The origins and conservation genetics of *Cirsium scariosum* in the Mingan Islands of Québec

By

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ABSTRACT

The Mingan Archipelago along the north shore of the Gulf of St. Lawrence is well known for the high diversity and rarity of its vascular plants. This diversity is often explained by a combination of the islands' temperate maritime climate and its peculiar regional geology where the islands are formed of calcareous sedimentary rock. Among the 80 or so species of rare plants on the islands, one in particular stands out, *Cirsium scariosum* (Meadow thistle), a species native to western North America, but with a few disjunct populations on the Mingan islands over 3200 km away. Initially recognized as a new species, *Cirsium minganense*, when it was first discovered in 1924 by Marie-Victorin, recent authors believe these Mingan populations of *Cirsium scariosum* are either the result of eastern migration during the Pleistocene or a contemporary anthropogenic introduction. Nevertheless, the question of how these populations arrived on the islands or whether they should be treated as a separate species has not been resolved. The eastern populations of *Cirsium scariosum* are now endangered and conservation efforts have been made to try and save this emblematic and historical plant. To determine whether the Mingan populations might represent a separate species and to better understand the plants' breeding system and genetic diversity for conservation, a phylogeny of closely related species and a population genetic analysis using genotyping by sequencing (GBS) was conducted. Results indicate that *C. minganense* is not closely related to Western Canadian *Cirsium scariosum* and that the subpopulations on the islands are not formed of uniform genotypes but are genetically distinct from one another.

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1. INTRODUCTION

1.1 AN INTRODUCTION TO THISTLES WITH EMPHASIS ON THE GENUS *CIRSIUM*

The word "Thistle" is a common name generally used to describe a group of flowering plants with sharp edged leaves armed with prickles and showy heads (a capitulate inflorescence) of mostly white, purple, or pink tubular flowers that are placed in the Asteraceae Bercht. & J. Presl or "sunflower" family. The term can either represent plants in the tribe Cardueae Cass. (~74 genera, 2500 species; Funk et al., 2005), namely species in the genera *Carduus* L., *Cirsium* Mill. and *Onopordum* L. or plants in other, distantly related sunflower tribes such as tribe Cichorieae Lamarck & de Candolle (~100 genera, 1550 species; Funk et al., 2009; Barres et al., 2013). In Canada, 46 species of thistles are found of which 16 are native (Desmet & Brouillet, 2013). Most native species are from the genera *Carduus* or *Cirsium* and are found in a wide array of habitats, but the remaining 30 species are members of the genus *Saussurea* Lam. & DC., which are non-spiny alpine and arctic species (Susanna et al., 1995). For the purposes of clarity, when the common name thistle is used alone in this thesis, it refers to the "true thistles" or species of the genus *Cirsium*.

The genus *Cirsium* is a large genus common almost everywhere in the temperate regions of Eurasia (~370 taxa; Werner, 1976; Ackerfield et al., 2020), eastern tropical Africa (~10 taxa; Beentje, 2000; Ackerfield et al., 2020), and North America (~118 taxa; Keil, 2006). *Cirsium* is one of nine genera in tribe *Cardueae* with its closest relative being the genus *Carduus*. Although morphologically similar, the two genera can be distinguished by the pappus or "egrets" attached to their fruits, bristle or feather-like structures for wind and animal dispersal that are assumed to be modified sepals when present in Asteraceae (Sheldon & Burrows, 1972). In Cirsium, the pappus is composed of feathery hairs (plumose), whereas in *Carduus* it is formed of simple or denticulate hairs (Kelch & Baldwin, 2003). Beyond this character, Cirsium and Carduus are extremely similar, each possessing species with winged stems, thorny leaves, tubular flowers mostly pink in hue, scales or tufts of hair between flowers and thorny bracts. The prickles found on these plants can occur anywhere from the stem to the surface of the leaves, and they are believed to act as a deterrent to herbivores (Ronel et al., 2009).

In North America, *Cirsium* species can be either annuals, biennials or perennials. They are found in a great variety of plant communities, such as prairies (e.g., *C. pumilum* (Nuttall) Sprengel var. *hillii* (Canby) B.Boivin, *C. carolinianum* (Walter) Fernald & B.G.Schubert), montane meadows (e.g., *C. scariosum* Nuttall), rocky desert canyons (e.g., *C. arizonicum* (A.Gray) Petrak and *C. neomexicanum* A.Gray), seeps from stream sides (e.g., *C. fontinale* (Greene) Jepson and *C. crassicaule* (Greene), Jepson), brackish marshes (e.g., *C. hydrophilum* var. *hydrophilum* (Greene), Jepson. and *C. scariosum* var. *loncholepis* (Petr.) D.J. Keil Peter, Ke.), forests (e.g., *C. brevistylum* Cronquist, Le. and *C. remotifolium* (Hooker) de Candolle, Ca.), coastal dunes (e.g., *C. rhothophilum* S.F.Blake, Wa.), lacustrine dunes (e.g*., C. pitcheri* (Torrey ex Eaton) Torrey & A.GrayTorrey & Gray) and openings in chaparral (e.g., *C. occidentale* var. *venustum* (Greene), Jepson) (Kelch and Baldwin, 2003).

1.2 TAXONOMY OF *CIRSIUM*

Even though *Cirsium* is one of the most diverse, and ecologically and economically (weeds; see below) important genera in North America, its taxonomy remains poorly studied. To this day, it remains one of the most confusing and taxonomically challenging groups of Asteraceae (Cronquist, 1994; Keil, 2006). The taxonomy of the genus remains confused largely due to its great diversity, low levels of genetic divergence among species, and for the propensity of its species to hybridise. Minimal genetic variation across species has hampered resolution of relationships in *Cirsium*. Even when showing drastically different morphological traits, *Cirsium* species are often able to hybridize (Bures et al., 2010). These combinations occasionally lead to reduced fertility, but more commonly a fertile population of hybrids is created (Bures et al., 2010). This shows there are few to no breeding barriers between *Cirsium* species, and a great potential for the emergence of new characters and character combinations in hybrids. The lack of satisfactory samples and observations can lead to inadequate hybrid recognition and to their classification under a distinct taxon or as a variant of non-hybrid taxa (Ownbey, 1951). These hybrid populations are usually more likely to be found in areas where the range of different *Cirsium* species overlap (Dabydeen, 1997). Ackerfield et al. (2020) pointed out five major factors that could explain this lack of clarity in the genus' taxonomy: (i) previously undescribed taxa, (ii) inadequate representation of taxa from herbarium specimens, (iii) phenotypic convergence, (iv) hybridization, and (v) incipient speciation. These factors also make it very difficult to properly protect species of *Cirsium* at risk as they are prone to misidentification.

The first attempt at a treatment for North American *Cirsium* was done by Gray (1874) in which he recognized 28 species and 5 varieties. Since then, many revisions to the genus have been made (Greene 1892; Petrak 1917; Rydberg 1917, 1922) culminating in the most recent treatment for the Flora of North America by Keil (2006) who recognized 62 species and 56 varieties. Many formerly recognized species were broadly circumscribed and synonymized with or treated as varieties of a more widespread taxon. In particular, the *C. arizonicum* (A. Gray)

Petrak, *C. clavatum* (M.E. Jones) Rydb., *C. eatonii* (A. Gray) B.L. Rob., and *C. scariosum* Nutt. species complexes underwent significant taxonomic changes (Ackerfield et al., 2020).

The *Cirsium scariosum* complex comprises eight infraspecific varieties *sensu* Keil (2006), namely vars. *americanum*, *citrinum*, *coloradense* (Rydb.) D.J. Keil, *congdonii*, *robustum* D.J. Keil, *scariosum*, *thorneae* S.L. Welsh, and *toiyabense* S.L. Welsh. In the past, these taxa were treated as species or erroneously treated as *C. drummondii* Torr. & A. Gray or *C. foliosum* (Hook.) DC. Four species were recognized in Moore & Frankton's (1967) detailed treatment of the complex: *C. acaulescens* (A. Gray) K. Schum., *C. coloradense* (Rydb.) Cockerell ex Daniels, *C. congdonii* R.J. Moore & Frankton, and *C. scariosum*. However, they later revised this treatment to acknowledge the prior binomial *C. tioganum* (Congdon) Petr. for *C. acaulescens*. Cronquist (1994) subsumed all variation within the group within a broadly delimited, polymorphic *C. scariosum*. Keil (2006) ultimately combined both Cronquist's (1994) and Moore & Frankton's (1967) treatments by subdividing *C. scariosum* into the eight varieties listed above.

Despite being polymorphic, taxa within the complex share morphological features such as a dense cluster of sessile heads typically overtopped by crowded, distal leaves. The complex is subdivided by habit (acaulescent vs. caulescent), with varieties *americanum* and *congdonii* being acaulescent, while varieties *coloradoense*, *thorneae*, and *toiyabense* are caulescent. However, two varieties (*citrinum* and *scariosum*) may have both acaulescent and caulescent plants within the same population. Five varieties (*americanum*, *coloradoense*, *scariosum*, *thorneae*, and *toiyabense*) are widespread in meadows and along streams throughout the Rocky Mountains and Intermountain regions. Three other varieties (*citrinum*, *congdonii*, and *robustum*) are narrowly distributed in California and adjacent Oregon.

1.3 PHYLOGENY OF *CIRSIUM*

Previous phylogenetic work on North American *Cirsium* has been limited (Ackerfield et al., 2020). The same reasons that make its taxonomy challenging can also apply to its phylogeny. Low levels of genetic divergence, high diversity and rampant hybridisation often mean that phylogenetic trees are unresolved and branch support is weak.

A molecular phylogenetic study on the ecological radiation of New World *Cirsium* using DNA sequences from the external and internal transcribed spacers (ETS and ITS) of nuclear ribosomal DNA (nrDNA; Kelch and Baldwin 2003) suggested that the incredible diversity of the genus in North America might result from a rapid evolutionary diversification following a single introduction from Eurasia. However, trees were poorly resolved and little divergence was discovered in ETS or ITS, two markers that are normally very effective at resolving infrageneric relationships. This suggested that diversification of *Cirsium* had either been very rapid or that ETS and ITS were mutating at a slower rate than what is typically seen in other plant lineages (Bodo Slotta et al., 2012).

Ackerfield et al. (2020) recently published a study attempting to infer a broadly sampled phylogeny of *Cirsium* in North America. The two main objectives of the study were to (i) test whether currently hypothesized species complexes (*C. arizonicum*, *C. clavatum*, *C. eatonii*, and *C. scariosum*) constitute monophyletic lineages, and to (ii) re-circumscribe any taxa that were identified as problematic. DNA sequence data from two nuclear ribosomal regions (ETS and ITS) and four plastid markers (*matK*, *ndhF*, *psbA-trnH*, and *trnL-trnF*) were used to infer evolutionary lineages and test species' delimitations. Eight species complexes were resolved as polyphyletic and these complexes were re-circumscribed. By doing so, evidence to support the recognition of six new taxa was uncovered. While the study provided evidence to support the re-circumscription of some species complexes, the *Cirsium scariosum* complex could not be resolved due to poor sampling. The question as to whether the Eastern Canadian population of *C. scariosum* could be a distinct species or variety was not addressed due to a lack of material, nor was any other taxonomic problem within the complex as the study included only two samples and they came from the same locality (Boise, Idaho).

Based on previous phylogenetic analyses of *Cirsium*, sequence variation among species for conventional nuclear (ITS, ETS) and plastid markers (e.g., *matK*) is insufficient to resolve relationships or to provide the statistical support necessary to fully reclassify the genus. When traditional markers fail to resolve phylogenies, a Next-Generation Sequencing (NGS) approach is generally taken as these techniques can provide the data necessary to resolve relationships among closely related species (e.g., Léveillé-Bourret et al., 2020), even at the intra-specific level (Ibrahim, 2010). Therefore, a genotyping by sequencing (GBS; Elshire et al., 2011) approach was taken in this thesis to get better tree support and resolution in *Cirsium*.

1.4 ECONOMIC RELEVANCE OF *CIRSIUM*

Cirsium species are known for being invasive troublesome weeds (Guggisberg et al., 2012), but the common idea that they represent a nuisance stems mostly from a small number of highly invasive Eurasian species such as *Cirsium vulgare* (Savi) Tenore (bull thistle, common thistle, or spear thistle) and *Cirsium arvense* L. (Scop.) (oddly known as "Canada thistle"), which are widely listed as noxious in Canada and the United States (USDA; Canada Thistle, 2015). *Cirsium arvense* is even considered to be among the world's worst weeds (Guggisberg, 2012). First identified in North America in 1795 (Guggisberg, 2012), it is now classified as a noxious weed in 49 states and provinces because in pastures dense growths of its spiny vegetation drive out forage plants and repel grazing animals from eating either thistles or neighbouring forage (Bullock et al., 1994). They are notorious for draining nutrients and water from the soil around them, thus becoming very damaging to soil quality, which increases the risk of erosion whilst drastically reducing the chance for survival in other plant speciess (Bendall, 1975). Consequently, its presence in agricultural fields can lead to economic losses because of reduced crop yields (Davis et al., 2018 ; Kazinczi et al, 2001)

1.5 ECOLOGICAL RELEVANCE OF *CIRSIUM*

Invasive *Cirsium* species can also put native plants at risk by overrunning an area and displacing native species (Haggar et al., 1986). Their success is mainly attributable to their diverse methods of dispersal (Heimann & Cussans, 1996), which means they can enter new areas by seed through wind (anemochory), water (hydrochory) or animal (zoochory; Klinkhamer et al., 1988) dispersal, and then firmly establish themselves locally by vegetative propagation (Donald, 1994; Hutchison, 1992). *Cirsium arvense* takes advantage of the fact it is an allelopathic plant by secreting chemicals from its roots that are toxic to surrounding vegetation. Bendall (1975) showed that in certain regions of south Tasmania, the growth of several annual plant species (e.g. *Lolium perenne* L., *Hordeum distichon* L., *Carduus pycnocephalus* L., *Silybum marianum* (L.) Gaertn.) is restricted to areas lacking *C. arvense*, and that change from a monospecific *C. arvense* population to a mixed plant species population could occur over just two metres.

Despite their reputation as noxious weeds, most *Cirsium* species hold a crucial role in ecosystems and such species may play important and unexpected roles in ecological dynamics. First, studies of *Cirsium* species dynamics and interactions have greatly contributed to the basic ecological insights of how biological interactions can structure, limit, and influence the abundance and distribution of native plants in various habitats (e.g. Louda et al. 1990, 1992; Louda and Potvin 1995; Guretzky and Louda 1997; Jackson 1998; Bevill et al. 1999). Analogous studies have also improved our understanding of the role of interactions in plant rarity (Louda and McEachern 1995; Stanforth et al. 1997; Bevill and Louda 1999; Bevill et al., 1999).

Secondly, *Cirsium* species are key to the support of a broad array of animal species in North America. They provide important habitat and food sources for native fauna (Louda & Rand, 2003). These species range from microscopic plant parasites (e.g. rust fungus, *Puccinia punctiformis*) to many macroscopic species (Friedli & Bacher, 2001). *Cirsium* species are visited by various species of arthropods and have a suite of adapted insects such as picture-winged flies (Tephritidae), weevils (Curculionidae), moths (Pyralidae, Pterophoridae), lacebugs (Tingidae), aphids (Aphididae), and sucking bugs (Hemiptera: Cicadellidae, Membracidae, Miridae, Pentatomidae) (Zwolfer 1965, 1988; Lamp and McCarty 1979). They are also commonly visited by various species of pollinators such as native bees and are strongly favored by many butterflies such as monarchs (*Danaus plexippus*), fritillaries (*Speyeria* species), skippers (*Hesperiidae* species), and the various types of tiger swallowtail (*Papilio* species). These species rely heavily on the flowers for pollen and nectar (Swartz et al., 2015) These nectar sources help support healthy populations of pollinators and can help to increase yields for many valuable crops in these areas (Cole et al., 2017). In fact, monarch butterflies rely heavily on native *Cirsium* species (e.g. *Cirsium flodmanii, Cirsium undulatum*) more than any other wildflower in some regions (North Dakota, US) during their migration back to Mexico (Antonson et al., 2021). Finally, *Cirsium* species also provide important sustenance through their seeds for other larger animal species (e.g., American Goldfinch, *Spinus tristis*) and their predators (e.g. raptors; Louda et al., 1998).

Lastly, the presence of native *Cirsium* in ecosystems makes it harder for aggressive, non-native thistles to invade an area as native thistles support beneficial indigenous insects that also consume non-native thistles (Eckberg, 2013). This is a preferable approach to the introduction of non-native insects to control invasive thistles as it seems introduced insects consume native thistles at least as much as desired targets (Wiggins et al., 2010). It is also worth noting that *Cirsium* species are not necessarily interchangeable in their ecosystem functions. For example, an early flowering species (e.g. *Cirsium canescens* Nutt.) and its earlier-feeding insects would not be equivalent to a late-flowering one (e.g. *Cirsium altissimum* (L.) Hill) whose insects are providing some resistance to an invasive species like *C. vulgare*. Unlike biotic resistance provided by plant competitors, where it is expected to be a substantial amount of functional redundancy among plant species, the role occupied by specific plants that serve as reservoirs of coevolved natural enemies is likely to be sensitive to the elimination of a specific plant species (Louda & Rand, 2003).

Despite the significance and importance of native thistles to our ecosystems (e.g. food source, habitat, etc.), these plants are often eradicated alongside invasive thistles leading to a situation where many native species are now threatened or at risk of extinction. Thirty-five *Cirsium* taxa in North America are considered rare or of conservation concern (Keil, 2006), while six of these taxa are also listed as federally threatened or endangered under the U.S. Endangered Species Act (United States, 1983) and two (*Cirsium pumilum* var. *hillii* (Canby) B. Boivin; *Cirsium* *pitcheri* (Torrey ex Eaton) Torrey & A. Gray*)* are listed as threatened or of special concern by COSEWIC (Canada, 2003).

1.6 OVERVIEW OF *CIRSIUM* SCARIOSUM

1.6.1 GENERAL DESCRIPTION

Cirsium scariosum is a native North American thistle. It is a rosette-forming monocarpic perennial which establishes from seeds, and the plants usually grow as rosettes for 3 to 20 years before flowering and dying. The plants range from acaulescent rosettes with a tight cluster of sessile heads, to tall, erect, unbranched plants reaching up to 200 cm, or even mound-like, more or less openly branched herbs (eFloras, 2021). When there is a stem, it is usually fleshy, ridged, and woolly in texture. The leaves are sharply toothed or cut into toothed lobes, lined with spines, and up to 40 centimetres at their longest near the base of the plant. The inflorescence holds several flower heads, each up to 4 cm long and 5 cm wide. The flowering head is subtended by phyllaries that may have spines and teeth, and the head is filled with white to purple disc florets but no ray florets. The fruit is a compressed achene a few millimeters long topped with a pappus that may be 3 centimeters in length (eFloras, 2021) (Figures 1.1 and 1.2).

