8.26"N	-57°11`48.13"W	29m
Astragalus robbinsii var. *fernaldii*	Sokoloff 13 (P0133)	593776	QC	Cap Crow	51°25`8.26"N	-57°11`48.13"W	29m
Astragalus robbinsii var. *fernaldii*	Sokoloff 14 (P0134)	593775	QC	Cap Crow	51°25`6.9"N	-57°11`50.2"W	29m
Astragalus robbinsii var. *fernaldii*	Sokoloff 15 (P0137)	593712	QC	Cap Crow	51°25`8.26"N	-57°11`48.13"W	29m
Astragalus robbinsii var. *fernaldii*	Sokoloff 16 (P0138)	593782	QC	Mont Parent	51°25`29.23"N	-57°9`44.83"W	93m
Astragalus robbinsii var. *fernaldii*	Sokoloff 17 (P0139)	593783	QC	Mont Parent	51°25`22.6"N	-57°9`41.6"W	93m
Astragalus robbinsii var. *fernaldii*	Sokoloff 18 (P0140)	593781	QC	Mont Parent	51°25`25.22"N	-57°9`40.21"W	93m
Astragalus robbinsii var. *fernaldii*	Sokoloff 19 (P0141)	593778	QC	Mont Parent	51°25`21.45"N	-57°9`27.25"W	93m
Astragalus robbinsii var. *fernaldii*	Sokoloff 20 (P0143)	593771	QC	Mont Parent	51°25`10.78"N	-57°9`24.84"W	93m
Astragalus robbinsii var. *fernaldii*	Sokoloff 21 (P0152)	593774	QC	Québec Labrador	51°25`0.6"N	-57°6`13.67"W	69m
Astragalus robbinsii var. *fernaldii*	Sokoloff 22 (P0157)	593772	QC	Québec Labrador	51°25`1.15"N	-57°6`14.08"W	69m
Astragalus robbinsii var. *fernaldii*	Sokoloff 23 (P0158)	593768	QC	Québec Labrador	51°25`1.76"N	-57°6`15.27"W	69m
Astragalus robbinsii var. *fernaldii*	Sokoloff 24 (P0164)	593773	QC	Île-au-Bois	51°23`28.54"N	-57°8`41.49"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 25 (P0166)	593762	QC	Île-au-Bois	51°23`27.92"N	-57°8`43.14"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 26 (P0168)	593763	QC	Île-au-Bois	51°23`27.08"N	-57°8`41.12"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 27 (P0169)	593713	QC	Île-au-Bois	51°23`27.08"N	-57°8`41.12"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 28 (P0170)	593761	QC	Île-au-Bois	51°23`25.4"N	-57°8`41.38"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 29 (P0172)	593720	QC	Île-au-Bois	51°23`28.54"N	-57°8`41.49"W	6m

Taxon	Collection # (DNA#)	CAN #	Prov.	Site Name	Lat	Long	Elev. (m)
Astragalus robbinsii var. *fernaldii*	Sokoloff 30 (P0173)	593719	QC	Île-au-Bois	51°23`27.92"N	-57°8`43.14"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 31 (P0174)	593718	QC	Île-au-Bois	51°23`27.08"N	-57°8`41.12"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 32 (P0175)	593717	QC	Île-au-Bois	51°23`27.08"N	-57°8`41.12"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 33 (P0176)	593764	QC	Île-au-Bois	51°23`25.4"N	-57°8`41.38"W	6m
Astragalus robbinsii var. *fernaldii*	Sokoloff 34 (P0177)	593765	QC	Mont Bonenfant	51°25`44.19"N	-57°7`29.06"W	76m
Astragalus robbinsii var. *fernaldii*	Sokoloff 35 (P0178)	593766	QC	Mont Bonenfant	51°25`34.65"N	-57°7`24.53"W	76m
Astragalus robbinsii var. *fernaldii*	Sokoloff 35.5 (P0181)	593770	QC	Mont Bonenfant	51°25`31.12"N	-57°7`23.48"W	76m
Astragalus robbinsii var. *fernaldii*	Sokoloff 36 (P0182)	593769	QC	Mont Bonenfant	51°25`29.15"N	-57°7`20.37"W	76m
Astragalus robbinsii var. *fernaldii*	Sokoloff 36.1 (P0179)	593767	QC	Mont Bonenfant	51°25`31.12"N	-57°7`23.48"W	76m
Astragalus robbinsii var. *fernaldii*	Sokoloff 37 (P0184)	593715	QC	Mont Bonenfant	51°25`44.19"N	-57°7`29.06"W	76m
Astragalus robbinsii var. *fernaldii*	Sokoloff 38 (P0185)	593716	QC	Mont Bonenfant	51°25`34.65"N	-57°7`24.53"W	76m
Astragalus robbinsii var. *fernaldii*	Sokoloff 39 (P0186)	593714	QC	Mont Bonenfant	51°25`29.15"N	-57°7`20.37"W	76m
Astragalus robbinsii var. *minor*	Sokoloff 40 (P0188)	593734	NL	Battery Trail	51°32`27.74"N	-56°46`19.76"W	53m
Astragalus robbinsii var. *minor*	Sokoloff 41 (P0189)	593735	NL	Battery Trail	51°32`27.74"N	-56°46`19.76"W	53m
Astragalus robbinsii var. *minor*	Sokoloff 42 (P0192)	593736	NL	Battery Trail	51°32`26.32"N	-56°46`20.18"W	53m
Astragalus robbinsii var. *minor*	Sokoloff 43 (P0193)	593732	NL	Battery Trail	51°32`25.55"N	-56°46`20.55"W	53m
Astragalus robbinsii var. *minor*	Sokoloff 44 (P0195)	593733	NL	Battery Trail	51°32`21.86"N	-56°46`21.66"W	53m
Astragalus robbinsii var. *minor*	Sokoloff 45 (P0196)	593730	NL	Battery Trail	51°32`20.34"N	-56°46`21.72"W	53m
Astragalus alpinus	Sokoloff 46 (P0197)	593731	NL	Battery Trail	51°32`20.34"N	-56°46`21.72"W	53m
Astragalus robbinsii var. *minor*	Sokoloff 47 (P0198)	593729	NL	Battery Trail	51°32`19.59"N	-56°46`22.08"W	53m
Astragalus robbinsii var. *minor*	Sokoloff 48 (P0200)	593728	NL	Battery Trail	51°32`17.33"N	-56°46`23.24"W	53m
Astragalus alpinus	Sokoloff 49 (P0201)	593711	NL	Battery Trail	51°32`17.33"N	-56°46`23.24"W	53m
Astragalus robbinsii var. *minor*	Sokoloff 50 (P0203)	593748	NL	Pointe Amour	51°27`40.81"N	-56°51`22.88"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 51 (P0204)	593727	NL	Pointe Amour	51°27`40.81"N	-56°51`22.88"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 52 (P0205)	593726	NL	Pointe Amour	51°27`40.81"N	-56°51`22.88"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 53 (P0206)	593722	NL	Pointe Amour	51°27`40.94"N	-56°51`22.06"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 54 (P0207)	593724	NL	Pointe Amour	51°27`41.93"N	-56°51`37.96"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 55 (P0209)	593721	NL	Pointe Amour	51°27`42.44"N	-56°51`36.15"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 56 (P0210)	593709	NL	Pointe Amour	51°27`42.73"N	-56°51`34.9"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 57 (P0211)	593725	NL	Pointe Amour	51°28`12.5"N	-56°50`29.97"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 58 (P0212)	593749	NL	Pointe Amour	51°28`12.63"N	-56°50`29.92"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 59 (P0213)	593750	NL	Pointe Amour	51°28`13.11"N	-56°50`30.38"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 60 (P0214)	593751	NL	Pointe Amour	51°28`10.58"N	-56°50`23.19"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 61 (P0216)	593752	NL	Pointe Amour	51°28`9.74"N	-56°50`23.86"W	16m

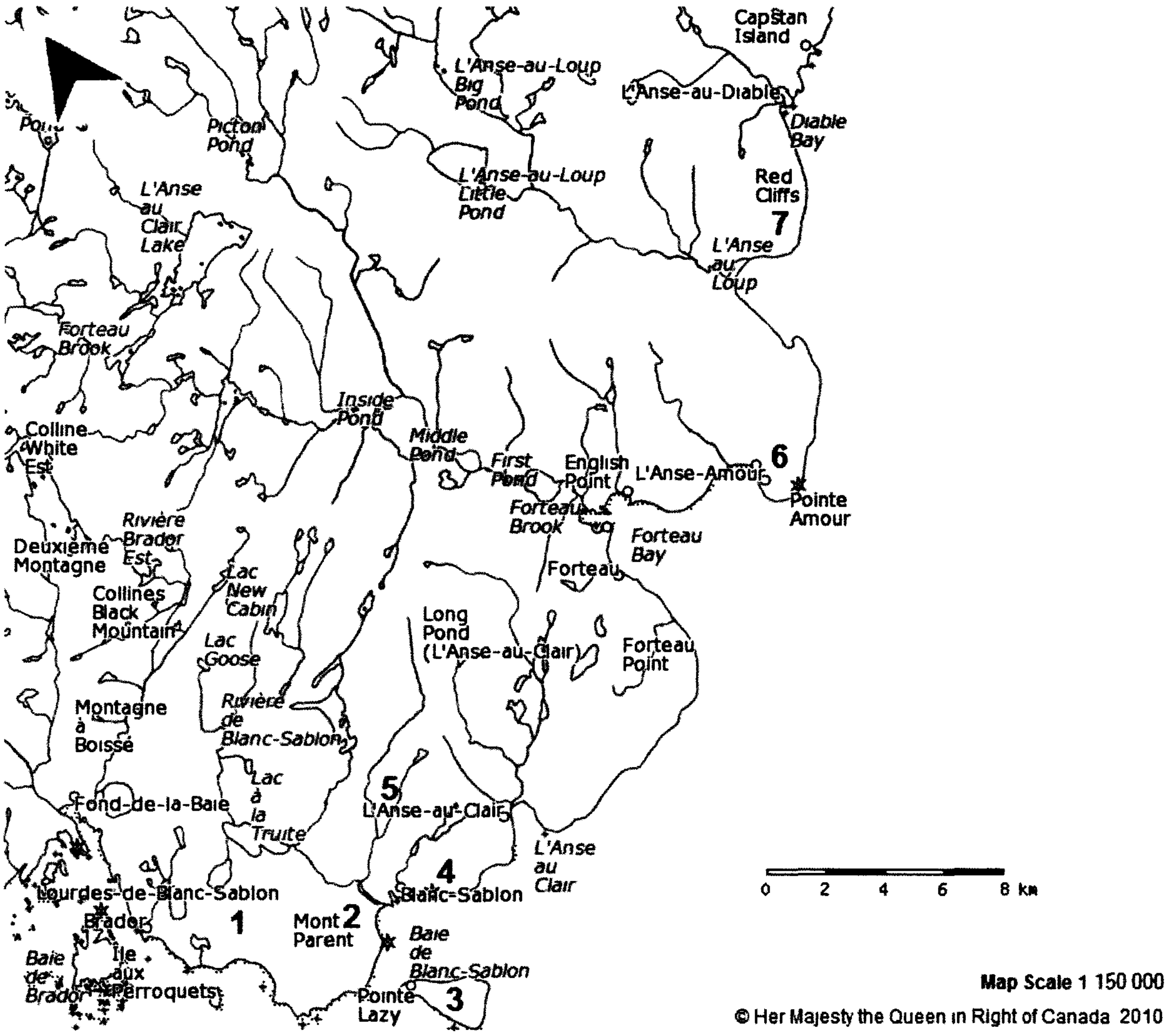
Taxon	Collection # (DNA#)	CAN #	Prov.	Site Name	Lat	Long	Elev. (m)
Astragalus robbinsii var. *minor*	Sokoloff 62 (P0217)	593753	NL	Pointe Amour	51°28`0.17"N	-56°52`23.67"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 63 (P0218)	593754	NL	Pointe Amour	51°27`59.17"N	-56°52`23.62"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 64 (P0219)	593755	NL	Pointe Amour	51°27`58.55"N	-56°52`23.72"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 65 (P0220)	593756	NL	Pointe Amour	51°27`57.94"N	-56°52`23.78"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 66 (P0222)	593723	NL	Pointe Amour	51°28`30"N	-56°52`9.07"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 67 (P0225)	593758	NL	Pointe Amour	51°28`30"N	-56°52`9.07"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 69 (P0221)	593757	NL	Pointe Amour	51°28`30"N	-56°52`9.07"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 70 (P0226)	593760	NL	Pointe Amour	51°28`30"N	-56°52`9.07"W	16m
Astragalus robbinsii var. *minor*	Sokoloff 71 (P0227)	593759	NL	Pointe Amour	51°28`30"N	-56°52`9.07"W	16m
Astragalus eucosmus	Sokoloff 100a (P0345)	593802	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100b (P0346)	593804	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100c (P0347)	393798	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100d (P0348)	593807	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100e (P0349)	593706	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100f (P0350)	593811	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100g (P0351)	593707	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100h (P0352)	593812	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100i (P0353)	593797	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100j (P0354)	593801	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100k (P0355)	593806	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100l (P0056)	593799	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100m(P0357)	593796	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100n (P0358)	593805	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100o (P0359)	593808	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100p (P0360)	593803	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100q (P0361)	593809	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100r (P0362)	593800	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus eucosmus	Sokoloff 100s (P0363)	593810	NB	Restigouche River	47°57`43"N	-66°56`27"W	28m
Astragalus alpinus	Sokoloff 101a (P0364)	593786	NB	Restigouche River	47°53`26"N	-66°57`17"W	18m
Astragalus alpinus	Sokoloff 101b (P0365)	593789	NB	Restigouche River	47°53`26"N	-66°57`17"W	18m
Astragalus alpinus	Sokoloff 101c (P0366)	593784	NB	Restigouche River	47°53`26"N	-66°57`17"W	18m
Astragalus alpinus	Sokoloff 101d (P0367)	593788	NB	Restigouche River	47°53`26"N	-66°57`17"W	18m
Astragalus alpinus	Sokoloff 101e (P0368)	593790	NB	Restigouche River	47°53`26"N	-66°57`17"W	18m
Astragalus alpinus	Sokoloff 101f (P0369)	593785	NB	Restigouche River	47°53`26"N	-66°57`17"W	18m