To date, eight intraspecific varieties of *C. scariosum* are officially recognized *sensu* Keil (2006): *americanum, citrinum*, *coloradense congdonii*, *robustum*, *scariosum*, *thorneae*, and *toiyabense* (data.canadensys.net). Four of the varieties (*americanum*, *coloradoense*, *thorneae*, and *toiyabense*) are widespread in meadows and along streams throughout the Rocky Mountains and Intermountain regions. Three other varieties (*citrinum*, *congdonii*, and *robustum*) are narrowly distributed in California and adjacent Oregon. Finally, *Cirsium scariosum var. scariosum* is widespread in meadows and along streams throughout the Rocky Mountains and

Intermountain region, but it is also disjunct in the Mingan Archipelago of Québec (Calvo et al., 2016). Because of the confusing nature of *Cirsium* and the difficulties classifying the genus, these varieties have been considered at the species level in the past, and it is not uncommon for specimens of the varieties to be misidentified as other thistle species (e.g. *C. drummondii* Torr. & A. Gray or *C. foliosum* (Hook.) DC.; Ackerfield et al., 2020). All the varieties of *Cirsium scariosum* share a morphological similarity which is the presence of a dense cluster of sessile heads typically overtopped by crowded, distal leaves. However, *C. scariosum* is a polymorphic group and the species is normally divided by a major characteristic in its habit, the state of being either acaulescent (no stem) vs. caulescent (with a stem). Varieties *americanum* and *congdonii* are acaulescent, while varieties *coloradoense*, *thorneae*, and *toiyabense* are caulescent. Interestingly, two varieties (*citrinum* and *scariosum*) are known to have acaulescent and caulescent plants within the same population (Cronquist, 1994; Keil, 2006). This makes their identification even more arduous and can lead to confusion and errors.

1.6.2 *CIRSIUM SCARIOSUM* MATING SYSTEMS

The genus *Cirsium* includes species with various reproductive strategies. *Cirsium* is reported as aposporous by Czapik (1996) without specific attribution. Apospory, which is considered a form of apomixis, bypasses the formation of spores and the meiotic process. It involves the direct development of a diploid sporophyte from a somatic cell of the parent plant, typically a cell within the ovule or embryo sac. This bypasses the need for sexual reproduction and results in the production of offspring that are genetically identical to the parent plant. Apospory can be advantageous for plants as it allows them to reproduce rapidly and maintain favorable traits without the genetic variation introduced through sexual reproduction although it also comes at the cost of reduced genetic variation in a population.

While *Cirsium* species are noted for hybridization and production of complex patterns of morphological variation (e.g., FNA 2006, 19: 95-97), their breeding systems are poorly known (Kelch and Baldwin 2003). Apomixis or the asexual production of seeds, is common in difficult taxonomic genera in Asteraceae that exhibit frequent hybridisation (e.g., *Crepis* L., *Bidens* L.; Noyes, 2007), but it is unknown in *Cirsium*, nor is it mentioned in the cytological literature for the genus where high ploidy numbers would point to its presence (Moore and Franklin 1969).The available evidence is consistent with the conclusion that while *Cirsium* may exhibit elements of apomictic reproduction, apomictic reproduction is not a regular part of the breeding system of the genus.

1.6.3 *CIRSIUM SCARIOSUM VAR. SCARIOSUM* ON THE ISLANDS OF THE MINGAN ARCHIPELAGO

As stated above, the distribution of *Cirsium scariosum var. scariosum* is highly disjunct (Calvo et al., 2016). The larger, western population of the species is found mainly in the United States in Idaho, Montana, Oregon, Utah, Washington and Wyoming, and also spans across the Canadian border to Alberta and British Columbia. The smaller eastern population is found in Québec on the islands of the Mingan Archipelago, almost 3500 km away from the western population (Figure 1.3).

Discovered for the first time in the Mingan Archipelago in 1924 by Marie-Victorin (Victorin, 1925), it was described as one of the most remarkable endemic plants of the Gulf of St-Lawrence (Flore laurentienne, 1935) and initially considered a new species named *C. minganense*

Victorin. The presence of these Mingan individuals is currently thought to be the result of either an eastern migration during the Pleistocene or a contemporary anthropogenic introduction from the United States (Golden et al., 2008). The recognition of the eastern population as a distinct species, *C. minganense* (= *C. foliosum* var. *minganense*), as originally proposed by Marie-Victorin (1925), has not been accepted by recent floras (e.g., Scoggan, 1979; Keil, 2006). For the sake of simplicity and clarity, the eastern population of *C. scariosum* var. *scariosum* will be hereafter referred to as *Cirsium minganense*, and the name *C. scariosum* var. *scariosum* will be used for the western population. As discussed below, *C. minganense* includes several geographically distinct subpopulations within the Mingan Islands.

Cirsium minganense is also of much interest because of the region where it is found. The Mingan Archipelago National Park Reserve (MANPR) constitutes an historical heritage of great value in Québec. It is part of the of the Gulf of St. Lawrence Territory and forms an exceptional location given its unique geology and flora (Dufour, 1979). The position of the islands in the Gulf of St. Lawrence, the postglacial geomorphologic processes involved in their formation, as well as the maritime seasonal temperatures and cycles, have enabled the creation of various unique ecological environments. The Ordovician limestone cuestas formations (hills or ridges with a gentle slope on one side, and a steep slopes on the other) that comprise the archipelago slowly rose out of the sea and their erosion led to a high calcium concentration in the soil. The descriptions of Twenhofel (1926) and of Waddington (1948) show that the islands consist of almost pure limestone (95% calcium carbonate [$CaCO_3$] and less than 2% silica [SiO_2]).

Biodiversity and species richness usually follow a latitudinal diversity gradient and decrease from the tropics towards the poles (Lawrence, E. R., & Fraser, D. J., 2020); however, exceptions have been recorded. For example, different regions of Canada are known to harbour a high rate of local endemism because of calciferous soils. For example, the limestone barrens of Newfoundland, regions of the Great Lakes in Ontario like the Bruce Peninsula, and alvars around Belleville, Ontario, tend to display high levels of diversity and endemism that are associated with the highly calciferous soils of these locations (Catling et al., 2014). These biodiversity hotspots are often home to strange and unique plant communities where it is not unusual to find new species (e.g., *Carex juniperorum* or *Braya fernaldii*; Catling et al., 1993; Fernald, 1950). Following that same pattern, the biodiversity on the islands is incredibly rich in comparison to the rest of the province of Québec. In addition to a vegetation typical of the boreal zone, the reserve is home to shrub and herbaceous vegetation typical of the Subarctic and Arctic zones (Belland et al., 1992).

Known for the high level of endemism (Couillard et al., 1987) that can be observed in the area, the geological and climatic particularities of the archipelago have allowed the establishment of more than 80 species of rare plants (Couillard, 1987). These species range from infrequently found plants, generally represented by a small number of individuals and occupying a restricted geographic distribution area (e.g. *Lobelia kalmii* L.), to highly specialized plants, with very specific needs such as *Taraxacum laurentianum* Fernald with its only known population worldwide being found in the Gulf of St. Lawrence (Couillard, 1987).

The strange distribution of *Cirsium scariosum* var. *scariosum* has attracted the interest of many botanists over the years (Golden et al., 2008; Moore & Frankton, 1967), but it is still unclear whether *C. minganense* should be treated as a distinct variety of *C. scariosum* or even as a separate species within the genus. By doing a molecular analysis of *C. minganense* and western individuals of *C. scariosum* and *C. hookerianum*, Golden et al. (2008) showed that the Québec individuals harbour the highest percentage of unique alleles and the lowest percentage of polymorphic loci. The chloroplast region showed a single bp difference unique to *C. hookerianum*, while the combined ETS/ITS region revealed 2 bp changes, unique to Mingan *C. scariosum*. These results were consistent with those of Kelch and Baldwin (2003) who found levels of sequence divergence ranging from 0 to 5.2% in ETS and 0 to 6.7% in ITS in their much broader analysis of New World *Cirsium* and deemed the 2(-4) bp difference (ETS + ITS) between eastern and western *C. scariosum* to be an important indicator of divergence between populations of these two regions, especially given the similar or lower level of sequence divergence between distinct species, *C. hookerianum* and western *C. scariosum*. They concluded that western *C. scariosum* is molecularly more similar to *C. hookerianum* from the same region than it is to *C. minganense*.

These data favour a Pleistocene origin hypothesis for the Mingan population and suggest either that hybridization among western species has had a homogenizing effect or that Mingan *C. scariosum* originated from *C. scariosum* populations genetically different from those found in Alberta. Unfortunately, because of the limitations associated with this study, such as the genetic complexity of the species and the nature of the methods used, it remained impossible to decide if the two populations were in fact different species or varieties of *C. scariosum.*

The *Cirsium minganense* subpopulations located on the islands of the Mingan Archipelago National Park Reserve have likely been isolated from one another for thousands of years (Golden et al., 2008). Each island on which the species are found are separated by distances ranging from 1 to 3 kilometres (Figure 1.4). Combined with a weak dispersal rate (Pers. Comm. Nancy Dénommée, Parks Canada), and an extremely small number of individuals reaching anthesis per year (a total of 18 for 2018, per comm. Nancy Dénommée) it is expected that a great part of the species' reproduction is achieved through self-pollination (Schoen et al., 1996). Because of this, the populations are probably comprised of homozygotic individuals and low genetic diversity (Herlihy & Eckert, 2002). All these factors could result in an unhealthy or weaker gene pool (Burns, 2005; Richards, 1996).

Leeuwen (1981) showed that in two other species of *Cirsium* (*C. palustre* (L.) Scop. and *C. vulgare*), the absence of cross-pollination resulted in reduced achene production and when the achenes were produced, they were heavier than those resulting from cross-pollination. This led to a shorter distance of establishment in these wind-dispersed species causing a higher level of in-breeding and reduced genetic diversity within populations. Being a monocarpic species, *C. scariosum* flowers once in its lifetime and dies. For that reason, population size and distribution, and pollinator numbers, have a greater impact on reproduction because the probability of cross pollination is reduced (Brys et al., 2011). In some species that are particularly sensitive to inbreeding depression, the negative effects of selfing may outweigh the benefits of gene-transmission. This means that selfing can be disadvantageous, as the reduced fitness and genetic abnormalities resulting from inbreeding depression can ultimately harm the population (Jarne & Charlesworth. In such cases, outcrossing, or cross-fertilization with unrelated individuals, may be a more favorable reproductive strategy, as it can increase genetic diversity and reduce the risk of inbreeding depression (e.g., Lande and Schemske, 1985). However, selfing may still be favoured despite strong inbreeding depression because opportunities for outcrossing pollination are uncommon (Lloyd, 1992). Gauthier et al. (2010) showed that *Cirsium pitcheri* (Torrey ex Eaton) Torrey & A. Gray), another threatened species of monocarpic *Cirsium*, endemic to calcareous regions of the Great Lakes in Canada, was greatly affected by an overall low level of connectivity between populations and small fluctuating population sizes. This led to a low level of genetic diversity and high levels of inbreeding. The results from that study can most likely be inferred to our species of interest. The monocarpic nature of *C. scariosum* var. *scariosum* makes it vulnerable to smaller population sizes because the number of individuals per population needed to ensure cross pollination is higher (Halsey et al., 2017).

Unfortunately, the population number and size of subpopulations of *C. minganense* found in the MANPR are dwindling rapidly and have been since 1995 (per. comm. Nancy Dénommée). Over the period from 1995 to 2018, four out of the 11 aggregations found in the MANPR went extinct while the remaining seven have seen the number of individuals reduced by a mortality average of 50.62% (Park Canada, 2018). The reasons behind this significant decrease in individuals in the remaining aggregations is not clear, but the most probable causes are habitat loss due to storms, forest progression, and a lack of cross-pollination (Per. Comm. Nancy Dénommée).

The groupings of individuals have been divided in four levels in this thesis. First off, all groupings of individuals under the species *Cirsium scariosum* were labeled as a species population. The species population refers to the total number of individuals belonging to a particular species within a defined geographic area or ecosystem. Secondly, the groupings of all the individuals of the species *C. scariosum* from the Western (Waterton National Park) and the Eastern (Mingan Archipelago National Park) were deemed as being populations. In an effort to make things explicit when it came to determining what exactly what would be considered a population, I followed the guidelines stated by Wells & Richmond (1995). By their standards, a species population includes all individuals of a species and is a group of conspecific individuals that is demographically, genetically, or spatially disjunct from other groups of individuals while a subpopulation can be an arbitrary spatially-delimited subset of individuals from within a population. In the case of this study, it was decided that each spatial groupings of individuals on different islands of the Mingan Archipelago National Park were going to be referred to as subpopulations (a spatially clustered group of individuals) while groupings located on the same island were considered an aggregation only if it was as more than 500 meters from its nearest continuous neighbour; otherwise, it is part of the same aggregation of individuals with the subpopulation of the island.

Different initiatives have been undertaken to maintain the numbers of *C. minganense* in the Park. Since 1995, Parks Canada has been monitoring plant numbers on each of the islands to understand population dynamics and to detect any significant decrease in numbers. Each plant has been given a specific number and tag for identification purposes. For this reason, the locations of all the *C. minganense* individuals in the Park are known to the Parks Canada team. This allowed for the recovery of many plants after large storms completely buried most of the *C. minganense* populations in 2010, 2015 and 2016. In order to increase the survival rate of seeds in colonies, 10 000 seeds have been harvested from mature individuals and planted in theoretically more favorable growing zones on the islands since 2001 (Figure 1.5.). A germination rate of 47% has been observed. After 2010, the abundance of plants in flower was drastically reduced, thus limiting the number of available seeds. Combined with poor growing conditions, environmental limitations such as forest progression and habitat loss, low survival rates and the many years needed for a plant to flower, the restoration project through seeding has become unsustainable. Since 2017, harvested seeds were sent to the Montreal Biodome to promote faster growth and the production of stronger seedlings that can eventually be reintroduced in the Park. Seeds were also sent to the Gosling Research Institute for Plant Preservation (GRIPP) for micro-propagation purposes. In 2017, *C. minganense* was proposed as a COSEWIC candidate species to ensure its conservation (cosewic.ca).It is now essential to determine if *Cirsium minganense* is in fact a species or variety of its own, as this issue bears on its priority for conservation management in the Mingan Archipelago.

This study's main objective was to assess the genetic diversity of *Cirsium minganense* subpopulations on the MANPR islands. A genetic analysis using genotyping-by-sequencing (GBS; Elshire et al., 2011) of the subpopulations found on the islands of the Mingan Archipelago allowed me to better quantify genetic variability within *Cirsium minganense*, to understand whether plants on the islands reproduce mainly by self-pollination or cross-pollination (Poland & Rife, 2012), and to determine if there is any genetic exchange between subpopulations located on different islands. A secondary objective of this study was to determine if the western populations of *Cirsium scariosum* and *C. minganense* are distinct species or varieties. Golden et al. (2008) suggested that the western population of *C. scariosum* have engaged in hybridization with *C. hookerianum*. Their proposal is based on the observation that the Western population of *C. scariosum* may either be identical to *C. hookerianum* in the ETS/ITS regions or show a maximum difference of only 2 sites. Furthermore, significant genetic variations have been detected between the subpopulations of the Mingan archipelago and the western population in Waterton Lakes National Park.

Since the publication of this study, which utilized a limited number of plastid haplotype markers and the nrDNA region prone to concerted evolution, there have been considerable advancements in genetic analysis technologies.

2. MATERIALS AND METHODS

2.1. FIELD WORK AND SAMPLING

2.1.1. MINGAN ARCHIPELAGO PARK RESERVE (MANPR), QUEBEC

Cirsium minganense is distributed on four of the eight islands of the MANPR. Grosse Île au Marteau, Île du Havre and Île du Fantôme are each home to two aggregations while 3 aggregations are found on Île Niapiskau. Even though the aggregations found on the same islands could potentially show genetic exchange, the probability of that are extremely low for two reasons. First, except for the two aggregations located on the island Niapsikau, the distance between aggregations ranges from 1500 meters to more than 2 kilometers, exceeding the foraging distance of most pollinators (USDA, 2008). Second, the yearly census done by Parks Canada has shown that since 1995, there have only been a handful of instances where plants in different aggregations on the same island flowered at the same time, making pollination between subpopulations almost impossible (Table 1.1.). For these reasons, each aggregation was interpreted as a distinct population in this study. Unfortunately, 4 of the aggregations found in the Mingan Archipelago National Park were excluded from the study either because of a lack of individuals (less than 5) or simply because the aggregation has been completely extirpated from the Park. Sample collection took place in the last week of August 2019.

The islands of the Mingan archipelago are home to three other *Cirsium* species: *Cirsium vulgare, Cirsium arvense* and *Cirsium muticum* Michx, with the latter being the only species native to North America. *Cirsium muticum* is very rare in the Park and only occurs in marshes near the middle of islands, whereas *C. minganense* is found close to the sea shore (Pers. Comm. Nancy Dénommée, Parks Canada). The risks of misidentification were thus non-existent. The other two non-native *Cirsium* species are found in low numbers mostly in disturbed areas and prairies with only one co-occurrence known for *C. arvense* with *C. minganense.* The species were easily distinguished by their distinctive morphological features. *Cirsium vulgare* has adaxial leaf faces with slender appressed bristle-like prickles (efloras.org) whereas *C. minganense* has adaxial leaf faces thinly arachnoid tomentose and soon glabrescent. Both species of invasive *Cirsium* also exhibit branching stems while in *C. minganense*, the stem is either absent in vegetative plants with only a basal rosette of leaves, or simple and erect when in flower (Keil, 2006). Hybridization was also unlikely as interspecific hybridization between the introduced European *Cirsium* species and the native North American *Cirsium* species has never been observed (Slotta et al., 2012; Kelch & Baldwin, 2003). A total of 153 individuals were collected from seven colonies across the MANPR and seven samples that were also collected from plants originating from Niapsikau maintained *ex situ* at the Montreal Biodome following the same protocol as described below (Table. 2.1). To avoid excessive damage to the plants, three criteria had to be met in order to take a sample from an individual: i) fresh leaf samples were harvested only from individuals characterized as 'grandes rosettes' (large vegetative individuals ≥ 11.3 cm in diameter) or plants in flower, ii) the individual needed to have at least 5 leaves, and iii) the plant to be sampled had to be healthy (e.g. no yellowing leaves). The number of individuals that were big enough for sampling varied greatly between aggregations. To ensure that maximum genetic diversity was included in our samples, 25 individuals per aggregation were sampled (Hale et al., 2012) when possible. For colonies with less than 25 individuals, a sample of all the plants that met the criteria in that aggregation were taken. Fresh 5 cm X 5 cm leaf samples were harvested and conserved in silica gel at room temperature until DNA was extracted (see below).

2.1.2. SAMPLING IN WATERTON LAKES NATIONAL PARK, ALBERTA

Sampling was done by Peter Achuff during a census of *C. scariosum* in the Waterton Lake National Park. The same protocol was followed for the collection of samples in that area. In addition, *Cirsium hookerianum* Nutt. samples were also collected because they are known to hybridize with *Cirsium scariosum* in certain locations where the two species' distribution overlap. Furthermore, it has been pointed out by previous research (Golden et al., 2008) that the western Canadian *C. scariosum* population might share more similarities to *C. hookerianum* than with *C. minganense.* If two or more species were present within a location, we would expect morphological and/or genomic data to distinguish them.

Additionally, three samples of *Cirsium undulatum* (Nutt.) Spreng. were collected. A representative voucher specimen was taken from each site sampled. The samples are currently kept at the National Herbarium of Canada (CAN).

Of the 25 sites analysed (Cisc – *Cirsium* scariosum, Ciho – *Cirsium hookerianum*, Cixx – Mixed/hybrid population), two appeared to be mixed – (F12, [Cisc & Cixx] and SLR1, [Ciho & Cixx]) – as indicated by morphology. Geographically, Cisc (*Cirsium* scariosum) and Ciho (*Cirsium hookerianum*) sites mostly occur separately and Cixx sites usually occur where Cisc and Ciho are in close proximity. This is not always true though; in a few places Cisc and Ciho are close together (found in the same 100m radius) but no Cixx were detected or Cixx occurred without both Cisc and Ciho being near-by. The Cixx pattern may be influenced by sampling intensity but the separate ranges of Cisc and Ciho seem well supported. A detailed list of the samples collected in the Waterton Lakes National Park can be found in Table 2.2.