Taxon	Collection # (DNA#)	CAN #	Prov.	Site Name	Lat	Long	Elev. (m)
Astragalus alpinus	Sokoloff 101g (P0370)	593787	NB	Restigouche River	47°53`26"N	-66°57`17"W	18m
Astragalus eucosmus	Sokoloff 102a (P0371)	593793	NB	Hammond River	45°25`37"N	-65°53`35"W	10m
Astragalus eucosmus	Sokoloff 102b (P0372)	593791	NB	Hammond River	45°25`37"N	-65°53`35"W	10m
Astragalus eucosmus	Sokoloff 102c (P0373)	593794	NB	Hammond River	45°25`37"N	-65°53`35"W	10m
Astragalus eucosmus	Sokoloff 102d (P0374)	593795	NB	Hammond River	45°25`37"N	-65°53`35"W	10m
Astragalus eucosmus	Sokoloff 102e (P0375)	593792	NB	Hammond River	45°25`37"N	-65°53`35"W	10m

Appendix 2: Geographic distribution of *Astragalus* populations sampled for cpDNA and AFLP studies, Newfoundland and Labrador and the Strait of Belle Isle



Map 1: *Astragalus* populations sampled in Newfoundland and Labrador for AFLP and cpDNA analysis, and accompanying morphological vouchers. Populations sampled include [1]: Bishop's Falls (*A. eucosmus*), [2]: Grand Falls (*A. eucosmus*), [3]: Indian River (*A. eucosmus*), [4]: Corner Brook (*A. eucosmus*), [5]: Barr'd Harbour Hill/Doctor's Brook (*A. r.* var. *minor*), [6]: Cape Mauve (*A. eucosmus*), [7]: Cook's Harbour (*A. eucosmus/A. bodinii*), [8]: St. Paul's (*A. r.* var. *minor*), [9]: Little Sacred Island (*A. eucosmus*), [10]: HaHa Mountain (*A. eucosmus*), [11]: Burnt Cape Ecological Reserve (*A. eucosmus*). Inset is represented in Map 2. Map courtesy of Natural Resources Canada.



Map 2: *Astragalus* populations sampled in the Strait of Belle Isle region, Newfoundland and Labrador and Québec, for cpDNA, AFLP and morphometric studies. Populations sampled include [1]: Cap Crow (*A. r.* var. *fernaldii* – type), [2]: Mont Parent (*A. r.* var. *fernaldii*), [3]: Île-au-Bois (*A. r.* var. *fernaldii*), [4]: Québec/Labrador Border (*A. r.* var. *fernaldii*), [5]: Mont Bonenfant *A. r.* var. *fernaldii*), [6]: Pointe Amour (*A. r.* var. *minor*), [7]: Battery Trail (*A. r.* var. *minor* /*A. alpinus*). Map courtesy of Natural Resources Canada.

Appendix 3: *Astragalus* Specimens Examined for Morphometric Analysis, including Sokoloff et al. samples, Claudia Hanel samples, herbarium specimens (CAN, NYBG, GH, UBC, UAC, MT, QFA) and corresponding DNA samples.

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus alpinus	CAN	ON	-	MT	-	10099	-
Astragalus alpinus	CAN	NF	Battery Trail	CAN	593731	Sokoloff 46	P0197
Astragalus alpinus	CAN	NF	Battery Trail	CAN	593711	Sokoloff 49	P0201
Astragalus alpinus	CAN	NB	Restigouche River	CAN	593784	Sokoloff 100t	P0366
Astragalus alpinus	CAN	NB	Restigouche River	CAN	593785	Sokoloff 100u	P0369
Astragalus alpinus	CAN	NB	Restigouche River	CAN	593790	Sokoloff 100v	P0368
Astragalus alpinus	CAN	NB	Restigouche River	CAN	593786	Sokoloff 100w	P0364
Astragalus alpinus	CAN	NB	Restigouche River	CAN	593787	Sokoloff 100x	P0370
Astragalus alpinus	CAN	NB	Restigouche River	CAN	593785	Sokoloff 100y	P0369
Astragalus alpinus	CAN	NB	Restigouche River	CAN	593789	Sokoloff 100z	P0365
Astragalus australis	CAN	NT	Aulavik National Park	CAN	582412	Aiken 99-035	P0286
Astragalus australis	CAN	NT	Banks Island	CAN	583696	Elven 199908-12	P0285
Astragalus australis	CAN	NT	Okpilik Lake	CAN	586860	Bandringa 308	P0290
Astragalus australis	U.S.A.	AK	Yukon	CAN	454489	Murray 6154	P0293
Astragalus bodinii	CAN	NF	Cook's Harbour	CAN	593747	Sokoloff 9	P0111
Astragalus eucosmus	U.S.A.	AK	-	CAN	511083	Lepage 20-439	-
Astragalus eucosmus	U.S.A.	AK	-	CAN	271590	Raymond 412	-
Astragalus eucosmus	U.S.A.	AK	-	CAN	76307	Anderson 8537	-
Astragalus eucosmus	U.S.A.	AK	-	CAN	76308	Anderson 8778	-
Astragalus eucosmus	CAN	NF	-	CAN	204331	Lepage 21-611	-
Astragalus eucosmus	CAN	NF	-	CAN	76255	-	-
Astragalus eucosmus	CAN	NF	-	CAN	566880	Brouillet 92310	P0259
Astragalus eucosmus	CAN	AB	-	UAC	16401	-	-
Astragalus eucosmus	CAN	BC	-	UAC	48616	UAC 48616	P0304
Astragalus eucosmus	CAN	AB	-	UAC	53264	MacDonald 16b2	P0305
Astragalus eucosmus	CAN	AB	-	UAC	59490	Crack 74-43	P0308
Astragalus eucosmus	CAN	AB	-	UAC	57804	Strong s.n.	P0309
Astragalus eucosmus	CAN	AB	-	UAC	40493	Stewart 124b	P0310
Astragalus eucosmus	CAN	AB	-	UAC	16394	Moss 12270	P0311
Astragalus eucosmus	CAN	AB	-	UAC	40492	Stewart 104	P0312
Astragalus eucosmus	CAN	BC	-	UAC	43210	Long 1-83-48	P0313
Astragalus eucosmus	CAN	BC	-	UAC	45418	Chinnappa 1305	P0314
Astragalus eucosmus	CAN	BC	-	UAC	45419	Chinnappa 1277	P0315
Astragalus eucosmus	CAN	BC	-	UAC	45420	Chinnappa 1274	P0316

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus eucosmus	CAN	BC	Beatton River	CAN	280948	Correll 10430	-
Astragalus eucosmus	CAN	BC	Beatton River	CAN	257445	Taylor 62	-
Astragalus eucosmus	CAN	NF	Bishop's Falls	CAN	593740	Sokoloff 2	-
Astragalus eucosmus	CAN	ON	Black Duck	CAN	260455	Moir 2091	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496383	Deshaye J94-1350	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496353	Deshaye J94-259	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496352	Deshaye J94-244	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	495216	Deshaye J94-981	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496385	Deshaye J94-1387	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496384	Deshaye J94-1336	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496011	Deshaye J94-346	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	495107	Deshaye J94-1193	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496187	Deshaye J94-1264	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	495859	Deshaye J94-773	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	495911	Deshaye J94-845	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496291	Deshaye J94-1705	-
Astragalus eucosmus	CAN	NF	Blanc Sablon	QFA	496290	Deshaye J94-1706	-
Astragalus eucosmus	CAN	AB	Bragg Creek	UAC	48389	Chinnappa s.n.	-
Astragalus eucosmus	CAN	ON	Brant River	CAN	320625	Cowell 1075	P0261
Astragalus eucosmus	CAN	NF	Breakfast Head	CAN	205188	Tuomikoski 233	-
Astragalus eucosmus	CAN	NF	Breakfast Head	CAN	545191	Brouillet 87591	P0258
Astragalus eucosmus	CAN	AB	Cadomin	CAN	555056	Welsh 24585	P0266
Astragalus eucosmus	CAN	NF	Cape Ardoise	-	-	Hanel 0707261	CH26-1
Astragalus eucosmus	CAN	NF	Cape Ardoise	-	-	Hanel 0707269	CH26-9
Astragalus eucosmus	CAN	NF	Cape Ardoise	CAN	545192	Hay 87301	-
Astragalus eucosmus	CAN	NF	Cape Mauve	CAN	593770	Cape Mauve	-
Astragalus eucosmus	CAN	NF	Cook's Harbour	CAN	593708	Sokoloff 8	-
Astragalus eucosmus	CAN	NF	Cook's Harbour	-	-	Hanel 0707241	CH24-2
Astragalus eucosmus	CAN	NF	Cook's Harbour	CAN	545194	Brouillet 87115	-
Astragalus eucosmus	CAN	NF	Cook's Harbour	CAN	566849	Brouillet 92045	-
Astragalus eucosmus	CAN	NF	Corner Brook, TCH	CAN	593737	Sokoloff 6	-
Astragalus eucosmus	CAN	NF	Corner Brook, TCH	CAN	593705	Sokoloff 6.1	-
Astragalus eucosmus	CAN	NF	Corner Brook, TCH	MT	-	Mann896	-
Astragalus eucosmus	CAN	NF	Corner Brook, TCH	MT	-	Vincent 21g	-

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus eucosmus	CAN	ID	Custer County	CAN	553806	Blake s.n.	-
Astragalus eucosmus	CAN	ON	Fawn River	CAN	250457	Moir 661	-
Astragalus eucosmus	CAN	ON	Fort Severn	CAN	242685	Hustich 1458	-
Astragalus eucosmus	CAN	NF	Grand Falls	CAN	593738	Sokoloff 3	-
Astragalus eucosmus	CAN	NF	Grand Falls, Trail	CAN	593741	Sokoloff 4	-
Astragalus eucosmus	CAN	NF	HaHa Bay	GH	58731	Fernald 28588	-
Astragalus eucosmus	CAN	NF	HaHa Bay	CAN	205190	Tuomikoski 285	-
Astragalus eucosmus	CAN	NF	HaHa Mountain	-	-	Hanel 07072531	CH25-3
Astragalus eucosmus	CAN	NB	Hammond River	CAN	593794	Sokoloff 101a	P0373
Astragalus eucosmus	CAN	NB	Hammond River	CAN	594793	Sokoloff 101b	P0371
Astragalus eucosmus	CAN	NB	Hammond River	CAN	593795	Sokoloff 101c	P0374
Astragalus eucosmus	CAN	ON	Hudson Bay	CAN	414272	Baldwin 7551	-
Astragalus eucosmus	CAN	NF	Indian River	CAN	593739	Sokoloff 5	-
Astragalus eucosmus	CAN	NF	Indian River	CAN	593746	Sokoloff 5.1	-
Astragalus eucosmus	CAN	NF	Indian River	CAN	593745	Sokoloff 5.2	-
Astragalus eucosmus	CAN	NF	Indian River	CAN	593742	Sokoloff 1	-
Astragalus eucosmus	CAN	NT	Iqualuit	CAN	585790	Aiken 02-064	P0256
Astragalus eucosmus	CAN	ON	James Bay	CAN	76272	Dutilly 16-440	-
Astragalus eucosmus	CAN	ON	James Bay	CAN	219691	Dutilly 30-796	-
Astragalus eucosmus	CAN	ON	Jigsaw Island	CAN	414356	Baldwin 7636	-
Astragalus eucosmus	CAN	NF	L'Anse aux Meadows	CAN	240291	Rouleau 497	-
Astragalus eucosmus	CAN	NF	L'anse aux Sauvages	-	-	Hanel 07072516	CH25-17
Astragalus eucosmus	CAN	BC	Liard Crossing	CAN	264041	Porsild 22240	-
Astragalus eucosmus	CAN	NF	Little Quirpon	-	-	Hanel 0707259	CH25-10
Astragalus eucosmus	CAN	NF	Little Quirpon	-	-	Hanel 0707259	CH25-10
Astragalus eucosmus	CAN	NF	Mountain	-	-	Hanel 07072532	CH25-3
Astragalus eucosmus	CAN	SK	Nipawin River	CAN	437624	Baldwin 11047	P0264
Astragalus eucosmus	CAN	SK	Nipawin River	CAN	418553	Baldwin 11044	P0265
Astragalus eucosmus	U.S.A.	MT	Park County	CAN	176559	Hitchcock 16683	-
Astragalus eucosmus	CAN	NF	Portland Head	CAN	385665	Bouchard 74038	-
Astragalus eucosmus	CAN	YK	Quartet Lake	UBC	V223033	Bennet 05-01177	P0332
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593802	Sokoloff 100a	P0345
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593804	Sokoloff 100b	P0346
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593807	Sokoloff 100d	P0348