2.1.3. HERBARIUM SPECIMEN SAMPLING

Species included in analyses were chosen using phylogenetic trees of tribe Cardueae (Kelch & Baldwin, 2003) and the genus *Cirsium* (Bodo Slotta et al, 2012). They were selected for their genetic relatedness to the main species of interest. These include *Cirsium scariosum* Nuttall var. *itcherum* (Gray) Keil (Gillett & Mosquin, 80977, DAO; Gillett & Crompton, 80987, DAO), *Cirsium scariosum* Nuttall var. *scariosum* (Grondin, 783278, 783277, 783278, DAO; Hamel & Généreux, 81016, DAO), *Cirsium scariosum* Nuttall var. *coloradense* (Rydb.) Keil (Mosquin, 700045, DAO; Gardner, 305303, DAO), *Cirsium edule* Nuttall (Calder & Mackay, 866279, DAO; Calder & Saville, 866302, DAO), *Cirsium faucium* Petrak (Rzedowski, 831277, DAO), *Cirsium flodmanii* (Rydb.) Arthur (Boivin, 866387, DAO; Marshall, 854295, DAO), *Cirsium occidentale* (Nutt.) Jepson (Pollard, 633949, DAO; Pollard, 633963, DAO), *Cirsium occidentale* (Nutt.) Jeps. Var. *venustum* (Gree.) Jepson (Rose, 248859, MT), *Cirsium itcher* (Torr.) T. & G. (Cody et al., 159549, DAO; Oldham, 660785, DAO), and *Cirsium wheeleri* (Gray) Petrak (Parmelee et al., 870484, DAO; Parmelee et al., 870480, DAO). *Cirsium vulgare* (Savi) Tenore (Cody, 128067, DAO) was chosen as the outgroup as interspecific hybridization between the introduced European *Cirsium* species and the native North American *Cirsium* species is not likely, and it was found in a clade closely related to native North American species of *Cirsium* in previous phylogenetic analyses (Slotta et al., 2012; Kelch & Baldwin, 2003). A list of the herbarium specimens sampled for this project can be found in Table 2.3.

2.2. DNA extraction

DNA extraction from leaf samples was done following the standard protocol given for the Qiagen® Dneasy® plant kit. First, ≤0.05 g of silica dried leaf tissue was put in microcentrifuge tubes with a tungsten carbide bead. A working lysis solution (400 µl) was added into each microtube and grinded using a TissueLyser for a period of 1.5 minutes at 30 Hz. The tubes were then rotated in the racks and ground for another 1.5 min at 30 Hz to ensure that the samples were thoroughly pulverized. Buffer AP1 (400 µl) and 4 µl RNase A were added to the tubes and vortexed until the solution was homogenous and deemed exempt of clumps. The mixture was then incubated for 10 min at 65°C and mixed 2–3 times during incubation to lyse the cells. Buffer P3 (130 µl) was added to the lysate, mixed, and incubated for 5 min on ice in order to precipitate detergent, proteins, and polysaccharides. The lysate was centrifuged for 5 min at 20,000 x g (14,000 rpm) as some of the plant materials seemed to generate very viscous lysates and large amounts of precipitates. The lysate was pipetted into QIAshredder Mini spin columns and placed in a 2 ml collection tube before being centrifuged for 2 min at 20,000 x g (14,000 rpm). The flow-through fraction was transferred into a new tube without disturbing the cell-debris pellet and 1.5 volumes of Buffer AW1 was added to the cleared lysate. The mixture (650 µl) was then transferred into a DNeasy Mini spin column and placed in a 2 ml collection tube that was centrifuged for 1 min at ≥6000 x g. The flow-through was discarded afterward. This step was repeated with the remaining mixture. The DNeasy Mini spin column was placed into a new 2 ml collection tube and 500 µl of Buffer AW2 was added and centrifuged for 1 min at ≥6000 x g (≥8000 rpm). The flow-through was discarded. Buffer AW2 (500 µl) was added to the DNeasy Mini spin column and centrifuged for 2 min at 20,000 x g (14,000 rpm) to dry the membrane and transferred to a 1.5 ml microcentrifuge tube. Buffer AE (100 µl) was pipetted directly onto the DNeasy membrane. The tubes were incubated for 5 min at room temperature, and then centrifuged for 1 min at ≥6000 x g (≥8000 rpm) to ensure elution. The last step was repeated a second time. The samples were then conserved at 4 C°. DNA quantification was done using a spectrophotometer (Nanodrop 1000, Fisher Scientific) and a fluorometric quantification method (Qubit, Csaikl et al., 1998). A total of 200 ng of genomic DNA per sample was used for the preparation of the GBS libraries.

2.3. GENOTYPING BY SEQUENCING

Genotyping-by-Sequencing (GBS) is a powerful tool for conservation genetics for several reasons (Poland et al., 2012):

1. Cost-effectiveness: GBS is a relatively inexpensive method compared to traditional genotyping techniques that can be time-consuming and costly. GBS allows for the genotyping of many individuals at a fraction of the cost of traditional methods, making it an attractive option for conservation genetics.

2. High-throughput: GBS can generate thousands of Single Nucleotide Polymorphisms (SNPs) across the genome, allowing for a high level of resolution in the study of genetic diversity, population structure, and gene flow. This high-throughput approach enables researchers to obtain a detailed understanding of the genetic makeup of populations and the factors that contribute to their vulnerability.

3. Non-invasiveness: GBS can be performed using non-invasive sampling techniques, such as extracting DNA from leaves and seeds without harming or disturbing the target species or their habitats.

4. Application across diverse taxa: GBS can be applied across a wide range of plant species, from crops to wild, non-model plants, making it a versatile tool for conservation genetics.

In summary, GBS is an efficient, cost-effective, and non-invasive method for genotyping large numbers of plant samples and obtaining detailed insights into genetic diversity, population structure, and gene flow. As such, it has become an increasingly popular tool for conservation genetics, helping to inform the development of effective conservation strategies for threatened plant species and their ecosystems.

Library preparation was done by IBIS at the University of Laval. The protocol used for NGS library preparation was largely inspired from the original procedure developed in the Poland Lab (Poland et al. 2012), with modifications described by Abed et al. (2019). This method consisted of (1) common and barcoded adapter preparation; (2) complexity reduction using enzymes; and (3) multiplexing using barcoded adapters. Genomic DNA was co-digested with the restriction enzymes PstI (CTGCAG), MspI (CCGG) and NSi1(ATGCAT). Barcoded adapters were then ligated to individual samples. Samples were pooled by plate into a single library and amplified by a polymerase chain reaction. Each 96-plex plate was sequenced on a single lane of Illumina HiSeq 6000 (Genome Quebec, University of McGill) using 256 bp paired-end reads.

2.4. DATA ANALYSIS USING THE STACKS PIPELINE

2.4.1. CLEANING THE DATA

Following sequencing, raw FASTQ files obtained from the Illumina sequencing were demultiplexed using the process_radtags program in STACKS (version 2.59) with options '-r' (rescue barcodes) and '-q' (quality filtering).

2.4.2 RUNNING THE CORE PIPELINE COMPONENTS

The main STACKS pipeline consists of six programs:— building single-end loci (ustacks), creating a catalog of loci (cstacks), matching samples back against the catalog (sstacks), transposing the data to be organized from sample to instead being organized by locus (tsv2bam), assembling the paired-end contig, calling variable sites in the population and genotyping each sample at those sites (gstacks) and finally, perform a population genomics analysis (populations).

ustacks — This program takes as input a set of short-read sequences and aligns them into exactly-matching stacks (or putative alleles), the stacks are then compared and a set of putative loci is formed. From that set, the program detects SNPs at each locus using a maximum likelihood framework. The program was run using the default command options except for -m — minimum depth of coverage required to create a stack for which a depth of two was used.

cstacks — Executing cstacks will build a catalog of all loci across the population from any set of samples processed by the ustacks program. It will create a set of consensus loci, merging alleles together. Loci are matched up across samples according to sequence similarity. The program was run using only the default command options and parallel execution with six threads was enabled.

sstacks — The sets of stacks (i.e. putative loci) that were created by the ustacks program can now be searched against a catalog produced by cstacks. Parallel execution with six threads was enabled.

tsv2bam — The data is then transposed so that it is oriented by locus, instead of by sample. This is executed by the tsv2bam program. Because our data was composed of pair-end reads, the tsv2bam program pulled in the set of paired-end reads that were associated with each single-end locus that was assembled de novo. The program was run using only the default command options except for the parallel execution which was set at 20 threads instead of one.

gstacks — The gstacks program examines a GBD data set one locus at a time, looking at all individuals in the aggregation for that locus. The program starts with the results of the core single-end pipeline (ustacks→cstacks→sstacks→tsv2bam), incorporates the paired-end reads that were previously fetched by tsv2bam, assembles the paired-end reads into a contig, merges the contig with the single-end locus, and finally aligns reads from individual samples to the locus.

It then identifies SNPs within the meta population for each locus and then genotypes each individual at each identified SNP. Once SNPs have been identified and genotyped, gstacks will phase the SNPs at each locus for each individual into a set of haplotypes. The program was run using only the default command options except for the parallel execution which was set at 20 threads instead of one.

populations — The populations program generates population-level summary statistics and exports data in a variety of formats by analyzing a population of individual samples. A population map specifying which individuals belong to which population was entered in the programs command which then calculates population genetic statistics such as expected/observed heterozygosity, π, and FIS at each nucleotide position.

Three separate analyses were run with the populations program to target specific samples and populations in the data. One run was done with all the *C. minganense*, *C. scariosum* and *C. hookerianum* samples but excluding the herbarium specimens and the other *Cirsium* species used in our analyses. A second run was executed including only *C. minganense*, and finally, a third run was done with all the individuals, including our field samples, herbarium specimens, and other *Cirsium* species available for analysis.

The three analyses were set to run with twenty threads in parallel sections of code (-t 20), a minimum of 90% of individuals across populations required to process a locus (--min-samples-overall 0.9), and with results exported in Variant Call Format (VCF), fstats, Structure, Genepop, Plink and Fasta formats. All other program commands were run with default settings.

2.5 TREE BUILDING IN R

The neighbor joining tree was reconstructed in R (v 4.1.1.). The dataset was first converted from vcfR format (a format that allows the user to manipulate and visualize VCF data in R) to a genlight object. The data was then filtered to remove outliers by taking out any samples with less than 20000 loci. A total of 13 samples were removed from the initial 287 samples after filtering. The tree was constructed using the aboot function in R which calculates a dendrogram with bootstrap support using any distance applicable to genlight objects. I used the `bitwise.dist` distance, which calculates the number of allelic differences between individuals, and set the cutoff parameter to 50. The rest of the parameters were set to default. Trees were rooted using an outgroup (Caetano-Anollés et al., 2018). I had originally intended to use *C. vulgare* as the outgroup. However, specimens of this species yielded relatively low numbers of loci. Consequently, I chose *C. undulatum*, based on its presumed sister-relationship to the *C. scariosum/C. hookerianum* group (Slotta et al., 2012), and the high number of loci retrieved for it.

Clade support for internal nodes in the tree was subjectively characterised as very poor or very weak (<55% BS), poor or weak (55%–64% BS), moderate (65% - 74% BS), good or well (75%–84% BS), very good or very well (85%–94% BS), and strong (95%–100% BS). The limits of these categories follow Starr et al. (2004) and are based on previous simulation studies (Hillis and Bull 1993; Huelsenbeck et al. (1996).

2.6 PRINCIPAL COMPONENTS ANALYSIS IN R

A PCA was executed for the whole dataset, the *C. minganense* dataset and the Western *C. scariosum* dataset. The function glPca in adegenet (v 2.0.1) in R was used to execute the analysis. This function implements Principal Component Analysis (PCA) for very large SNP datasets stored as a genlight object. The first PCA, which included the whole dataset, was filtered to remove outliers by taking out any samples with less than 20000 loci, while the other two PCA datasets were filtered to remove outliers by taking out any samples with less than 15000 loci as this was still enough data to reduce any noise in the results and the quality of the reads for the samples included in this particular analysis allowed for a more rigorous filtering threshold. All PCAs used only unscaled data.

2.7 ADMIXTURE ANALYSIS

I used ADMIXTURE analysis to assess population structure and potential gene flow among two groups: Western *C. scariosum* and *C. hookerianum* samples, and the Eastern *C. minganense* subpopulations from MANPR. The ADMIXTURE software (v 1.3.0) is a Bayesian clustering method used to better understand the population structure in our sample dataset. My protocol follows Liu et al. (2020) for running ADMIXTURE. The analysis was run with values of K from 1 to 20, and the resulting cross validation error rates were used to select the best K value for the dataset. A K value of 9 was chosen for the Eastern samples' dataset (Figure 2.1.) and a K value of 2 for the Western *C. scariosum var. scariosum* dataset (Figure 2.2.).

3. RESULTS

3.1. POPULATION STATISTICS

Table 3.1. shows the results for the population statistics analysis from STACKS for each aggregation of *C. minganense* in the Mingan Archipelago National Park Reserve. The number of private alleles is small in proportion to the number of calculated loci (3228 loci). Results for the allelic richness (mean = 1.00X), P (mean = 0.99X; frequency of the most frequent allele at each locus), observed heterozygosity (mean = 0.00X), expected heterozygosity (mean = 0.00X), Pi (mean value of π = <0.01, nucleotide diversity) indicate most loci are fixed within populations, and individuals are mostly homozygous. The inbreeding coefficient (FIS) observed was low for all aggregations (mean = < 0.001) while the fixation index (Fst) was 0.4728.

3.2. NEIGHBOUR JOINING TREE

Most internal nodes in the neighbour joining tree had strong support with bootstrap (BS) values >97% (Figures 3.1 and A.1.). Three basal nodes separated *Cirsium undulatum*, *C. pitcheri* and *C. occidentale* in turn from the rest of the species sampled (97-100% BS), while the next node separates all western Canadian samples of *C. scariosum var. scariosum*, *C. hookerianum*, and their putative hybrids into a very poorly supported clade where the species and their putative hybrids are intermingled and do not form natural groups. The terminal portion of the tree consisted of a clade (100%) where *C. edule* was sister to a monophyletic group (100% BS) comprising a strongly supported (100% BS) *C. minganense* clade sister to a single western *C. scariosum* var. *americanum* sample from Colorado. Additionally, strong support (BS values > 95%) was found on each branch representing a aggregation of *C. minganense* and a clear distinction can be seen between each island and colony. Interestingly, one sample (NIAP2-2633) originating from the Niapsikau island not only branched out from the subpopulation for that island, but also from the entirety of the Mingan Archipelago National Park branches. This sample went through the same filtering and was deemed to have reliable enough data to be included in the analysis. The seemingly uniqueness of this individual might be linked to a high number of unique alleles. For this reason, this individual might be heavily considered as a candidate for cross breeding in an eventual conservation program.

3.3. PRINCIPAL COMPONENT ANALYSIS

The PCA of the 91 genotypes for western *C. scariosum var. scariosum*, *C. hookerianum* and *C. scariosum x hookerianum* (Figure 3.2.) explained only a small portion of the variance for principal components 1 (8.96%) and 2 (3.63%). This reflects what was previously seen in the results for the neighbor joining tree and the admixture analysis with *C. scariosum* var. *scariosum* forming a mostly discrete cloud, while *C. hookerianum* overlapped substantially with the suspected hybrids.

The PCA plot of 265 genotypes of *C. scariosum var. scariosum*, *C. hookerianum*, *C. scariosum x hookerianum*, *C. edule*, *C. occidentale*, *C. pitcheri*, *C. scariosum var. Americanum, C. undulatum* and *C. minganense* (Figure 3.3.) resulted in an explained variance of 54.32% for PC1 and 3.16% for PC2. Western *C. scariosum* and *C. minganense* were clearly separated on the first axis, while the remaining taxa were distinguished along the second axis.

Principal co-ordinates analyses of 165 *C. minganense* genotypes (Figure 3.4.) exhibits similar results to previous analyses. Panel (A) shows the explained variance for principal components 1 and 2 (25.39% and 20.31% respectively) and panel (B) shows the explained variance for principal components 2 and 3 (14.21%). Fantome 4 and Fantome 5 are clearly separated on the first and second axes. Marteau 11 forms a distinct cluster on axis 3. Havre 6 and Havre 7 overlap on all three axes, as do Niapsikau 1 and Niapsikau 2 (mirroring their intermixture in the ADMIXTURE plot; see below). The two aggregations from Havre and the one from Marteau are intermixed clusters in the analysis suggesting a possible relatedness between them.

3.4. ADMIXTURE ANALYSIS

The admixture analysis plot for the *C. minganense* subpopulations seen in Figure 3.5 supports what was observed in the neighbor joining tree. Each aggregation is clearly distinct and only a very small amount of genetic information is shared between them. The two aggregations found on the island Niapsikau are an exception with three genetic clusters spread across the two locations. This was expected as these two aggregations are within 100 meters of one another. Interestingly, the aggregation found on the island of Marteau shows two genetic clusters, but they are not shared with any other location.

Figure 3.6. shows the admixture analysis plot done on the Western Canadian *C. scariosum* and *C. hookerianum*. *Cirsium hookerianum* is composed almost entirely of a single genetic cluster. *Cirsium scariosum* var scariosum is mostly a second cluster, but with substantial admixture with the *C. hookerianum* cluster. Putative hybrids show a mix of both clusters. This plot reveals a large amount of shared genetic information between the two species, and even more so in the suspected hybrid plants.

4. DISCUSSION

4.1. PHYLOGENY OF *CIRSIUM SCARIOSUM*

For the sake of its conservation, it is essential to determine whether *C. minganense* should be treated as a species distinct from *C. scariosum* or as a variety of *C. scariosum*. In order for a plant to be protected under COSEWIC, it either has to be an accepted taxonomic species, native to Canada or it must meet certain criteria regarding taxonomic validity, native origin, regularity of occurrence and dependence on Canadian habitat.

In certain cases, taxa below the species level (subspecies, varieties, or regional populations) may be considered for protection. For this to occur, they must be determined to be “Designatable Units”, which has two criteria: discrete, and significant.

Discrete requires evidence of distinguishing markers/characteristics that separate the taxon from other groups of the same species, and that this distinction is associated with a natural separation over a considerable amount of time. There are no set definitions for how distinct, and how long a time period, is required. Significance, the second criteria of a designable unit, states that the discrete group has unique markers/characteristics relative to other populations of the same species. It is thus not a species of its own yet, but it is currently going through a speciation process.

In the context of this project, the data showed that *C. minganense* is a separate species. The evidence for *Cirsium minganense* surpasses the standards needed for a group to fall under COSEWIC protection with the main argument being that the group is not a result of recent human introduction over the past century or so, but a natural disjunction dating back thousand of years. Moreover, the results showed substantial genetic divergence from *C. scariosum*. Lastly, even if the group was not to be considered a species, we can make the case that *C. minganense* meets the criteria for a designable unit, thus whether or not the conclusion that *C. minganense* is a species is accepted, the unit most definitely qualifies for COSEWIC listing.

4.1.1 Western population of *Cirsium scariosum* var. *scariosum*

The neighbor joining tree shows a single branch including both the Western *C. scariosum* populations and the *C. hookerianum* population combined with a large amount of hybridization between the two species. This is also represented in the Admixture analysis and it is clearly seen in the PCA. I can conclude from this that these two species might be more similar and closely related that previously thought by Golden et al. (2008). It has been shown that *Cirsium* species have a propensity to hybridise (Bures et al, 2004 & 2010; Wells, 1983; Dabydeen, 1987 & 1997), even when they possess drastically different morphological traits (Bures et al., 2010). A good example of that would be the morphological dissimilarity of hybrid *Cirsium palustre* × *C. rivulare* as compared to its parental species, *C. rivulare* and *C. palustre*. The resulting hybrid generally showcases fewer flower heads, more densely packed leaves with higher spike density and thinner leaf blades then either of its parents. *C. palustre displays* a tightly packed cluster of flower heads while *C. rivulare* has 5 flower heads pointed in all 5 axis (Bures et al., 2010). These combinations can lead to hybrids with reduced fertility, but the more common result is a population of fertile hybrids (Bures et al., 2010). This shows there are few to no breeding barriers between many *Cirsium* species (new world vs old world species are generally not able to hybridize), and a great potential for the emergence of new characters and character combinations in hybrids. It is thus logical to raise the question: Are the Western populations of *C. scariosum* still genetically pure enough to be considered as one species? The *C. scariosum* x *C. hookerianum* hybrid seems to have become quite prevalent is some areas where both species overlap, and the genetic similarity between the species is quite noticeable in our analyses. This might be a significant factor when considering how long these species have been exchanging genetic information and could suggest that certain populations of Western *C. scariosum* are potentially worth reconsidering as distinct varieties. And following that, if Western *C. scariosum* and *C. hookerianum* are closer to each other than to Eastern *C. scariosum*, is it logical to state that both populations of *C. scariosum* are the same species? Answering these questions would require a more detailed genetic analysis of the population genetics of *Cirsium scariosum* in Western Canada while also including samples from other *C. scariosum* populations from the United States.

4.1.2 WESTERN AND EASTERN POPULATION OF *CIRSIUM SCARIOSUM*

One hypothesis for the presence of *C. scariosum* on the Mingan Islands was an anthropogenic introduction by botanists who were collecting samples on the islands between

1914 and 1925; this would mean that the species occurrence in the Park would date back only a century (Moore & Frankton, 1967). If that theory was correct, I would expect that samples of *C. minganense* would be either nested within the clade for Western *C. scariosum* or at least sister to it. This is not what is seen in our neighbor joining tree. In fact, the Western population of *C. scariosum* is more closely related to *C. hookerianum* than it is to *C. minganense*. Moreover, our analysis suggests that *C. edule* is more closely related to *C. minganense* than Western *C. scariosum* var. *scariosum*, a result that is consistent with the phylogenetic analyses of tribe Cardueae (Kelch & Baldwin, 2003) and *Cirsium* (Bodo Slotta et al, 2012), where *Cirsium edule* is not placed as sister to *C. scariosum* var. *scariosum*. Even though support for branches is very poor in either Kelch & Baldwin (2003) or Slotta et al. (2012) (bootstrap support was found to be under 50), both analyses are consistent with this analysis in suggesting that *C. edule* is not genetically close to Western *C. scariosum* var. *scariosum.*

The PCA (Figure 3.8.) also supports a distant relationship between *C. scariosum* var. *scariosum* and *C. minganense*. These two groups are found tightly clumped at each extremity of the X axis, with *C. minganense* clearly isolated from all other species included in the analysis. The X axis shows the highest level of explained variance at 54.32% versus the Y axis at 3.16%. This would not be expected in a population that had been disjunct for less than 100 years. For reference, other species included in this analysis, such as *C. edule* and *C. pitcheri* show noticeably less distance (less variance) on the X axis, and they are officially regarded as true distinct species having diverged thousand of years ago. It would thus be logical that the appreciable variance associated to *C. minganense* and its Western counterpart would be representative of a divergence that would be measured in thousand of years rather than hundreds. The only outlier in the results is the remaining *C. scariosum* var. *americanum* herbarium specimen although its location in both the neighbor joining tree and PCA still shows an appreciable distance with C. *minganense*, supporting the later as its own distinct species. All other specimens of *C. scariosum* varieties from the United States had to be discarded because of low quality and less than 20 000 loci in the samples. As this sample was taken from a specimen collected in 1966 and represents the only other variety of *C. scariosum* that could be included in the study, an analysis that includes all the putative varieties of *C. scariosum* will be necessary to gain a complete picture of how *C. minganense* is related to the southern varieties of *C. scariosum*.

4.1.3. CIRSIUM MINGANENSE; *CIRSIUM SCARIOSUM* VAR. *MINGANENSE* OR *CIRSIUM SCARIOSUM*?

The overall results of this study clarifies taxonomy of the peculiar eastern population of *C. minganense*. All of the results show a definitive disjunction between *C. minganense* and its Western *C. scariosum* counterpart with the only exception to this being the single *C. scariosum* var. a*mericanum* sample from Colorado*.* Even then, the weight of the data supports *C. minganense* as a distinct species given its distant relationship to *C. scariosum* var. *scariosum* in the NJ tree, the long branch separating it from *C. scariosum* var. *americanum* (Figure 3.1) and its distance from all other samples in Principal Components analysis (Figure 3.8). For this reason, I propose that the Eastern population of *Cirsium scariosum* var. *scariosum* should be recognised as a distinct species, *Cirsium minganense.* For reference, other members of *Cirsium* have been labeled as their own species based on results that were far less conclusive than the data presented in this paper as reported by Ackerfield et al. (2020). For example, *Cirsium cymosum* var. *cymosum*, *C. cymosum* var. *canovirens*, *C. brevifolium*, and *C. inamoenum* form a taxonomically challenging group found in California, the Pacific Northwest, and the northern

Rocky Mountains (Lesica, 2012). These taxa share several morphological characteristics, such as solitary pedunculate heads, corollas ranging from white to pale lavender, and leaves that clasp or extend down the stem up to 3 cm (Keil, 2006). The distinctions between these taxa are subtle and often not well captured in herbarium specimens. For example, *C. cymosum* var. *cymosum* and *C. inamoenum* have a discreet glutinous dorsal ridge on their involucral bracts (Greene, 1897), whereas *C. brevifolium* and *C. cymosum* var. *canovirens* display a prominent dorsal ridge (Keil, 2006). However, identifying the glutinous dorsal ridge can be challenging with herbarium specimens, as it tends to dry and turn brown, making it difficult to use as a criterion after collection. For this reason, *Cirsium cymosum* and *C. inamoenum* were both described concurrently (as Carduus) by Greene (1897).

4.2. POPULATION GENETICS OF *CIRSIUM SCARIOSUM* IN THE MINGAN ARCHIPELAGO NATIONAL PARK RESERVE

The population genetics of *Cirsium minganense* is one of the main subjects of interest when it comes to conservation of the subpopulations seen in the Mingan Archipelago National Park Reserve. A good understanding of the overall gene pool of the population and the gene flow between aggregations is necessary to make informed decisions and to create an effective conservation plan (Kramer & Havens, 2009).

4.2.3 OVERALL GENETIC HEALTH OF *C. MINGANENSE* IN THE MINGAN ARCHIPELAGO NATIONAL PARK RESERVE

Cirsium minganense is genetically depauperate, most likely due to inbreeding, isolation, and past population bottlenecks. Our initial hypothesis stated that the subpopulations would be mainly comprised of homozygotic individuals with low genetic diversity (Herlihy & Eckert, 2002). This supposition was based on factors such as the weak dispersal rate of fruits and pollen (Pers. Comm. Nancy Dénommée, Parks Canada), an extremely small number of individuals reaching anthesis per year, which would lead to the hypothesis that successful reproduction would be achieved more often through self-pollination. Population statistics revealed that the population is nearly entirely comprised of fixed homozygotes. The Fst obtained (0.4728) also indicates highly differentiated aggregations (>0.25 would fit that description). This is consistent with the rest of the results collected throughout the analyses.

Given the data document these subpopulations are extremely genetically depauperate, the relatively low FIS value is surprising. This coefficient is a measure of the deviation of the observed genotype frequencies in a population from the expected genotype frequencies under random mating, based on allele frequencies observed in the subpopulation. It ranges from -1 (all individuals are heterozygous) to 1 (all individuals are homozygous).

An FIS value near zero implies that there is not an excess of homozygotic individuals in the population, meaning that the observed genotype frequencies are not too far off from the expected frequencies under random mating (Waples, 2015). However, a low FIS value does not necessarily mean that the population has high heterozygosity. In fact, a low FIS value can be consistent with past inbreeding or extreme bottleneck events (Miao, Y. C. et al., 2016). For example, if a population has experienced a severe bottleneck event, only a small subset of the original genetic variation would be present in the current population. Over time, this can lead to a decrease in heterozygosity and an increase in homozygosity, even if the population is not currently experiencing high levels of inbreeding.

Similarly, past inbreeding events can also lead to a decrease in heterozygosity and an increase in homozygosity over time. If a population has undergone inbreeding in the past, then the offspring of related individuals will have a higher probability of being homozygous at any given locus. This can reduce the overall genetic diversity of the population, leading to low heterozygosity even if the current level of inbreeding is low. Therefore, a low FIS value in a population can indicate the absence of current high levels of inbreeding, but it can also suggest that the population has experienced past inbreeding or bottleneck events, which have reduced its genetic diversity. The low inbreeding coefficient (F_{IS}) in our results implies there is not an excess of homozygotic individuals, when considering allele frequencies at the population level at present although those allele frequencies indicate the populations have almost no heterozygosity. This is consistent with past inbreeding or extreme bottleneck(s) or with both events occurring.

Fst is directly related to the variance in allele frequency among populations and, conversely, to the degree of resemblance among individuals within populations. If Fst is small, it means that the allele frequencies within each population are similar; if it is large, it means that the allele frequencies are different. When considering populations of plants that are clearly classified as the same species, FST values above 15% are regarded as indicating significant differentiation, while values below 5% indicate insignificant differentiation or only minor distinctions (Frankham et al., 2002). A value of zero suggests complete panmixis, meaning that the two populations freely interbreed. On the other hand, a value of one indicates that all genetic variation can be attributed to population structure, implying that the two populations have no shared genetic diversity. The high Fst value found in the results for *Cirsium minganense* reflects the high level of differentiation and isolation of the subpopulations found on the islands of the Mingan Archipelago National Park.

4.2.4 Genetic isolation of *C. minganense* in the Mingan Archipelago National Park Reserve

Following my observations and the current literature, we hypothesized that the subpopulations of *C. minganense* located on the islands of the Mingan Archipelago National Park Reserve (MANPR) had likely been isolated from one another for thousands of years (Golden et al., 2008).

Even with such limited diversity, the different subpopulations of *C. minganense* found on the islands of the Archipelago are clearly divergent from each other, except for the aggregations found on the same islands.

Results provided by the Admixture analysis support the conclusion that the subpopulations located on different islands have been isolated for a prolonged period. The two aggregations found on Niapsikau share the same 3 genotypes, which is not surprising as they are located less than a hundred meters from one another. The rest of the subpopulations found in the Park, excluding the one found on Marteau, are all quite distinct from each other, even though some of them are found on the same island. Both populations found on Havre have distinctive genotypes and the same pattern can be observed for the two subpopulations found on Fantôme. Interestingly, the only current aggregation on Marteau seems to include two distinct genotypes. These results might originate from a vestigial population that once shared genes through secondary contact with the current surviving subpopulation. Secondary contact, which refers to the reuniting of two previously isolated subpopulations of the same species can occur after a period of geographic isolation or genetic divergence and is often associated with hybridization and gene flow between the two subpopulations. Secondary contact can lead to changes in the genetic composition and phenotype of the populations and can have both positive and negative effects on the fitness and survival of individuals within the subpopulations (Schnitzler et al., 2020).

Alternatively, mutation or a higher rate of sexual reproduction in that subpopulation could explain these results (Leimu et al., 2006). The subpopulation size would likely not be related as other islands have similarly sized subpopulations that do not show the same pattern.

The results obtained through the Neighbor joining tree also supports the conclusion that subpopulations located on different islands have been isolated for a prolonged period. Each branch of the tree within *C. minganense* is very distinct and divided by aggregation, showing a good genetic distance between each aggregation, even those on the same island. The two subpopulations found on Havre are found in a common branching before splitting at the end of the tree, something that can also be seen with the two aggregations on Fantôme. This is expected as aggregations found on the same island are more likely to share similar genotypes. The only exception to this pattern is found in the two aggregations found on Niapsikau where both groups are found together on the same branch without any clear separation into distinct groups. The individuals of *C. scariosum* found on Niapsikau in the two aggregations could potentially be recognized as being part of the same aggregation, as there is no clear genetic difference between the two aggregations.

The PCAs also showed a close relationship between aggregations found on the same island except for the two aggregations found on Fantôme, which is also seen in the Admixture analysis. Interestingly, the results in Figure 7 show a strong relatedness between the two Havre aggregations and the Marteau subpopulation. This could suggest that the populations found on one of the islands was initially founded by the other island through migration. Alternatively, another potential explanation would be that the two islands were at some point part of the same piece of land and eventually divided, separating the original populations into two distinct subpopulations that evolved isolated from one another (Dufour, 1979).

Finally, population statistics show an interesting number of private alleles were detected in each aggregation found on the islands of the MANPR. This might suggest that migration between aggregations on each island is very low which fits with the hypothesis that gene flow between populations would be low (Schierup, 1998)

4.2.5. Outcrossing *C. minganense* in the Mingan Archipelago National Park Reserve

These results now make it possible to make recommendations as to whether the different aggregations' genes should be mixed to increase genetic diversity or kept isolated from one another to conserve the genetic uniqueness of the populations in the Park. Each option has its advantages and disadvantages.

It has been shown that cross pollination of different source populations increases genetic diversity of rare plant populations (St. Clair et al., 2020). The transfer of genetic material either in the form of seeds, plants or pollen between isolated populations has been shown to reduce inbreeding levels and increase genetic diversity in other plant species (Brzyski et al., 2018). The increased phenotypic variability within the resulting population generally leads to a higher survival rate and better adaptability to abiotic and biotic factors (Marli et al., 2015; Bengtsson, 2000). This could represent a viable option that is worth considering when creating a conservation plan for *C. minganense* in the Mingan archipelago.

Although the benefits of out crossing the aggregations are a tempting approach to reduce the negative effects that comes with small, isolated populations, the impact of doing so artificially must consider its possible detrimental effects (Sletvold et al., 2012). Artificial cross breeding of individuals coming from isolated populations could lead to outcrossing depression (OD): breaking up co-adapted gene complexes, undermining local adaptation (Barmentlo et al., 2018). It is possible that some of the Mingan subpopulations have evolved local adaptations to their microhabitat (Leimu & Fischer, 2008). For example, some are on North facing slopes while others face South, which could have large impacts on their tolerance to freezing temperatures or the amount of light they receive over a year. The type of soil found in each location also varies greatly, ranging from sandy, loose soil to more rocky, packed soil (Smith et al., 2012).

In practice, predicting the likelihood of OD has been challenging, as noted by Edmands (2002) and McClelland and Naish (2007). Edmands (2002) listed 35 species, including plants, invertebrates, and vertebrates, where OD was observed, but did not provide information on the species in which OD did not occur. Frankham et al. (2011 & 2015) indicate that the likelihood of OD occurring in crosses between populations with the same karyotype, which were separated within the last 500 years and inhabit similar environments, is low. In their view, the probabilities of OD are considerably smaller than the chances of population extirpation resulting from the negative effects of inbreeding depression and loss of genetic diversity in separate, small, isolated populations.

With this in mind, I recommend a cautious approach to outbreeding, validated with experimental data. Controlled outcrossing of individuals from different islands in a controlled environment such as the GRIPP (Gosling Research Institute for Plant Preservation). The fitness of the progeny from these crosses should then be evaluated, first ex situ (in the controlled environment), and then in situ at the location of one of the extirpated populations in the Mingan National Park. Ideally, this would be one of the far eastern islands in the Mingan Archipelago that exhibits similar habitat conditions to where the species is currently found. As such test sites would be many kilometers away from currently known subpopulations and in the direction of the prevailing wind, the possibility for unintended crossings via pollinators would be minimised. This would allow rigorous evaluation of the risks and benefits of genetic augmentation with no risk to the remaining wild subpopulations.

5. CONCLUSION

To conclude, the phylogenetic analysis of *Cirsium scariosum* and its Eastern population, *C. minganense*, raises questions about their taxonomic classification and genetic relationships. The analysis reveals a close genetic similarity and hybridization between Western Canadian *C. scariosum* populations and *C. hookerianum*, suggesting that the Western populations may not be comprised of only two species but also a new *C. scariosum x C. hookerianum* hybrid. Furthermore, the analysis indicates that *C. minganense* is more closely related to C. edule than to Western *C.*

scariosum, supporting the classification of the Eastern population as a distinct species. The population genetics analysis of *C. minganense* in the Mingan Archipelago National Park Reserve shows that the population is genetically depauperate, likely due to inbreeding, isolation, and past population bottlenecks. The subpopulations in the park are genetically distinct, indicating a prolonged period of isolation. The decision of whether to promote outcrossing between subpopulations to increase genetic diversity or to keep them isolated to preserve their uniqueness poses challenges. While outcrossing can enhance genetic diversity and adaptability, it may also lead to the loss of unique alleles and disrupt local adaptation, potentially causing outbreeding depression. Careful consideration is necessary when making conservation decisions for *C. minganense*, considering the potential benefits and risks associated with outcrossing.

6. SUPPORTING TABLES

Table 1.1. Number of *C. minganense* individuals per colony on each island of the Mingan Archipelago National Park Reserve in 2018. (Grande rosettes are large vegetative individuals ≥ 11.3 cm in diameter) (Parks Canada, 2018)

Island	**Aggregation**	**Grandes rosettes (diam. ≥11,3 cm)**	**Flowering plants**
Île Niapiskau	1	30	1
Île Niapiskau	2	123	14
Île du Fantôme	4	18	0
Île du Fantôme	5	108	0
Île du Havre	6	20	0
Île du Havre	7	19	0

Grosse Île au Marteau	11	29		1

Table 2. 1. List of *Cirsium minganense* samples collected in the Mingan Archipelago Park Reserve. Collector: Simon Piché-Mongeon.