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593706	Sokoloff 100e	P0349
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593811	Sokoloff 100f	P0350
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593707	Sokoloff 100g	P0351
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593812	Sokoloff 100h	P0352
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593801	Sokoloff 100j	P0354
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593806	Sokoloff 100k	P0355
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593805	Sokoloff 100n	P0358
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593808	Sokoloff 100o	P0359
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593809	Sokoloff 100q	P0361
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593800	Sokoloff 100r	P0362
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593810	Sokoloff 100s	P0363
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593791	Sokoloff 102b	P0372
Astragalus eucosmus	CAN	NB	Restigouche River	CAN	593792	Sokoloff 102e	P0375
Astragalus eucosmus	CAN	BC	Robb Lake	CAN	411087	Argus 10350	P0267
Astragalus eucosmus	CAN	NF	Savage Cove	CAN	566856	-	P0260
Astragalus eucosmus	CAN	NF	Savage Cove	CAN	545193	Brouillet 87168	-
Astragalus eucosmus	CAN	BC	Sikanni River	CAN	280947	Correll 10180	-
Astragalus eucosmus	CAN	BC	Sikanni River	CAN	280946	Correll 10287	-
Astragalus eucosmus	CAN	BC	Slikine River	UBC	V176879	Gorman 1423	P0336
Astragalus eucosmus	U.S.A.	WY	Sublette County	NYBG	812909	Shultz 2878	P0340
Astragalus eucosmus	U.S.A.	WY	Sublette County	CAN	563713	Atwood 16464	P0342
Astragalus eucosmus	U.S.A.	WY	Teton County	CAN	511103	Williams 839	-
Astragalus eucosmus	CAN	NT	Tuktoyuktuk	UBC	V177200	Sims 6261a	-
Astragalus eucosmus	CAN	ON	Winisk River	CAN	414389	Baldwin 7669	-
Astragalus eucosmus	CAN	ON	Wubuk Point	CAN	414524	Baldwin 7793	-
Astragalus eucosmus f. leucocarpus	U.S.A.	AK	-	CAN	511088	LePage 23-155	-
Astragalus eucosmus var. *facinorum*	CAN	NF	Grand Falls	GH	58732	Fernald 5795	-
Astragalus eucosmus var. *facinorum*	CAN	NF	Grand Falls	NYBG	5415	Fernald 5795	-
Astragalus robbinsii	CAN	AB	Assiniboine River	UAC	55570	MacDonald. 040717d3	-
Astragalus robbinsii var. *alpiniformis*	U.S.A.	OR	Wallawa Mountains	GH	58623	Cusick 2103	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Blanc Sablon	CAN	76189	Fernald 3632	-
Astragalus robbinsii var. *fernaldii*	CAN	QC	Cap Crow	CAN	593779	Sokoloff 10	P0115
Astragalus robbinsii var. *fernaldii*	CAN	QC	Cap Crow	CAN	593780	Sokoloff 11	P0126

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus robbinsii var. *fernaldii*	CAN	QC	Cap Crow	CAN	593777	Sokoloff 12	P0131
Astragalus robbinsii var. *fernaldii*	CAN	QC	Cap Crow	CAN	593776	Sokoloff 13	P0133
Astragalus robbinsii var. *fernaldii*	CAN	QC	Cap Crow	CAN	593775	Sokoloff 14	P0134
Astragalus robbinsii var. *fernaldii*	CAN	QC	Cap Crow	CAN	593712	Sokoloff 15	P0137
Astragalus robbinsii var. *fernaldii*	CAN	NF	Cap Crow	CAN	593743	Sokoloff 36	P0179
Astragalus robbinsii var. *fernaldii*	CAN	NF	Cap Crow	CAN	593767	Sokoloff 35.5	P0181
Astragalus robbinsii var. *fernaldii*	CAN	QC	Cap Crow	CAN	593710	Sokoloff 12.5	P0132
Astragalus robbinsii var. *fernaldii*	CAN	NF	Cap Crow	NYBG	6099	Fernald 3632	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Cap Crow	MT	-	100	-
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593773	Sokoloff 24	P0164
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593762	Sokoloff 25	P0166
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593763	Sokoloff 26	P0168
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593713	Sokoloff 27	P0169
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593761	Sokoloff 28	P0170
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593720	Sokoloff 29	P0172
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593719	Sokoloff 30	P0173
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593718	Sokoloff 31	P0174
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593717	Sokoloff 32	P0175
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	593764	Sokoloff 33	P0176
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	76190	Lewis 131855	-
Astragalus robbinsii var. *fernaldii*	CAN	QC	Île-au-Bois	CAN	338485	Cleghorn 2213	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	NF	Île-au-Bois	MT	-	Lewis s.n.	-
Astragalus robbinsii var. *fernaldii*	CAN	QC	Mont Bonenfant	CAN	593765	Sokoloff 34	P0177
Astragalus robbinsii var. *fernaldii*	CAN	QC	Mont Bonenfant	CAN	593766	Sokoloff 35	P0178
Astragalus robbinsii var. *fernaldii*	CAN	QC	Mont Bonenfant	CAN	593769	Sokoloff 36	P0182

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus robbinsii var *fernaldii*	CAN	QC	Mont Bonenfant	CAN	593715	Sokoloff 37	P0184
Astragalus robbinsii var *fernaldii*	CAN	QC	Mont Bonenfant	CAN	593716	Sokoloff 38	P0185
Astragalus robbinsii var *fernaldii*	CAN	QC	Mont Bonenfant	CAN	593714	Sokoloff 39	P0186
Astragalus robbinsii var *fernaldii*	CAN	QC	Mont Parent	CAN	593782	Sokoloff 16	P0138
Astragalus robbinsii var *fernaldii*	CAN	QC	Mont Parent	CAN	593783	Sokoloff 17	P0139
Astragalus robbinsii var *fernaldii*	CAN	QC	Mont Parent	CAN	593781	Sokoloff 18	P0140
Astragalus robbinsii var *fernaldii*	CAN	QC	Mont Parent	CAN	593778	Sokoloff 19	P0141
Astragalus robbinsii var *fernaldii*	CAN	QC	Mont Parent	CAN	593771	Sokoloff 20	P0143
Astragalus robbinsii var *fernaldii*	CAN	NF	Mont Parent	QFA	516895	Garneau 94-798m	-
Astragalus robbinsii var *fernaldii*	CAN	QC	QC Labrador Border	CAN	593774	Sokoloff 21	P0152
Astragalus robbinsii var *fernaldii*	CAN	QC	QC Labrador Border	CAN	593772	Sokoloff 22	P0157
Astragalus robbinsii var *fernaldii*	CAN	QC	QC Labrador Border	CAN	593768	Sokoloff 23	P0158
Astragalus robbinsii var *fernaldii*	CAN	NF	QC Labrador Border	-	-	Hanel 0807181	P0300
Astragalus robbinsii var *fernaldii*	CAN	NF	QC Labrador Border	-	-	Hanel 0807183	P0302
Astragalus robbinsii var *fernaldii*	CAN	NF	QC Labrador Border	-	-	Hanel 0807182	P0301
Astragalus robbinsii var *harringtonii*	U S A	AK	Kenai Lake	CAN	278692	Beaman 568	-
Astragalus robbinsii var *harringtonii*	U S A	AK	Kuskakwim River	CAN	404429	Drury 4061	-
Astragalus robbinsii var *harringtonii*	U S A	AK	Palmer County	CAN	76237	Anderson 7015	-
Astragalus robbinsii var *harringtonii*	U S A	AK	Palmer County	CAN	204330	Dutilly 20-110	-
Astragalus robbinsii var *jesupi*	U S A	VT	Conneticut River	CAN	176790	Herb 3606	-
Astragalus robbinsii var *minor*	CAN	AB	-	CAN	511073	s n	-
Astragalus robbinsii var *minor*	CAN	NS	-	QFA	516893	Oldham 14989	-
Astragalus robbinsii var *minor*	U S A	AK	Anchorage	CAN	511095	Dutilly 21-071	-
Astragalus robbinsii var *minor*	CAN	BC	Assiniboine River	CAN	245430	Scamman 6666	P0277
Astragalus robbinsii var *minor*	CAN	AB	Banff	CAN	76219	Macoun 4271	-
Astragalus robbinsii var *minor*	CAN	AB	Banff	CAN	197495	Momoit s n	-
Astragalus robbinsii var *minor*	CAN	AB	Banff	CAN	409387	Beder s n	P0270
Astragalus robbinsii var *minor*	CAN	AB	Banff	CAN	236427	Moss 7355	-
Astragalus robbinsii var *minor*	CAN	AB	Banff	CAN	288057	Porsild 12250	-
Astragalus robbinsii var *minor*	CAN	NF	Barr'd Harbour Hill	CAN	593744	Sokoloff 7	-
Astragalus robbinsii var *minor*	CAN	NF	Barr'd Harbour Hill	NYBG	104357	Wiegand 28597	-
Astragalus robbinsii var *minor*	CAN	NF	Barr'd Harbour Hill	MT	-	Wiegand 28597	-
Astragalus robbinsii var *minor*	CAN	NF	Battery Trail	CAN	593734	Sokoloff 40	P0188
Astragalus robbinsii var *minor*	CAN	NF	Battery Trail	CAN	593735	Sokoloff 41	P0189