Identification number	**Origin**	**Aggregation**	**Date of collection**	**Coordinates**
0106	Niapsikau Island, Quebec	1	29/07/2019	N50 13.300 W63 44.793
1004	Niapsikau Island, Quebec	1	30/07/2019	N50 13.283 W63 44.820
1011	Niapsikau Island, Quebec	1	29/07/2019	N50 13.287 W63 44.813
0341	Niapsikau Island, Quebec	1	30/07/2019	N50 13.293 W63 44.802
1014	Niapsikau Island, Quebec	1	30/07/2019	N50 13.287 W63 44.813
0342	Niapsikau Island, Quebec	1	30/07/2019	N50 13.293 W63 44.802
1109	Niapsikau Island, Quebec	1	30/07/2019	N50 13.300 W63 44.797
0923	Niapsikau Island, Quebec	1	30/07/2019	N50 13.293 W63 44.802
1017	Niapsikau Island, Quebec	1	30/07/2019	N50 13.290 W63 44.804
1129	Niapsikau Island, Quebec	1	30/07/2019	N50 13.287 W63 44.814
0736	Niapsikau Island, Quebec	1	30/07/2019	N50 13.300 W63 44.796
1115	Niapsikau Island, Quebec	1	30/07/2019	N50 13.293 W63 44.802
1069	Niapsikau Island, Quebec	1	30/07/2019	N50 13.287 W63 44.813
2927	Niapsikau Island, Quebec	2	29/07/2019	N50 13.268 W63 44.902
3046	Niapsikau Island, Quebec	2	30/07/2019	N50 13.256 W63 44.913
2931	Niapsikau Island, Quebec	2	29/07/2019	N50 13.261 W63 44.912
2928	Niapsikau Island, Quebec	2	29/07/2019	N50 13.267 W63 44.907
2306	Niapsikau Island, Quebec	2	29/07/2019	N50 13.270 W63 44.905
3048	Niapsikau Island, Quebec	2	29/07/2019	N50 13.265 W63 44.906
2935	Niapsikau Island, Quebec	2	30/07/2019	N50 13.275 W63 44.917
2942	Niapsikau Island, Quebec	2	29/07/2019	N50 13.269 W63 44.899
2633	Niapsikau Island, Quebec	2	29/07/2019	N50 13.264 W63 44.913
3149	Niapsikau Island, Quebec	2	29/07/2019	N50 13.268 W63 44.913
1550	Niapsikau Island, Quebec	2	29/07/2019	N50 13.257 W63 44.914
3065	Niapsikau Island, Quebec	2	29/07/2019	N50 13.263 W63 44.909
3054	Niapsikau Island, Quebec	2	29/07/2019	N50 13.263 W63 44.914
2619	Niapsikau Island, Quebec	2	30/07/2019	N50 13.269 W63 44.915
3069	Niapsikau Island, Quebec	2	30/07/2019	N50 13.270 W63 44.919
3050	Niapsikau Island, Quebec	2	29/07/2019	N50 13.268 W63 44.901
3047	Niapsikau Island, Quebec	2	29/07/2019	N50 13.257 W63 44.914
3052	Niapsikau Island, Quebec	2	29/07/2019	N50 13.268 W63 44.906
3053	Niapsikau Island, Quebec	2	29/07/2019	N50 13.265 W63 44.911
3068	Niapsikau Island, Quebec	2	29/07/2019	N50 13.269 W63 44.913
3070	Niapsikau Island, Quebec	2	30/07/2019	N50 13.270 W63 44.922
3056	Niapsikau Island, Quebec	2	29/07/2019	N50 13.267 W63 44.909
3154	Niapsikau Island, Quebec	2	29/07/2019	N50 13.264 W63 44.917
2934	Niapsikau Island, Quebec	2	29/07/2019	N50 13.265 W63 44.913
3042	Niapsikau Island, Quebec	2	29/07/2019	N50 13.268 W63 44.919

2945	Niapsikau Island, Quebec	2	30/07/2019	N50 13.272 W63 44.917
3090	Niapsikau Island, Quebec	2	30/07/2019	N50 13.276 W63 44.879
3043	Niapsikau Island, Quebec	2	29/07/2019	N50 13.266 W63 44.909
3045	Niapsikau Island, Quebec	2	29/07/2019	N50 13.260 W63 44.918
3049	Niapsikau Island, Quebec	2	29/07/2019	N50 13.262 W63 44.916
3067	Niapsikau Island, Quebec	2	29/07/2019	N50 13.267 W63 44.915
1413	Fantôme island, Quebec	4	29/07/2019	N50 14.209 W63 40.763
1377	Fantôme island, Quebec	4	29/07/2019	N50 14.183 W63 40.756
1378	Fantôme island, Quebec	4	29/07/2019	N50 14.183 W63 40.755
1453	Fantôme island, Quebec	4	29/07/2019	N50 14.183 W63 40.755
1451	Fantôme island, Quebec	4	29/07/2019	N50 14.211 W63 40.767
1450	Fantôme island, Quebec	4	29/07/2019	N50 14.212 W63 40.765
1398	Fantôme island, Quebec	4	29/07/2019	N50 14.205 W63 40.765
0080 (GRIPP)	Fantôme island, Quebec	4	29/07/2019	N50 14.211 W63 40.764
1379	Fantôme island, Quebec	4	29/07/2019	N50 14.182 W63 40.756
1455	Fantôme island, Quebec	4	29/07/2019	N50 14.180 W63 40.755
1914	Fantôme island, Quebec	5	30/07/2019	N50 13.819 W63 40.498
1240	Fantôme island, Quebec	5	30/07/2019	N50 13.810 W63 40.506
1666	Fantôme island, Quebec	5	30/07/2019	N50 13.807 W63 40.502
1425	Fantôme island, Quebec	5	30/07/2019	N50 13.809 W63 40.503
653	Fantôme island, Quebec	5	30/07/2019	N50 13.810 W63 40.498
2624	Fantôme island, Quebec	5	29/07/2019	N50 13.809 W63 40.502
2897	Fantôme island, Quebec	5	30/07/2019	N50 13.808 W63 40.502
0243	Fantôme island, Quebec	5	30/07/2019	N50 13.815 W63 40.499
1545	Fantôme island, Quebec	5	29/07/2019	N50 13.803 W63 40.506
2839	Fantôme island, Quebec	5	29/07/2019	N50 13.807 W63 40.502
0493	Fantôme island, Quebec	4	30/07/2019	N50 14.181 W63 40.756
3091	Fantôme island, Quebec	5	29/07/2019	N50 13.803 W63 40.504
2616	Fantôme island, Quebec	5	30/07/2019	N50 13.809 W63 40.501
0266	Fantôme island, Quebec	5	30/07/2019	N50 13.820 W63 40.499
0279	Fantôme island, Quebec	5	30/07/2019	N50 13.808 W63 40.503
0271	Fantôme island, Quebec	5	30/07/2019	N50 13.805 W63 40.502
1522	Fantôme island, Quebec	5	30/07/2019	N50 13.815 W63 40.499
1922	Fantôme island, Quebec	5	30/07/2019	N50 13.818 W63 40.497
0250	Fantôme island, Quebec	5	29/07/2019	N50 13.813 W63 40.498
2058	Fantôme island, Quebec	5	30/07/2019	N50 13.804 W63 40.502
1449	Fantôme island, Quebec	4	30/07/2019	N50 14.211 W63 40.766
2072	Fantôme island, Quebec	5	30/07/2019	N50 13.806 W63 40.503
2047	Fantôme island, Quebec	5	29/07/2019	N50 13.804 W63 40.505
0625	Fantôme island, Quebec	5	29/07/2019	N50 13.802 W63 40.506
2258	Fantôme island, Quebec	5	29/07/2019	N50 13.813 W63 40.499
2609	Fantôme island, Quebec	5	30/07/2019	N50 13.811 W63 40.500
3501	Fantôme island, Quebec	5	29/07/2019	N50 13.806 W63 40.502
2581	Fantôme island, Quebec	5	29/07/2019	N50 13.805 W63 40.505
1674	Fantôme island, Quebec	5	30/07/2019	N50 13.806 W63 40.501

1935	Fantôme island, Quebec	5	30/07/2019	N50 13.817 W63 40.497
1430	Fantôme island, Quebec	4	30/07/2019	N50 14.210 W63 40.763
1375	Fantôme island, Quebec	4	30/07/2019	N50 14.183 W63 40.756
1413	Fantôme island, Quebec	4	30/07/2019	N50 14.210 W63 40.764
1578	Fantôme island, Quebec	5	30/07/2019	N50 13.813 W63 40.501
0644	Fantôme island, Quebec	5	29/07/2019	N50 13.807 W63 40.502
3941	Havre island, Quebec	6	31/07/2019	N50 13.309 W63 35.700
2625	Havre island, Quebec	6	31/07/2019	N50 13.309 W63 35.702
2292	Havre island, Quebec	6	31/07/2019	N50 13.309 W63 35.700
1480	Havre island, Quebec	6	31/07/2019	N50 13.310 W63 35.700
1479	Havre island, Quebec	6	31/07/2019	N50 13.310 W63 35.701
2500	Havre island, Quebec	6	31/07/2019	N50 13.298 W63 35.733
1494	Havre island, Quebec	6	31/07/2019	N50 13.319 W63 35.799
1489	Havre island, Quebec	6	31/07/2019	N50 13.319 W63 35.803
1490	Havre island, Quebec	6	31/07/2019	N50 13.319 W63 35.802
1000	Havre island, Quebec	6	31/07/2019	N50 13.320 W63 35.806
1317	Havre island, Quebec	6	31/07/2019	N50 13.321 W63 35.812
1003	Havre island, Quebec	6	31/07/2019	N50 13.322 W63 35.811
2608	Havre island, Quebec	6	31/07/2019	N50 13.326 W63 35.830
2290	Havre island, Quebec	6	31/07/2019	N50 13.326 W63 35.845
1652	Havre island, Quebec	6	31/07/2019	N50 13.325 W63 35.830
2291	Havre island, Quebec	6	31/07/2019	N50 13.326 W63 35.844
1493	Havre island, Quebec	6	31/07/2019	N50 13.318 W63 35.800
1488	Havre island, Quebec	6	31/07/2019	N50 13.318 W63 35.798
1435	Havre island, Quebec	7	31/07/2019	N50 13.036 W63 36.167
0735	Havre island, Quebec	7	31/07/2019	N50 13.018 W63 36.175
1458	Havre island, Quebec	7	31/07/2019	N50 13.023 W63 36.172
1439	Havre island, Quebec	7	31/07/2019	N50 13.034 W63 36.166
1394	Havre island, Quebec	7	31/07/2019	N50 13.020 W63 36.172
1382	Havre island, Quebec	7	31/07/2019	N50 13.020 W63 36.173
2810	Havre island, Quebec	7	31/07/2019	N50 13.018 W63 36.173
1434	Havre island, Quebec	7	31/07/2019	N50 13.033 W63 36.167
1386	Havre island, Quebec	7	31/07/2019	N50 13.021 W63 36.173
1440	Havre island, Quebec	7	31/07/2019	N50 13.033 W63 36.166
1497	Havre island, Quebec	7	31/07/2019	N50 13.025 W63 36.173
3990	Havre island, Quebec	7	31/07/2019	N50 13.021 W63 36.172
1395	Havre island, Quebec	7	31/07/2019	N50 13.020 W63 36.171
1483	Havre island, Quebec	7	31/07/2019	N50 13.035 W63 36.166
1452	Havre island, Quebec	7	31/07/2019	N50 13.029 W63 36.171
3986	Havre island, Quebec	7	31/07/2019	N50 13.018 W63 36.174
1381	Havre island, Quebec	7	31/07/2019	N50 13.020 W63 36.173
1441	Havre island, Quebec	7	31/07/2019	N50 13.033 W63 36.169
1392	Havre island, Quebec	7	31/07/2019	N50 13.019 W63 36.176
3152	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.885 W63 32.983
2829	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.885 W63 32.978

0563	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.885 W63 32.982
2008	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.843 W63 33.063
3051	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.886 W63 32.981
0568	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.885 W63 32.983
3146	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.887 W63 32.983
3136	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.885 W63 32.985
1503	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.847 W63 33.056
3005	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.842 W63 33.064
0593	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.841 W63 33.064
3809	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.885 W63 32.987
2126	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.884 W63 32.976
3808	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.883 W63 32.990
3150	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.884 W63 32.984
1588	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.841 W63 33.063
1821	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.836 W63 33.071
2225	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.899 W63 32.934
1940	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.838 W63 33.065
2585	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.887 W63 32.972
1516	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.840 W63 33.065
1605	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.840 W63 33.068
0596	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.842 W63 33.064
2765	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.887 W63 32.972
3125	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.884 W63 32.988
3801	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.884 W63 32.989
3802	Grosse Île au Marteau, Quebec	11	31/07/2019	N50 12.884 W63 32.988
2153	Montreal Biodome (GRIPP), Niapsikau	2	17/09/2019	
0699	Montreal Biodome (GRIPP), Niapsikau	2	17/09/2019	
B1363	Montreal Biodome (GRIPP), Fantôme	4	17/09/2019	
3805	Montreal Biodome (GRIPP), Fantôme	4	17/09/2019	
2081	Montreal Biodome (GRIPP), Niapsikau	2	17/09/2019	
B587	Montreal Biodome (GRIPP), Marteau	11	17/09/2019	
B2925	Montreal Biodome (GRIPP), Niapsikau	2	17/09/2019	

Table 2. 2. List of samples collected in the Waterton Lakes National Park, Alberta. Collector : Peter Achuff

Identification number	Species	Location	Latitude	Longitude	Elevation (Feet)	Date of collection
8329	*Cirsium undulatum* (Nutt.) Spreng.	Waterton National Park, Alberta	49.067480	-113.884838	1290	2019/9/11
8327	*Cirsium undulatum* (Nutt.) Spreng.	Waterton National Park, Alberta	49.067480	-113.884838	1290	2019/9/11
8325	*Cirsium undulatum* (Nutt.) Spreng.	Waterton National Park, Alberta	49.067480	-113.884838	1290	2019/9/11
8182	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.402435	-114.340224	1340	2019/9/10
8181	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.402435	-114.340224	1340	2019/9/10

8180	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.402435	-114.340224	1340	2019/9/10
8179	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.402435	-114.340224	1340	2019/9/10
8184	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.058690	-113.475000	1375	2019/9/11
8187	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.058690	-113.475000	1375	2019/9/11
8185	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.058690	-113.475000	1375	2019/9/11
8183	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.058690	-113.475000	1375	2019/9/11
8237	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.023683	-113.686566	1380	2019/9/28
8239	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.023683	-113.686566	1380	2019/9/28
8241	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.023683	-113.686566	1380	2019/9/28
8238	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.023683	-113.686566	1380	2019/9/28
8287	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.357830	-114.629630	1480	2019/8/2
8286	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.357830	-114.629630	1480	2019/8/2
8283	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.357830	-114.629630	1480	2019/8/2
8285-	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.357830	-114.629630	1480	2019/8/2
8285	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.357830	-114.629630	1480	2019/8/2
8284	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.357830	-114.629630	1480	2019/8/2
8275	*Cirsium scarioum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.327579	-114.572889	1480	2019/8/1
8272	*Cirsium scarioum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.327579	-114.572889	1480	2019/8/1
8271	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.302647	-114.572725	1470	2019/8/1
8269	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.302647	-114.572725	1470	2019/8/1
8267	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.302647	-114.572725	1470	2019/8/1
8299	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.082125	-114.191366	1465	2019/8/3
8303	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.082125	-114.191366	1465	2019/8/3
8301	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.082125	-114.191366	1465	2019/8/3
8280	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	49.398680	-114.643270	1555	2019/8/2
8277	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	49.398680	-114.643270	1555	2019/8/2
8315	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.343820	-114.118270	1710	2019/8/18
8309	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.343820	-114.118270	1710	2019/8/18
8318	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	49.343820	-114.118270	1710	2019/8/18
8312	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.343820	-114.118270	1710	2019/8/18
8225	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.232060	-114.230270	1555	2019/7/27
8177	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.232060	-114.230270	8329	2019/7/27
8226	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.232060	-114.230270	1555	2019/7/27
8223	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.232060	-114.230270	1555	2019/7/27
8224	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.232060	-114.230270	1555	2019/7/27
8202-	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.495190	-114.496150	1490	2019/7/26
8199	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.495190	-114.496150	1490	2019/7/26

8201	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.495190	-114.496150	1490	2019/7/26
8172	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.333550	-114.332980	8329	2019/7/19
8212	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.333550	-114.332980	1445	2019/7/28
8204	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.333550	-114.332980	1445	2019/7/28
8208	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.333550	-114.332980	1445	2019/7/28
8213	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.311490	-114.303790	1450	2019/7/27
8220	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.311490	-114.303790	1450	2019/7/27
8219	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.311490	-114.303790	1450	2019/7/27
8216	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.311490	-114.303790	1450	2019/7/27
8222	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.311490	-114.303790	1450	2019/7/27
8290	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.141810	-114.190570	1690	2019/8/3
8289	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.141810	-114.190570	1690	2019/8/3
8288	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.141810	-114.190570	1690	2019/8/3
8292-	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.141810	-114.190570	1690	2019/8/3
8248-	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.114573	-114.113548	1715	2019/7/29
8251	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.114573	-114.113548	1715	2019/7/29
8249	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.114573	-114.113548	1715	2019/7/29
8247	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.114573	-114.113548	1715	2019/7/29
8259	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.649952	-114.698695	1375	2019/7/31
8258-	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.649952	-114.698695	1375	2019/7/31
8261	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.649952	-114.698695	1375	2019/7/31
8257	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.649952	-114.698695	1375	2019/7/31
8264	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.657970	-114.729750	1385	2019/7/31
8262	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.657970	-114.729750	1385	2019/7/31
8266	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.657970	-114.729750	1385	2019/7/31
8192	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.496810	-114.397770	1780	2019/7/26
8191	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.496810	-114.397770	1780	2019/7/26
8193	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.496810	-114.397770	1780	2019/7/26
8189-	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.496810	-114.397770	1780	2019/7/26
8244	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.077178	-113.790414	1515	2019/7/28
8246	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.077178	-113.790414	1515	2019/7/28
8242-	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.077178	-113.790414	1515	2019/7/28
8243	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.077178	-113.790414	1515	2019/7/28
8308	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	51.064140	-115.348560	1395	2019/8/10
8306	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	51.064140	-115.348560	1395	2019/8/10
8304	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	51.064140	-115.348560	1395	2019/8/10
8324	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	50.574860	-114.963720	2105	2019/8/18

8320	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	50.574860	-114.963720	2105	2019/8/18
8322	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	50.574860	-114.963720	2105	2019/8/18
8228	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.307800	-114.404200	1425	2019/7/27
8236	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.307800	-114.404200	1425	2019/7/27
8235	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.307800	-114.404200	1425	2019/7/27
8233	*Cirsium scariosum* X *Cirsium hookerianum*	Waterton National Park, Alberta	49.307800	-114.404200	1425	2019/7/27
8256-	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	49.120313	-113.999263	1465	2019/7/29
8254	*Cirsium hookerianum Nutt.*	Waterton National Park, Alberta	49.120313	-113.999263	1465	2019/7/29
8198	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.444519	-114.415929	1395	2019/7/26
8195	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.444519	-114.415929	1395	2019/7/26
8197	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.444519	-114.415929	1395	2019/7/26
8296	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.151087	-114.298756	1505	2019/8/3
8298	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.151087	-114.298756	1505	2019/8/3
8294-	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.151087	-114.298756	1505	2019/8/3
8294	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.151087	-114.298756	1505	2019/8/3
8295-	*Cirsium scariosum* Nutt. var. *scariosum*	Waterton National Park, Alberta	49.151087	-114.298756	1505	2019/8/3

Table 2.3. List of *Cirsium* species samples collected in herbaria.