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus robbinsii var. *minor*	CAN	NF	Battery Trail	CAN	593736	Sokoloff 42	P0192
Astragalus robbinsii var. *minor*	CAN	NF	Battery Trail	CAN	593732	Sokoloff 43	P0193
Astragalus robbinsii var. *minor*	CAN	NF	Battery Trail	CAN	593733	Sokoloff 44	P0195
Astragalus robbinsii var. *minor*	CAN	NF	Battery Trail	CAN	593730	Sokoloff 45	P0196
Astragalus robbinsii var. *minor*	CAN	NF	Battery Trail	CAN	593729	Sokoloff 47	P0198
Astragalus robbinsii var. *minor*	CAN	NF	Battery Trail	CAN	593728	Sokoloff 48	P0200
Astragalus robbinsii var. *minor*	CAN	NF	Battery Trail	MT	-	NDC99690	-
Astragalus robbinsii var. *minor*	CAN	NF	Battery Trail	MT	-	NDC991042	-
Astragalus robbinsii var. *minor*	CAN	NF	Blanc Sablon	QFA	485706	Gervais 96-36a	-
Astragalus robbinsii var. *minor*	CAN	NF	Blanc Sablon	QFA	516890	Blondeau 05-105	-
Astragalus robbinsii var. *minor*	CAN	AB	Bow River	CAN	76225	Dawson 4273	-
Astragalus robbinsii var. *minor*	CAN	AB	Bow River	CAN	245216	Hermann 13271	P0271
Astragalus robbinsii var. *minor*	CAN	AB	Castle Mountain	CAN	76222	Macoun 76222	-
Astragalus robbinsii var. *minor*	CAN	BC	Chilcotin River	UBC	V174581	Maze 174581	P0334
Astragalus robbinsii var. *minor*	CAN	NF	Doctor's Brook	-	-	Hanel 0707271	CH27-2
Astragalus robbinsii var. *minor*	CAN	YK	Ferry Jill	CAN	412608	Gillett 485	P0268
Astragalus robbinsii var. *minor*	CAN	NF	Forteau	CAN	233202	s.n.	-
Astragalus robbinsii var. *minor*	CAN	NF	Fox Cove	-	-	Hanel 0808079	CH07-10
Astragalus robbinsii var. *minor*	U.S.A.	AK	Franklin	CAN	76235	Anderson 7165	-
Astragalus robbinsii var. *minor*	CAN	NF	Gros Morne	MT	-	Wiegand 95202	-
Astragalus robbinsii var. *minor*	CAN	BC	Hedley River	UBC	V176394	Rose aar51667	P0335
Astragalus robbinsii var. *minor*	CAN	AB	Jasper	CAN	236426	Moss 4901	-
Astragalus robbinsii var. *minor*	CAN	AB	Jasper	CAN	236424	Moss 4798	-
Astragalus robbinsii var. *minor*	U.S.A.	AK	Knick River	CAN	511093	Dutilly 21-110	-
Astragalus robbinsii var. *minor*	CAN	AB	Laggan	CAN	76223	Macoun 65-093	-
Astragalus robbinsii var. *minor*	U.S.A.	WY	Lincoln County	NYBG	694917	Harrison 473	P0341
Astragalus robbinsii var. *minor*	CAN	BC	Manning Park	CAN	392961	Chuang 853	P0275
Astragalus robbinsii var. *minor*	CAN	BC	Manning Park	CAN	392661	Chuang 213	P0276
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593748	Sokoloff 50	P0203
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593727	Sokoloff 51	P0204
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593726	Sokoloff 52	P0205
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593722	Sokoloff 53	P0206
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593724	Sokoloff 54	P0207
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593721	Sokoloff 55	P0209