Identification number	**Species**	**Collector(s)**	**Country**	**State/Province**	**Year of collection**
DAO-854297	Cirsium vulgare	H.H. Marshall	Canada	Manitoba	1987
DAO-128067	Cirsium vulgare	W.J. Cody	Canada	Ontario	1975
DAO-783278	Cirsium scariosum var. scariosum	G. Baillargeon, Nicole Charest, Robert Gauthier	Canada	Québec	1977
DAO-660785	Cirsium pitcheri	Michael J. Oldham, Mireille Delisle-Oldham	Canada	Ontario	1989
DAO-831277	Cirsium faucium	J. Rzedowski	Mexico	Sultepec	1973
DAO-81016 (10558)	Cirsium scariosum var. scariosum	A. Hamel, H. Généreux	Canada	Québec	1957
DAO-633963	Cirsium occidentale	Henry M. Pollard	USA	California	1967
DAO-633949	Cirsium occidentale	Henry M. Pollard	USA	California	1958
DAO-159549	Cirsium pitcheri	W.J. Cody	Canada	Ontario	1956
DAO-870480	Cirsium wheeleri	J.A. Parmelee	USA	Arizona	1965
DAO-870484	Cirsium wheeleri	J.A. Parmelee	USA	Arizona	1965

MT-00248859	Cirsium occidentale	Lewis S. Rose	USA	California	1966
DAO-854295	Cirsium flodmanii	H. H. Marshall	Canada	Manitoba	1971
DAO-866387	Cirsium flodmanii	Unknown	Canada	Manitoba	1996
DAO-866302	Cirsium edule	J.A. Calder	Canada	British Columbia	1954
DAO-866279	Cirsium edule	J.A. Calder	Canada	British Columbia	1961
DAO-80987	Cirsium scariosum var. americanum	J.M. Gillett	USA	California	1966
DAO-80977	Cirsium scariosum var. americanum	J.M. Gillett	USA	Colorado	1963
DAO-700045	Cirsium scariosum var. coloradense	T. Mosquin	USA	Utah	1962
DAO-305303	Cirsium scariosum var. coloradense	R. Gardner	USA	Wyoming	1971

Table 3.1. Number of private alleles, allelic diversity (mean number of alleles per locus), P (mean frequency of the most frequent allele at each locus), observed heterozygosity (HO), expected heterozygosity (HE), πPi (mean value of π, nucleotide diversity and FIS (inbreeding coefficient) for each aggregation of *C. minganense* in the MANPR calculated across 3228 loci.

Aggregation	**n**	**Private alleles**	**Allelic richness**	**P**	H_O	H_E	**π**	F_{IS}
Niapsikau 1	13	8	1.0098	0.9943	0.0089	0.00699	0.00728	-0.00253
Niapsikau 2	32	34	1.0128	0.99354	0.00873	0.0083	0.00844	0.00107
Fantôme 4	15	5	1.0080	0.99297	0.01082	0.00775	0.00803	-0.0054
Fantôme 5	29	9	1.0041	0.99698	0.00576	0.00332	0.00338	-0.00413
Havre 6	18	27	1.0113	0.994	0.0075	0.00754	0.00777	0.003
Havre 7	18	5	1.0060	0.99556	0.00693	0.00527	0.00543	-0.0024
Marteau 11	28	18	1.0129	0.9921	0.00843	0.01002	0.0102	0.01076

7. SUPPORTING FIGURES

Figure 1.1. *C. minganense* in flower on the Mingan Archipelago National Park Reserve on the Niapsikau Island.

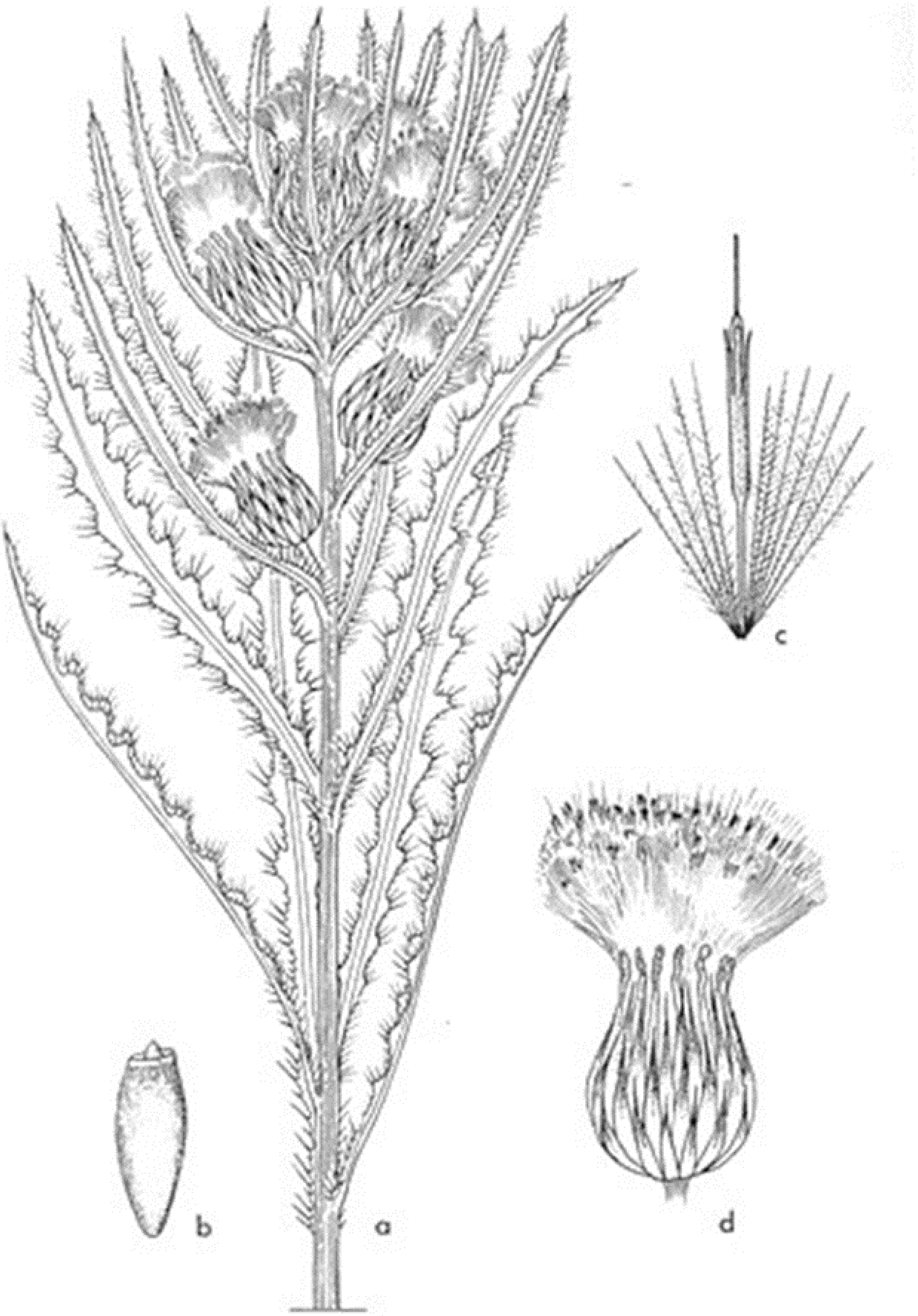

Figure 1.2. *Cirsium scariosum*. a – habit; b – achene; c – floret with pappus; d – head (adapted from Moore, 1974, p.40)

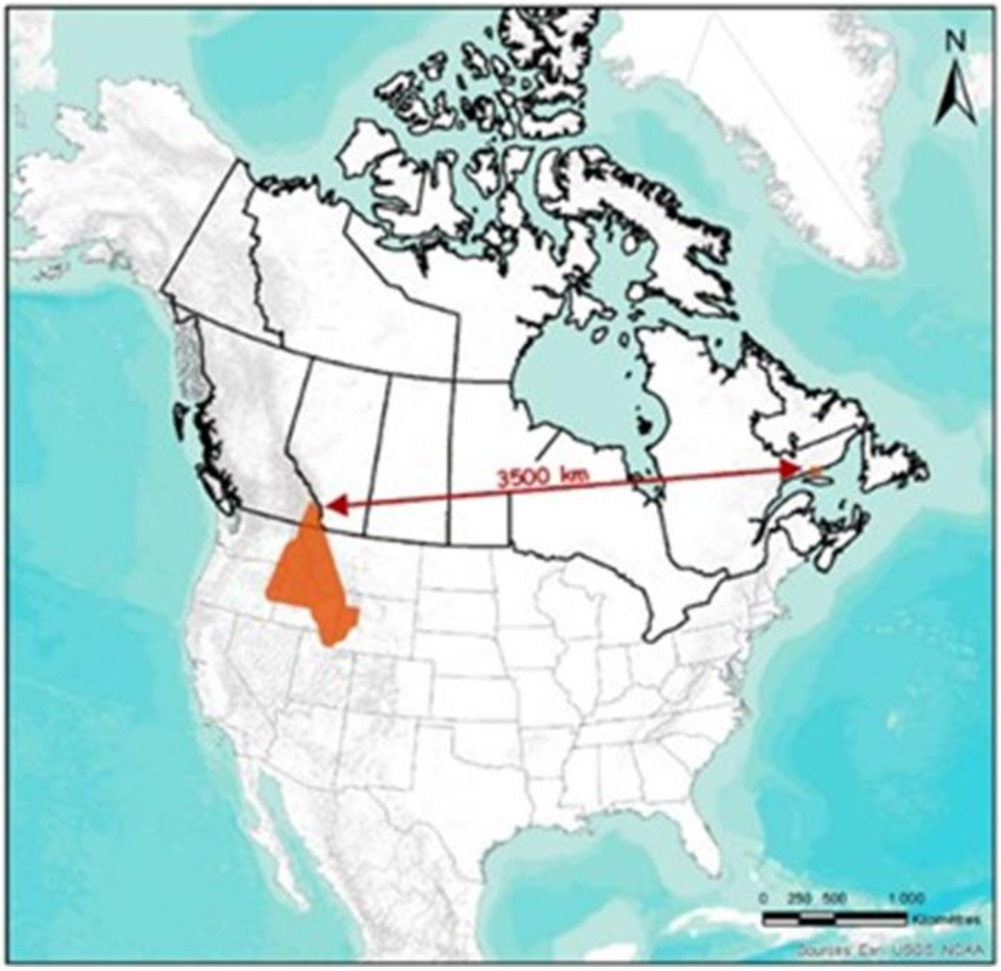

Figure 1.3. *C. scariosum* distribution in North America. (Parks Canada).

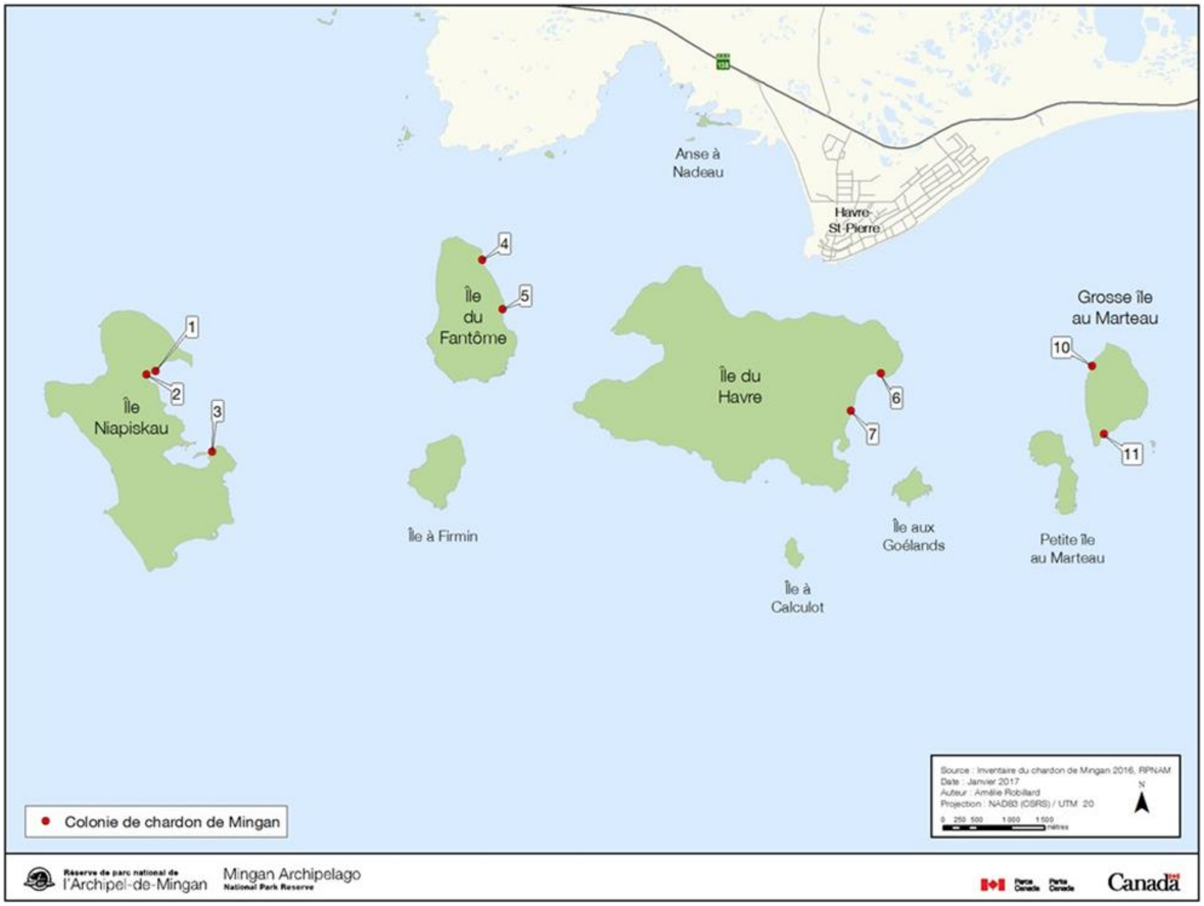

Figure 1.4. *C. minganense* aggregations distribution on the islands of the Mingan Archipelago National Park Reserve (Robillard, 2017). Only the sector of the Park where aggregations of *C. minganense* are found is illustrated.

Figure 3.6. *C. minganense* in the Mingan Archipelago National Park Reserve with mesh nets on the flowers to harvest the seeds. This particular individual was located on the Havre Island in the most northern aggregation and was sampled (sample HAV6-1439) on the 29th of July 2019.

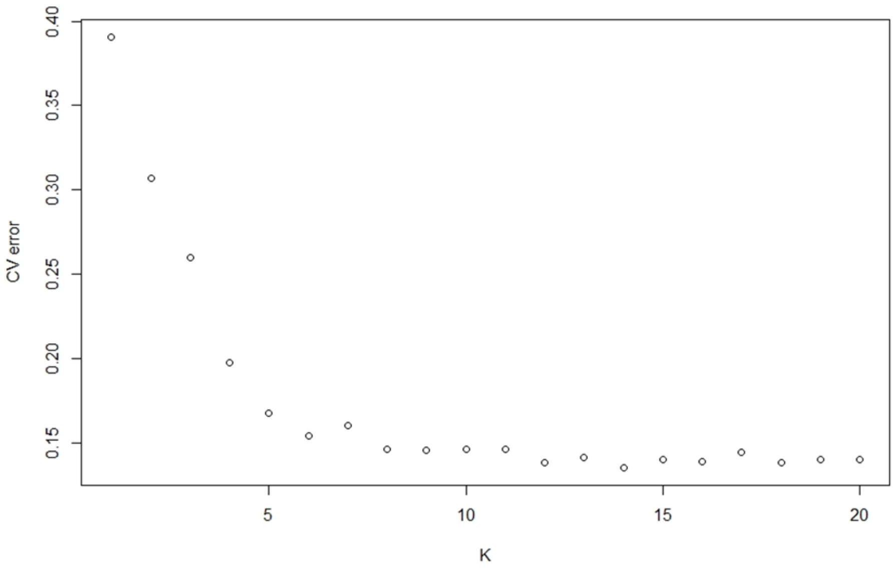

Figure 4.1. Cross-validation error as a function of K (K=1 to 20) for the Mingan populations dataset

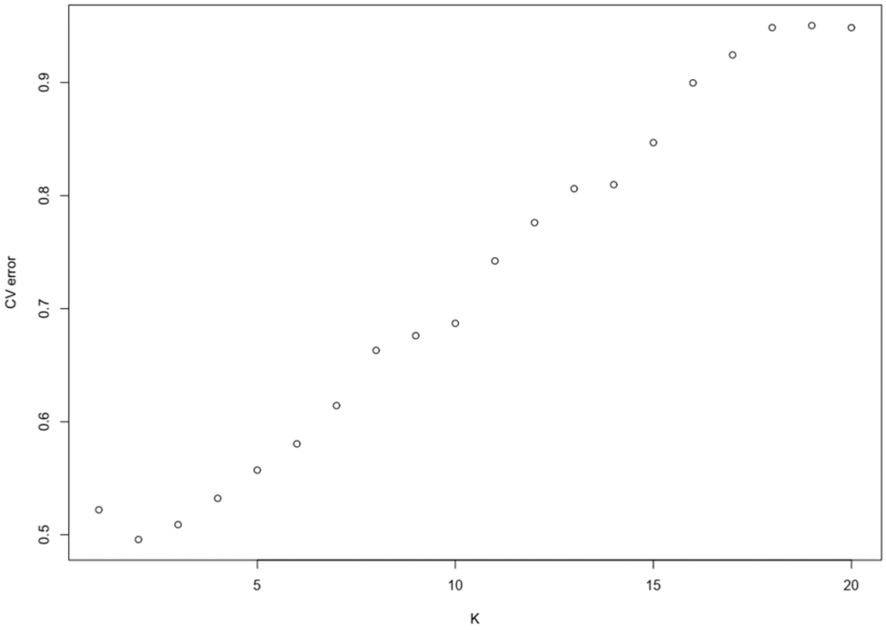

Figure 2.2. Cross-validation error as a function of K (K = 1 to 20) for *Cirsium scariosum* var. *scariosum*, *Cirsium hookerianum* and *Cirsium scariosum x hookerianum*

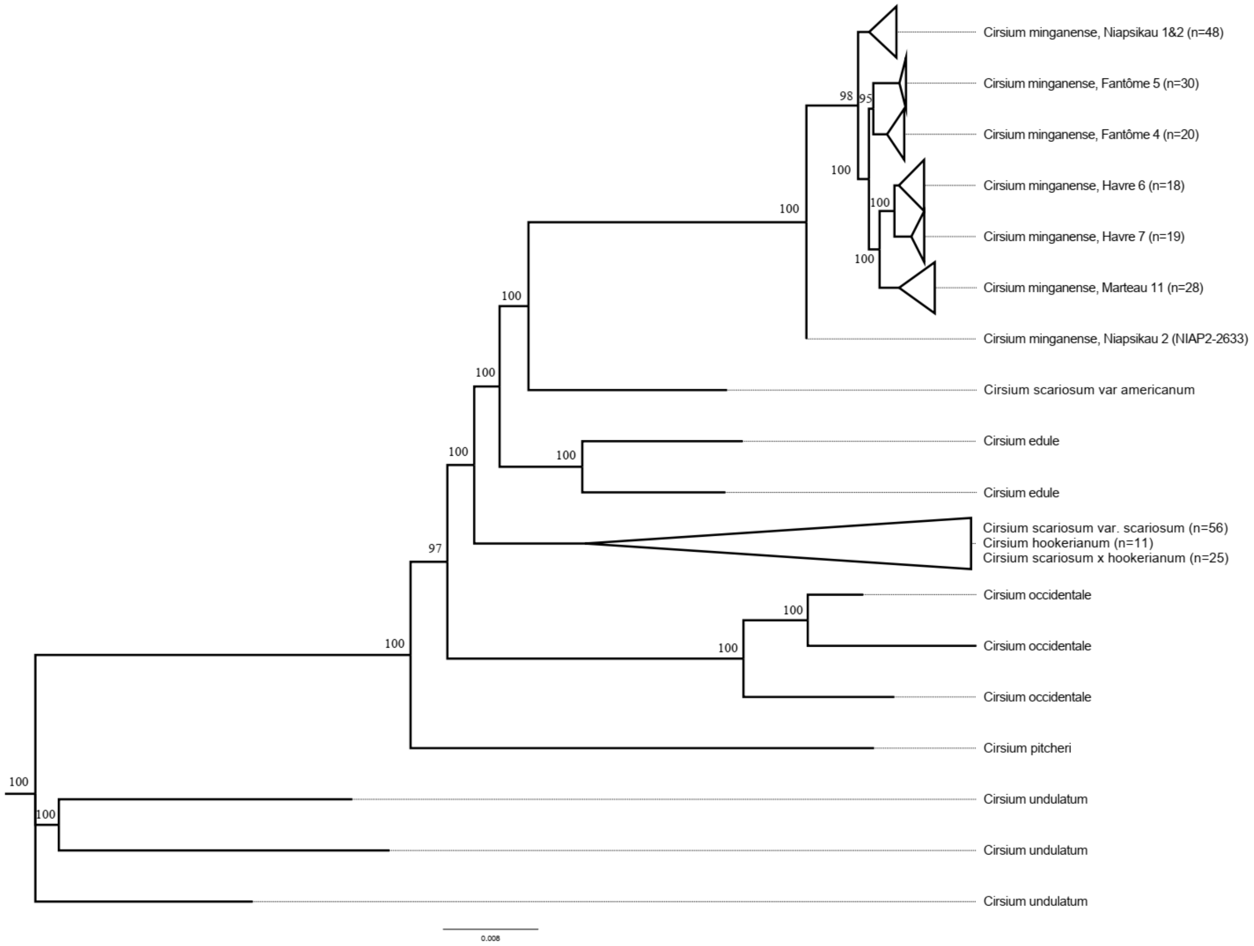

Figure 3.1. Simplified neighbor joining phylogenetic tree based on 266 samples and 25,843 binary SNPs. Branch support was assessed by 1000 standard bootstrap replicates in R. All bootstrap value below 50% are not given. Samples provenance for *C. minganense* are labeled as the island (Havre, Marteau and Fantôme) and followed by aggregation number if groups of individuals were separated by more than 500 meters.

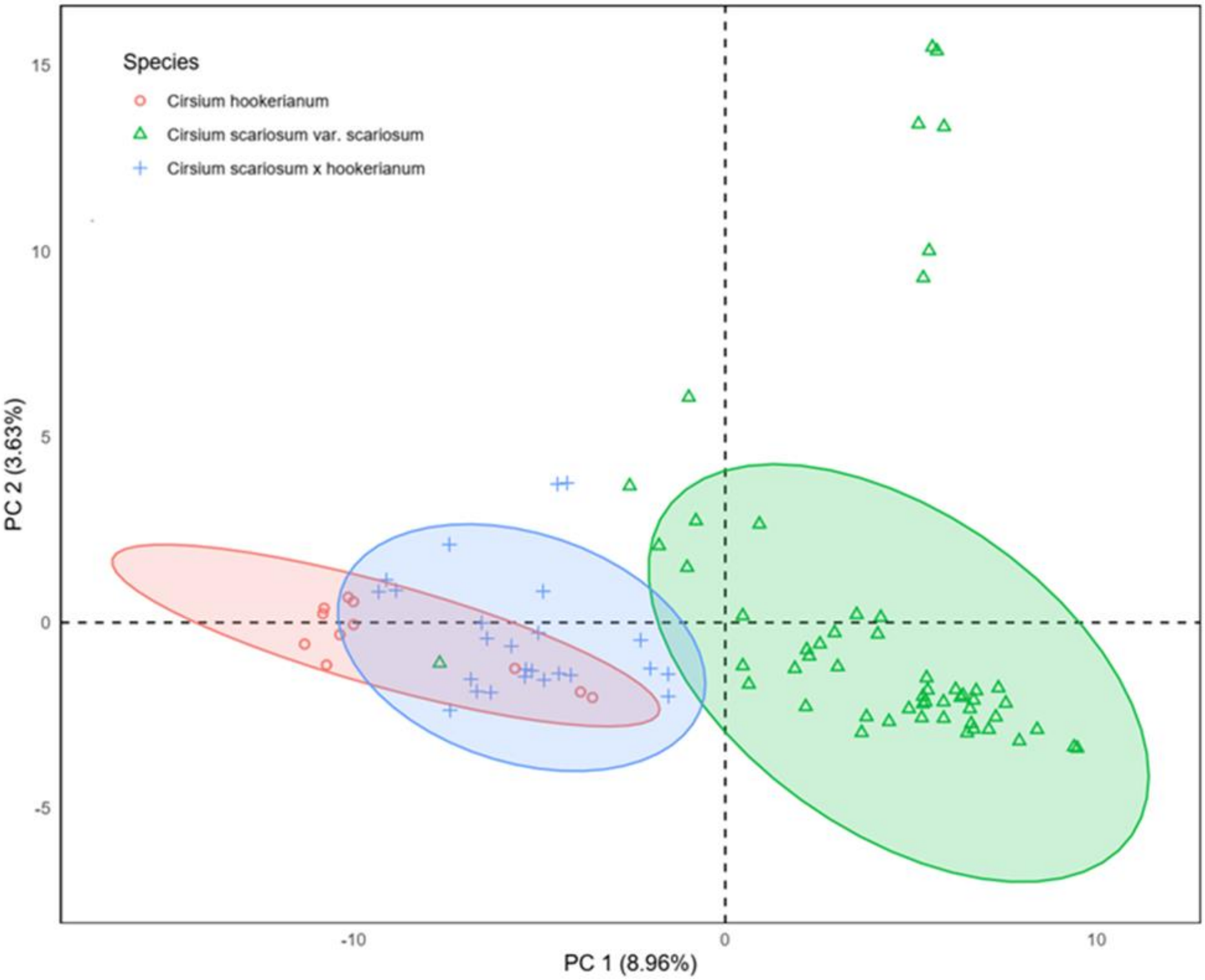

Figure 3.2. Principal co-ordinates analyses of 91 genotypes for *C. scariosum* var. *scariosum*, *C. hookerianum* and *C. scariosum x hookerianum* based on 25,843 binary SNPs obtained with genotyping-by-sequencing (GBS). The plot shows the explained variance for principal components 1 and 2. Colours and shapes represent different species as explained in the top left of the figure.

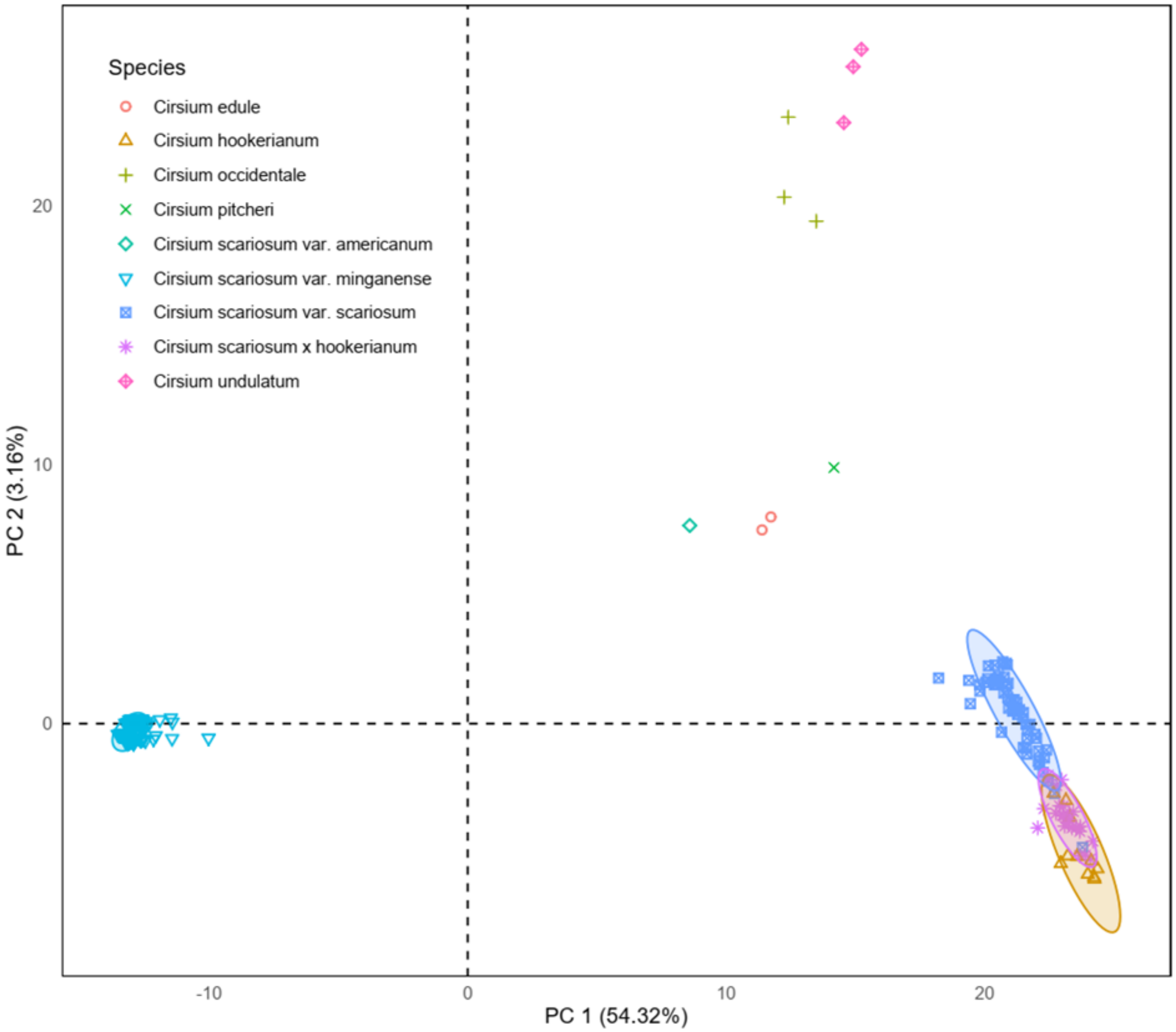

Figure 3.3. Principal co-ordinates analysis of 265 genotypes of different species and varieties of *Cirsium* based on 25,843 binary SNPs obtained with genotyping-by-sequencing (GBS) . Plot is showing the explained variance for principal components 1 and 2. Colours and shapes represent different species and varieties as noted at the top left of the figure.

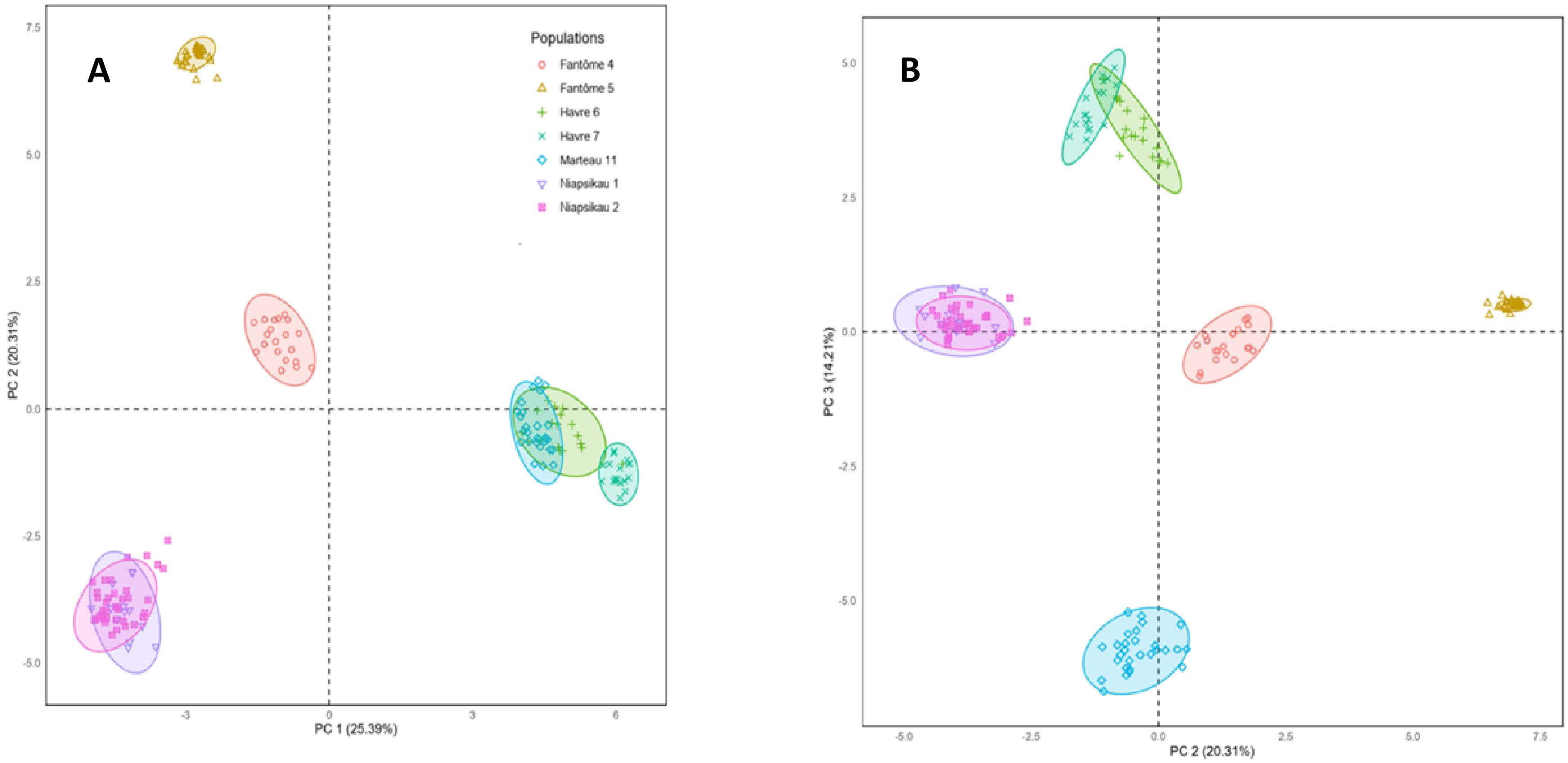

Figure 3.4. Principal co-ordinates analyses of 165 C. minganense genotypes based on 25,843 binary SNPs obtained with genotyping-by-sequencing (GBS). Panel (A) shows the explained variance for principal components 1 and 2, panel (B) shows the explained variance for principal components 2 and 3. Colours and shapes represent different populations as given in the top right of panel (A).

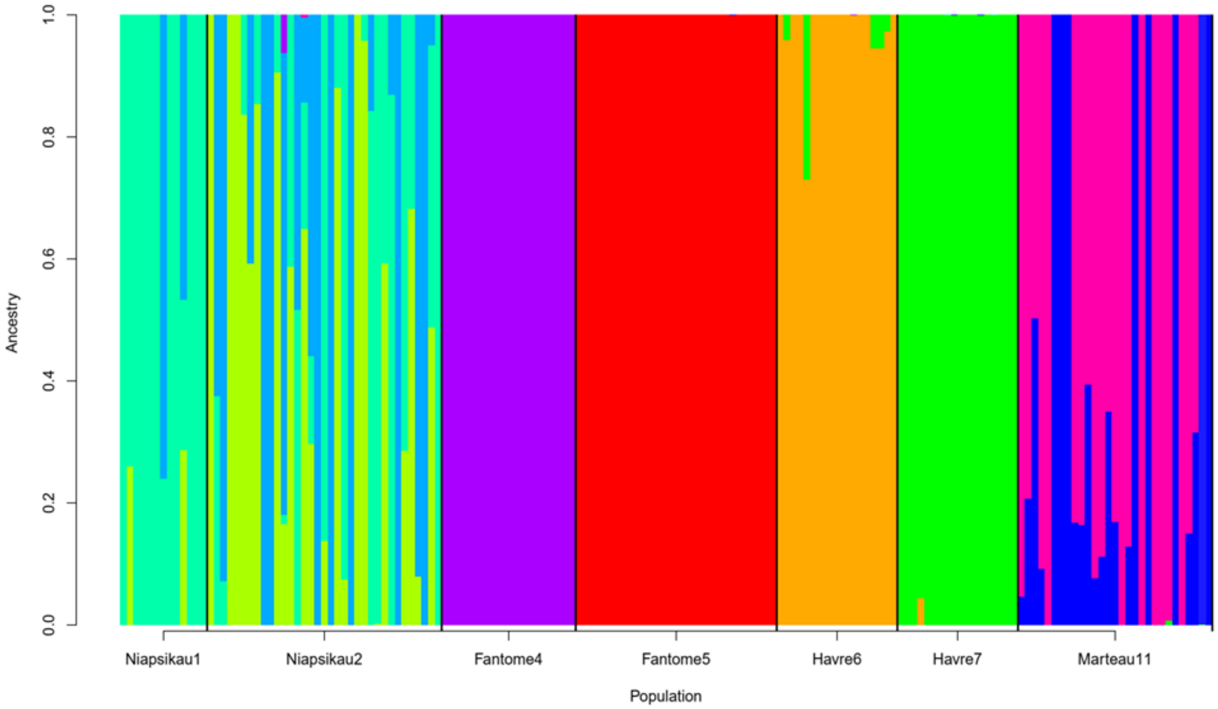

Figure 3.5. ADMIXTURE analysis barplot of the seven *C. minganense* aggregations found in the Mingan Archipelago National Park reserve. Each column represents an individual and each color represents a genotype. (K=9)

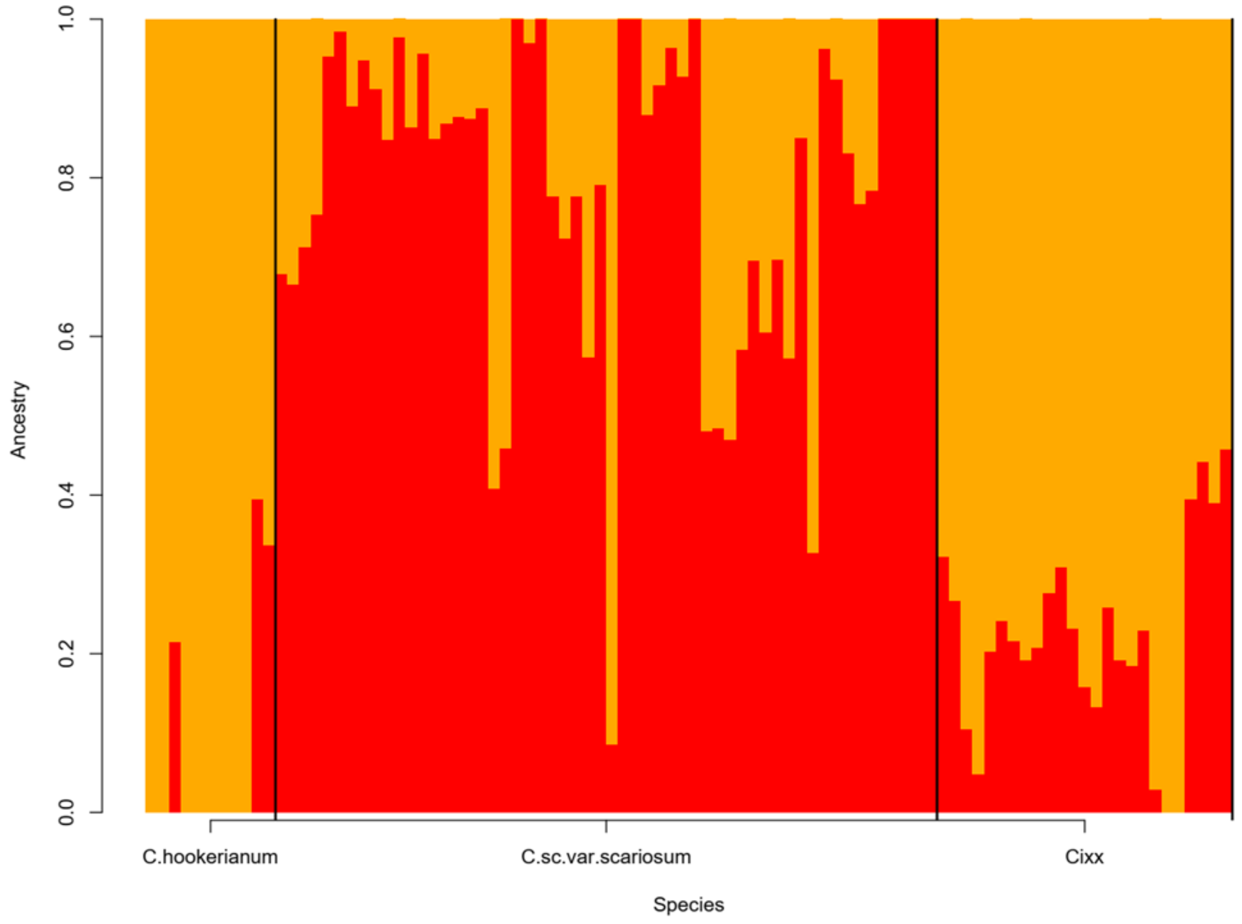

Figure 3.6. ADMIXTURE analysis barplot of *Cirsium scariosum* var. *scariosum*, *Cirsium hookerianum* and *Cirsium scariosum x hookerianum* (Cixx) collected in the Waterton National Park, Alberta. Each column represents an individual and each color represents a genotype. (K=2)