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593709	Sokoloff 56	P0210
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593725	Sokoloff 57	P0211
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593749	Sokoloff 58	P0212
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593750	Sokoloff 59	P0213
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593751	Sokoloff 60	P0214
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593752	Sokoloff 61	P0216
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593753	Sokoloff 62	P0217
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593754	Sokoloff 63	P0218
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593755	Sokoloff 64	P0219
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593756	Sokoloff 65	P0220
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593723	Sokoloff 66	P0222
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593758	Sokoloff 67	P0225
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593757	Sokoloff 69	P0221
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593760	Sokoloff 70	P0226
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	593759	Sokoloff 71	P0227
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	CAN	564963	Darbyshire 4224	-
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	MT	-	NDC99787	-
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	QFA	516892	Blondeau 05-093	-
Astragalus robbinsii var. *minor*	CAN	NF	Pointe Amour	QFA	516891	Blondeau 05-104	-
Astragalus robbinsii var. *minor*	CAN	BC	Rock Creek	UBC	V219091	Lomer 96-75	P0333
Astragalus robbinsii var. *minor*	U.S.A.	AK	Slana	CAN	76236	Anderson 10123	-
Astragalus robbinsii var. *minor*	CAN	AB	Spray Lake	QFA	516896	Dumais 7924	-
Astragalus robbinsii var. *minor*	CAN	AB	Spray River	UAC	16497	-	-
Astragalus robbinsii var. *minor*	CAN	AB	Spreading Creek	CAN	465734	McIsaac 914	P0274
Astragalus robbinsii var. *minor*	CAN	NF	St. Paul's Inlet	CAN	385667	Bouchard 74037	-
Astragalus robbinsii var. *minor*	CAN	NF	St. Paul's Inlet	MT	-	Bouchard 74037	-
Astragalus robbinsii var. *minor*	CAN	AB	Sunwapta Pass	CAN	236421	Moss 4862	-
Astragalus robbinsii var. *minor*	CAN	AB	Waterton Lakes	CAN	286421	Mate 295	-
Astragalus robbinsii var. *minor*	CAN	AB	Waterton Lakes	CAN	76221	Macoun 10205	-
Astragalus robbinsii var. *occidentalis*	U.S.A.	MT	Billingsley	CAN	557309	s.n.	-
Astragalus robbinsii var. *occidentalis*	U.S.A.	NV	Elko County	NYBG	694931	Atwood 7708	P0337
Astragalus robbinsii var. *occidentalis*	U.S.A.	NV	Elko County	NYBG	694922	Smith 2892	P0338
Astragalus robbinsii var. *occidentalis*	U.S.A.	NV	Elko County	NYBG	694923	Smith 2880	P0339
Astragalus robbinsii var. *occidentalis*	U.S.A.	NV	Elko County	NYBG	694928	Atwood 7708	-

Taxon	Count.	Prov/St.	Locality	Herb.	Accession #	Collector & #	DNA ID
Astragalus robbinsii var.*occidentalis*	CAN	AB	-	UAC	16509	-	P0321
Astragalus robbinsii var.*occidentalis*	CAN	AB	-	UAC	53272	-	P0319
Astragalus robbinsii var.*occidentalis*	CAN	AB	-	UAC	59453	-	P0317
Astragalus robbinsii var.*occidentalis*	CAN	AB	-	UAC	60031	-	-
Astragalus williamsii	CAN	YK	Lapie River	CAN	587146	Harris 3563	P0278

Appendix 4: Key to DNA samples used in chloroplast DNA and AFLP studies, missing samples as indicated by a dash are due to PCR failure or omission.

DNA ID	Taxon	Country	Prov.	Population	cpDNA	AFLP
GIL1494	*Astragalus robbinsii* var. *minor*	Canada	NF	St. Paul		X
GIL1495	*Astragalus robbinsii* var. *minor*	Canada	NF	St. Paul		X
GIL1537	*Astragalus eucosmus*	*Canada*	NF	Little Sacred Island		X
GIL1538	*Astragalus eucosmus*	*Canada*	NF	Little Sacred Island		X
GIL1583	*Astragalus eucosmus*	*Canada*	NF	HaHa Mountain		X
GIL1584	*Astragalus eucosmus*	*Canada*	NF	HaHa Mountain		X
GIL1642	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Doctor's Brook		X
GIL1643	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Doctor's Brook		X
GIL1681	*Astragalus eucosmus*	*Canada*	NF	Burnt Cape		X
P0001	*Astragalus eucosmus*	*Canada*	NF	Indian River	X	
P0003	*Astragalus eucosmus*	*Canada*	NF	Indian River	X	X
P0005	*Astragalus eucosmus*	*Canada*	NF	Indian River	X	
P0009	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills	X	
P0010	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills	X	
P0012	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills	X	
P0013	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills		X
P0015	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills	X	
P0017	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills		X
P0019	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills	X	
P0020	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills		X
P0021	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills	X	
P0023	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills		X
P0024	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills	X	
P0025	*Astragalus eucosmus*	*Canada*	NF	Bishop's Mills		X
P0027	*Astragalus eucosmus*	*Canada*	NF	Grand Falls		X
P0031	*Astragalus eucosmus*	*Canada*	NF	Grand Falls		X
P0034	*Astragalus eucosmus*	*Canada*	NF	Grand Falls	X	
P0036	*Astragalus eucosmus*	*Canada*	NF	Grand Falls	X	
P0037	*Astragalus eucosmus*	*Canada*	NF	Grand Falls	X	X
P0038	*Astragalus eucosmus*	*Canada*	NF	Grand Falls		X
P0041	*Astragalus eucosmus*	*Canada*	NF	Indian River	X	
P0043	*Astragalus eucosmus*	*Canada*	NF	Indian River	X	

DNA ID	**Taxon**	**Country**	**Prov.**	**Population**	**cpDNA**	**AFLP**
P0046	*Astragalus eucosmus*	*Canada*	NF	Indian River	X	X
P0048	*Astragalus eucosmus*	*Canada*	NF	Indian River	X	X
P0049	*Astragalus eucosmus*	*Canada*	NF	Corner Brook	X	
P0050	*Astragalus eucosmus*	*Canada*	NF	Corner Brook		X
P0051	*Astragalus eucosmus*	*Canada*	NF	Corner Brook	X	
P0053	*Astragalus eucosmus*	*Canada*	NF	Corner Brook		X
P0054	*Astragalus eucosmus*	*Canada*	NF	Corner Brook	X	
P0056	*Astragalus eucosmus*	*Canada*	NF	Corner Brook	X	
P0061	*Astragalus eucosmus*	*Canada*	NF	Corner Brook	X	X
P0064	*Astragalus eucosmus*	*Canada*	NF	Corner Brook		X
P0065	*Astragalus eucosmus*	*Canada*	NF	Corner Brook	X	
P0066	*Astragalus eucosmus*	*Canada*	NF	Corner Brook		X
P0068	*Astragalus eucosmus*	*Canada*	NF	Corner Brook	X	
P0069	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill		X
P0071	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0072	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0073	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0074	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0075	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0076	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0077	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	X
P0078	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill		X
P0080	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0082	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0083	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill		X
P0085	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill		X
P0087	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Barr'd Harbour Hill	X	
P0088	*Astragalus eucosmus*	*Canada*	NF	Cape Mauve	X	X
P0092	*Astragalus eucosmus*	*Canada*	NF	Cape Mauve	X	X
P0095	*Astragalus eucosmus*	*Canada*	NF	Cape Mauve	X	X
P0097	*Astragalus eucosmus*	*Canada*	NF	Cape Mauve		X
P0101	*Astragalus eucosmus*	*Canada*	NF	Cape Mauve	X	