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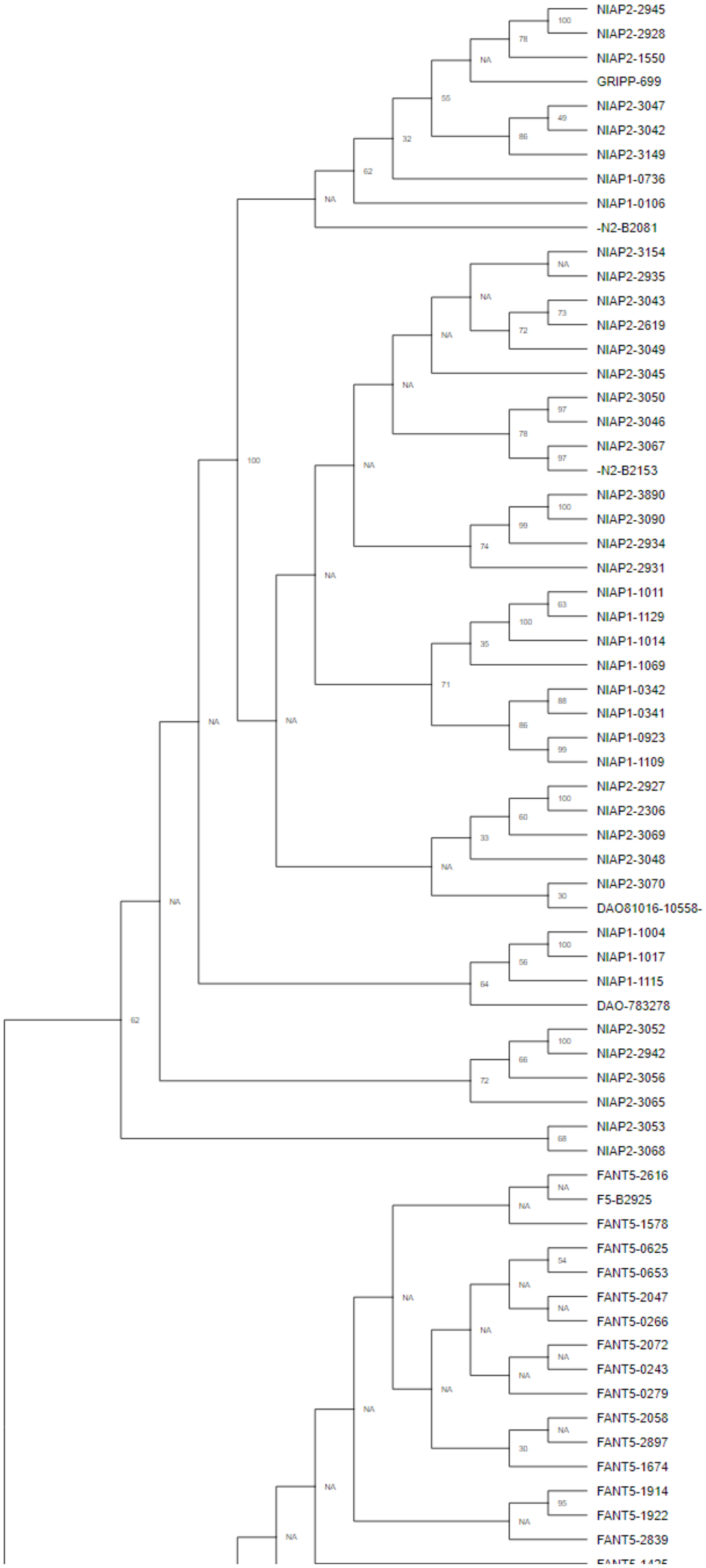
9. APPENDIX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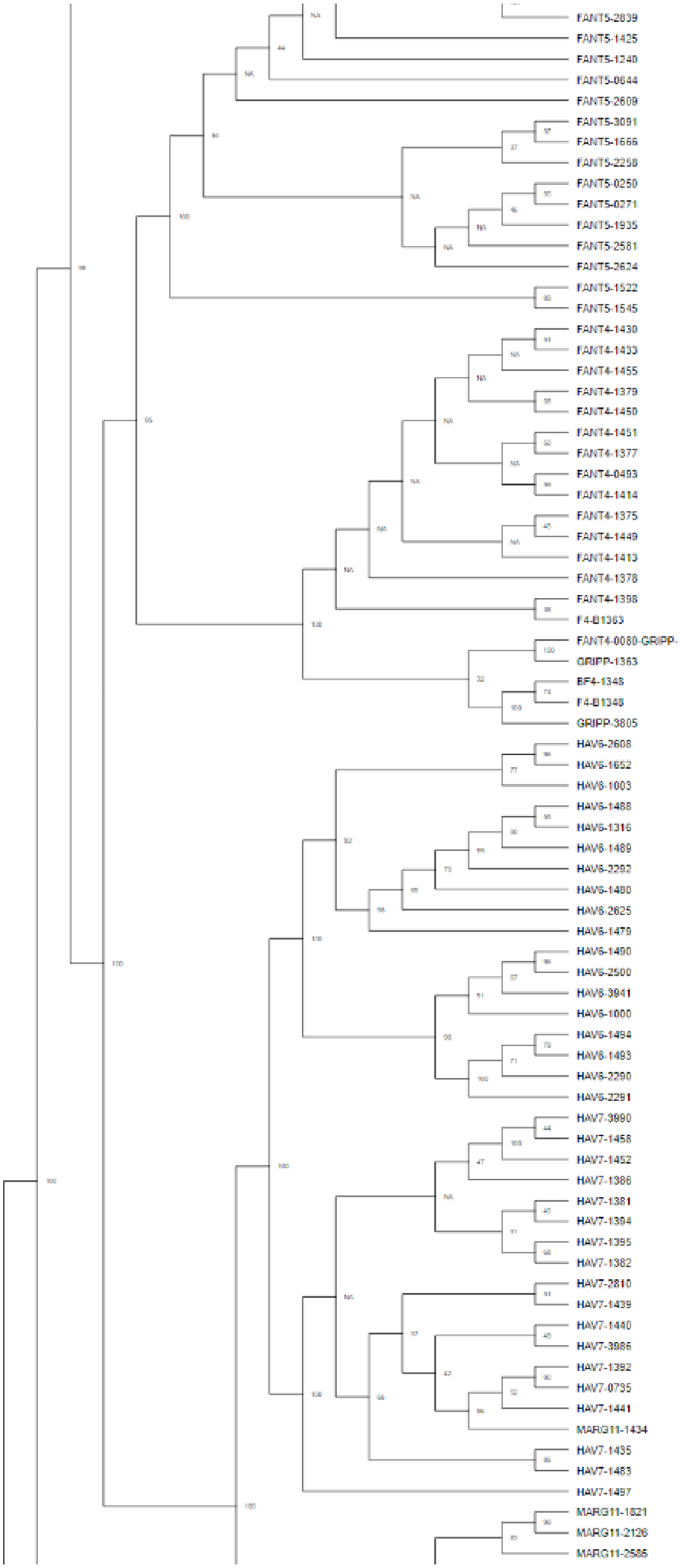
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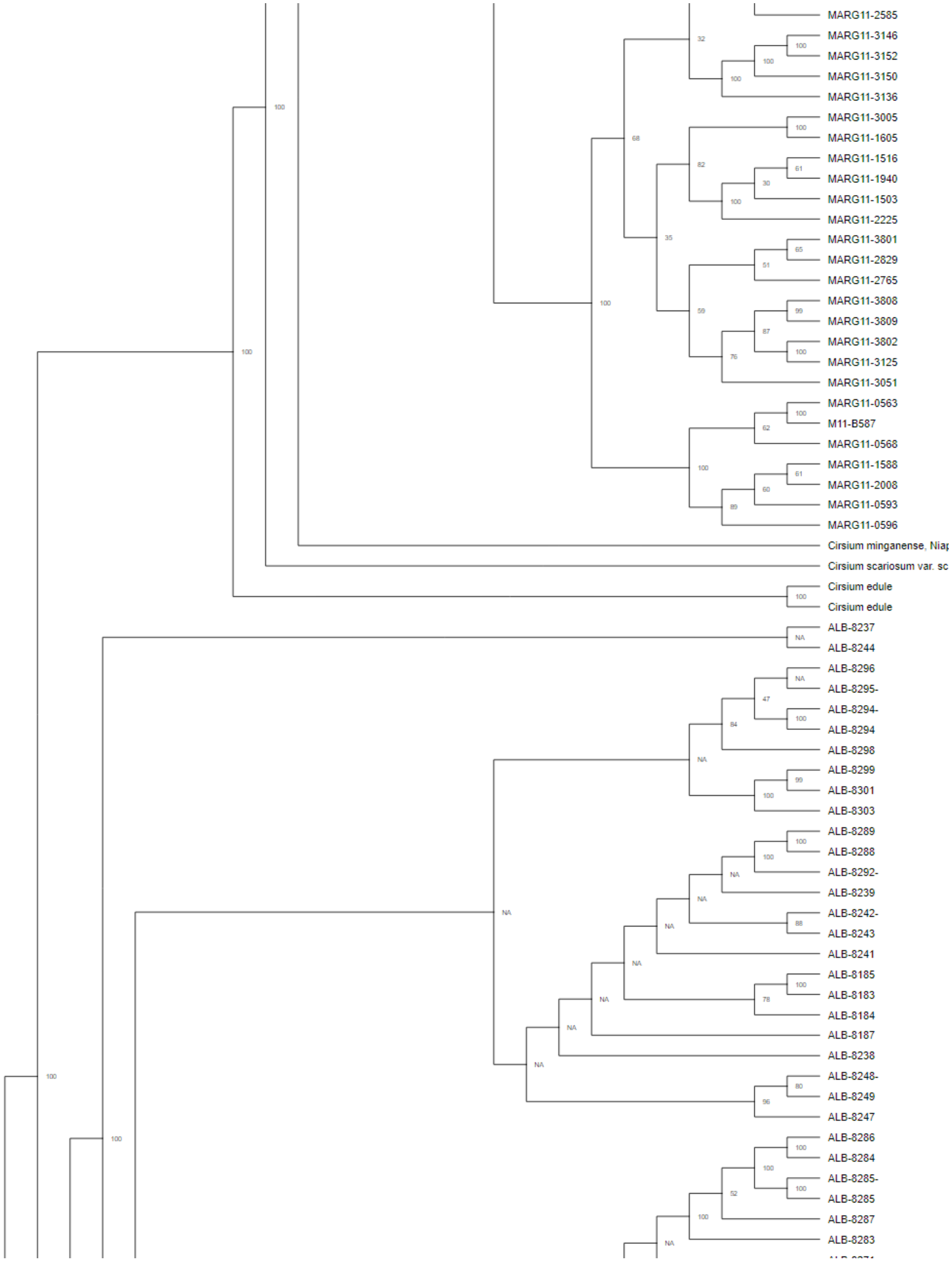

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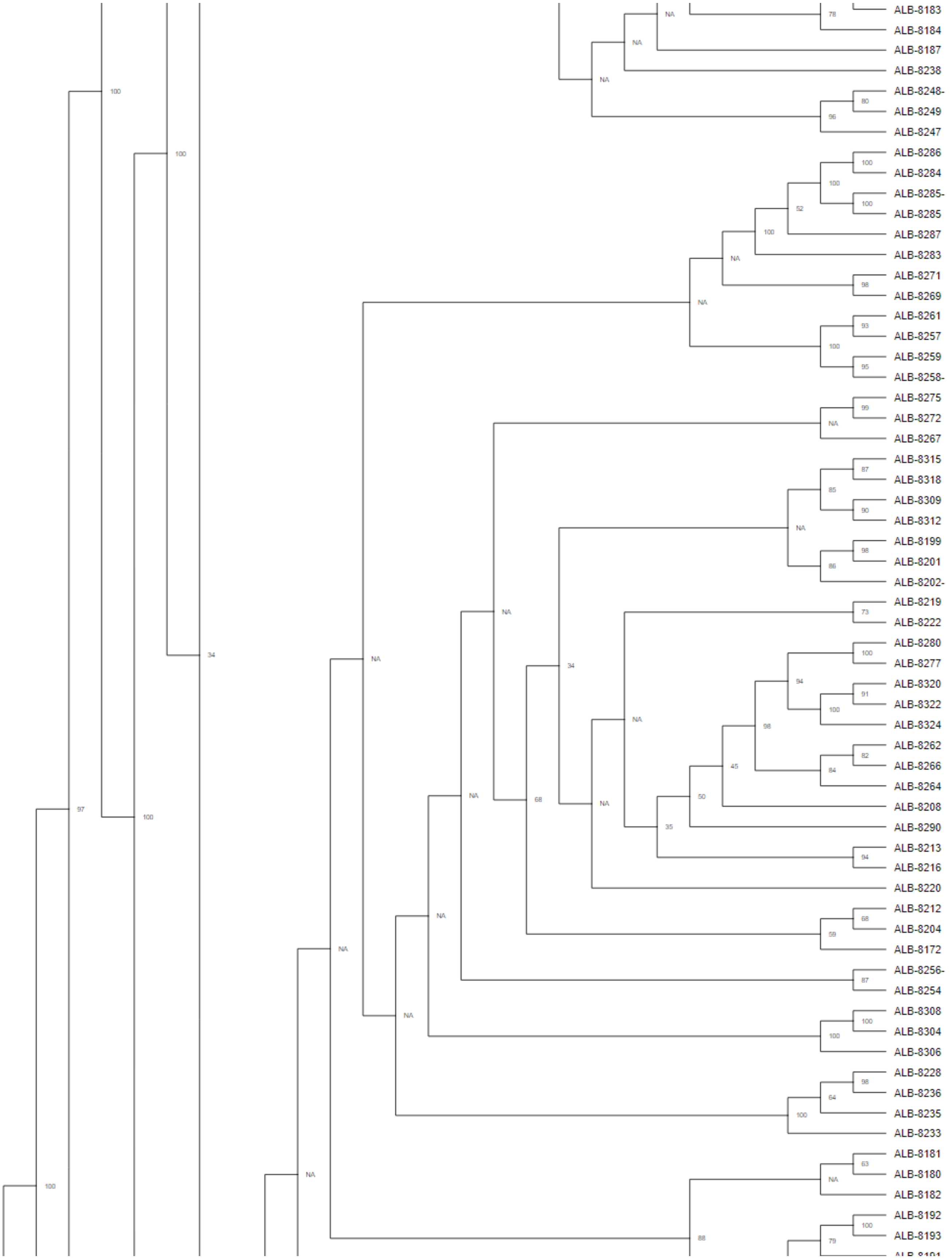
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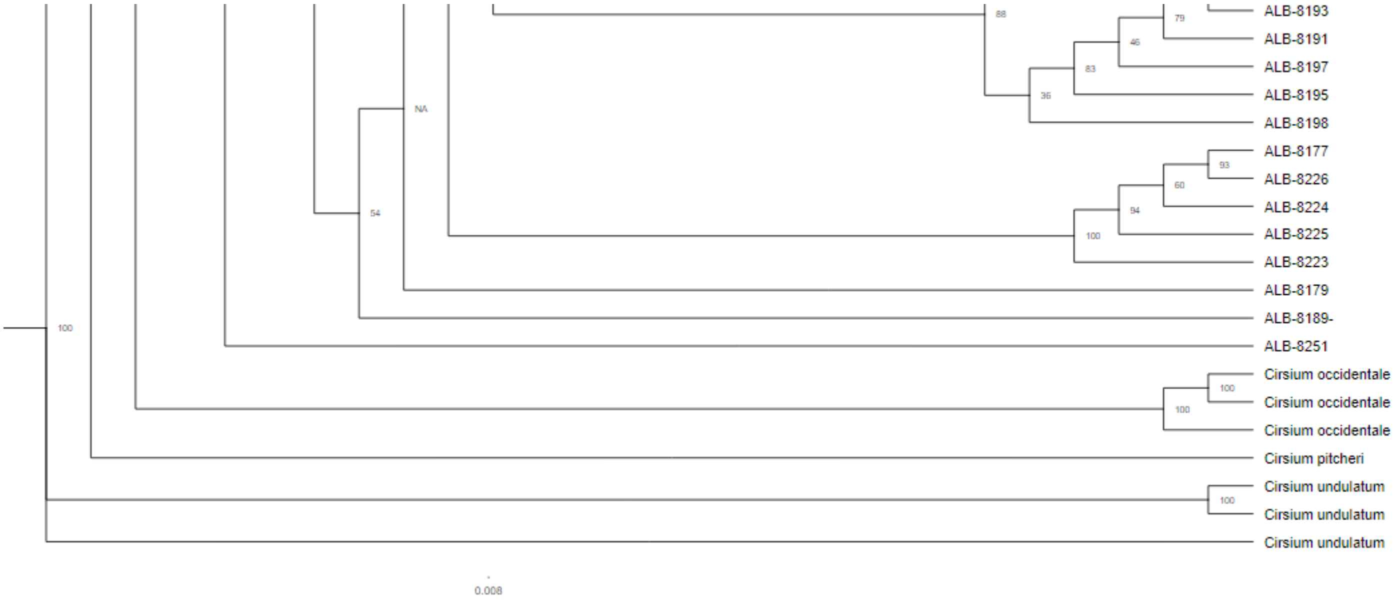

Figure A.1. Neighbor joining phylogenetic tree based on 266 samples and 25,843 binary SNPs. Branch support was assessed by 1000 standard bootstrap replicates in R. Samples provenance for *C. minganense* are labeled as the island (Havre, Marteau and Fantôme) and followed by subpopulation number if groups of individuals were separated by more than 500 meters.