DNA ID	Taxon	Country	Prov.	Population	cpDNA	AFLP
P0102	*Astragalus eucosmus*	*Canada*	NF	Cape Mauve	X	
P0103	*Astragalus eucosmus*	*Canada*	NF	Cape Mauve		X
P0109	*Astragalus eucosmus*	*Canada*	NF	Cook's Harbour		X
P0110	*Astragalus eucosmus*	*Canada*	NF	Cook's Harbour		X
P0111	*Astragalus bodinii*	*Canada*	NF	Cook's Harbour	X	X
P0113	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0114	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0115	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0116	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0117	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0118	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0120	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow		X
P0123	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0125	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow		X
P0126	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0128	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0129	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0130	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	X
P0131	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0132	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0134	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	X
P0135	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0137	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Cap Crow	X	
P0139	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Parent	X	
P0141	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Parent	X	X
P0142	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Parent	X	X
P0143	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Parent	X	
P0144	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Parent	X	X
P0145	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Parent	X	
P0146	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Parent		X
P0148	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border	X	
P0149	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border	X	X

DNA ID	Taxon	Country	Prov.	Population	cpDNA	AFLP
P0150	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border	X	
P0151	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border	X	X
P0153	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border	X	
P0154	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border	X	
P0155	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border	X	
P0156	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border		X
P0157	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Québec/Labrador Border	X	X
P0161	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Île-au-Bois	X	X
P0163	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Île-au-Bois	X	
P0164	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Île-au-Bois	X	X
P0166	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Île-au-Bois	X	X
P0168	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Île-au-Bois		X
P0171	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Île-au-Bois	X	X
P0172	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	X
P0174	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	
P0175	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	X
P0177	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	X
P0179	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	X
P0180	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant		X
P0182	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	
P0183	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	X
P0184	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	
P0185	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	X
P0187	*Astragalus robbinsii* var. *fernaldii*	*Canada*	QC	Mont Bonenfant	X	
P0188	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail	X	
P0189	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail	X	
P0192	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail		X
P0193	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail	X	
P0194	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail	X	
P0195	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail	X	X
P0196	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail		X
P0197	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail		X

DNA ID	Taxon	Country	Prov.	Population	cpDNA	AFLP
P0199	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail	X	X
P0201	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail	X	
P0202	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail		X
P0204	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Battery Trail		X
P0205	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0206	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0207	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0209	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0210	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour		X
P0211	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour		X
P0215	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0216	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0217	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0219	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	X
P0222	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0225	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour	X	
P0227	*Astragalus robbinsii* var. *minor*	*Canada*	NF	Pointe Amour		X
P0254	*?*	*Canada*	AB	?	X	
P0255	*?*	*Canada*	AB	?	X	
P0256	*Astragalus eucosmus*	*Canada*	NT	Iqaluit	X	
P0264	*Astragalus eucosmus*	*Canada*	SK	Nipawin River	X	
P0274	*Astragalus robbinsii* var. *minor*	*Canada*	AB	Spreading Creek	X	
P0278	*Astragalus williamsii*	*Canada*	YK	Lapie River	X	
P0293	*Astragalus australis*	*U.S.A.*	AK	Yukon River	X	
P0300	*Astragalus robbinsii* var. *fernaldii*	*Canada*	NF	Québec/Labrador Border	X	
P0301	*Astragalus robbinsii* var. *fernaldii*	*Canada*	NF	Québec/Labrador Border	X	X
P0302	*Astragalus robbinsii* var. *fernaldii*	*Canada*	NF	Québec/Labrador Border	X	
P0303	*Astragalus robbinsii* var. *fernaldii*	*Canada*	NF	Québec/Labrador Border	X	X
P0305	*Astragalus eucosmus*	*Canada*	AB	-	X	
P0309	*Astragalus eucosmus*	*Canada*	AB	-	X	
P0313	*Astragalus eucosmus*	*Canada*	AB	-	X	
P0314	*Astragalus eucosmus*	*Canada*	AB	-	X	

DNA ID	Taxon	Country	Prov.	Population	cpDNA	AFLP
P0318	*Astragalus robbinsii* var. *occidentalis*	*Canada*	AB	-	X	
P0345	*Astragalus eucosmus*	*Canada*	NB	Restigouche River	X	X
P0354	*Astragalus eucosmus*	*Canada*	NB	Restigouche River	X	X
P0362	*Astragalus eucosmus*	*Canada*	NB	Restigouche River	X	X
P0366	*Astragalus alpinus*	*Canada*	NB	Restigouche River	X	X
P0369	*Astragalus alpinus*	*Canada*	NB	Restigouche River	X	X
P0372	*Astragalus eucosmus*	*Canada*	NB	Hammond River	X	X
P0375	*Astragalus eucosmus*	*Canada*	NB	Hammond River	X	X
Woj164	*Astragalus corrugatus*	*Iran*	-	-		X
Woj170	*Astragalus siliquosus*	*Iran*	-	-		X

Appendix 5: P values and statistical output for interspecific comparisons of quantitative variables.

Variable	n (*A. eucosmus*)	n(*A. r.* var. *minor*)	n(*A. r.* var. *fernaldii*)	H Statistic	*p* Value
Leaf Length	102	79	65	8.75	0.013*
Leaflet Length	102	79	65	5.85	0.053
Peduncle Length FL	45	55	46	15.46	0.0004*
Raceme Axis Length FL	45	55	46	10.24	0.006*
Peduncle Length FR	30	47	18	5.70	0.06
Raceme Axis Length FR	30	47	18	5.64	0.06
Calyx Length	102	79	65	107.3	9.49e-14*
Teeth Length	102	79	65	13.37	0.001*
Banner Petal Length	45	55	46	54.72	1.4e-12*
Stipe Length	30	47	18	65.09	9.16e-15*
Pod Length	30	47	18	76.94	1.98e-17*
Pod Diameter	30	47	18	0.80	0.67
Beak Length	30	47	18	60.44	8.63e-14*
Stipule Length	102	79	65	9.25	0.01
Flower Length	45	55	46	62.36	2.94e-14*

*Statistically significant ($p>0.001$, $p>0.05$ for leaf length). Results of post-hoc Mann-Whitney U presented in Fig. X

Appendix 6: Morphology Principal Components Analysis Loadings on PC1 and PC2

Variable	**Loading on PC 1**	**Loading on PC 2**
Leaf Length	-0.314	0.3
Leaflet Length	-0.328	0.27
Abaxial Leaf Pubescence	0.252	-0.07
Adaxial Leaf Pubescence	0.14	-0.09
Flowering Peduncle Length	-0.28	0.3
Flowering Raceme Length	-0.2	0.27
Fruiting Peduncle Length	-0.19	0.27
Fruiting Raceme Length	-0.169	0.27
White Calyx Pubescence	0.05	0.182
Black Calyx Pubescence	0.08	0.07
Calyx Length	-0.234	-0.265
Calyx Teeth Length	-0.188	-0.11
Petal Colour	0.159	0.03
Banner Petal Length	-0.271	-0.13
Stipe Length	-0.253	-0.3
Pod Length	-0.312	-0.24
Pod Width	-0.15	0.02
Beak Length	-0.22	-0.342
White Pod Pubescence	0.11	0.21
Black Pod Pubescence	0.11	0.2
Stipule Length	-0.055	0.1
Flower Length	-0.264	-0.18