# Genetic analysis of *Pardosa* wolf spiders (Araneae: Lycosidae) across the northern Nearctic

Kathrin A. Sim

Department of Natural Resource Sciences
McGill University, Montreal

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**Abstract**

An analysis of the genetic structure of northern Nearctic wolf spiders from the genus *Pardosa* (Lycosidae) was conducted. Wolf spiders are common and abundant across the Nearctic, but despite their ecological importance, little is known about their phylogeography and taxonomically uncertain species exist. Wolf spiders were collected from sites across northern Canada to examine the phylogeographic history of *Pardosa glacialis* (Thorell) and taxonomic status of the Nearctic members of the *Pardosa lapponica* species-group. Results show that the high Arctic species *P. glacialis* occupied two major glacial refugia during the Wisconsin glaciation: Beringia and a lesser-known high Arctic refugium. Additionally, analyses support a *P. glacialis* population expansion in Beringia during the Wisconsin glaciation, likely facilitated by an increase in habitat due to suitable climate in the region. Post-glacial dispersal from the Beringian and high Arctic refugia produced a secondary contact zone in central high Arctic Canada. With regards to taxonomy, morphometric analyses of *P. lapponica* (Thorell) and *P. concinna* (Thorell), the only two members of the *P. lapponica* species-group in the Nearctic, revealed no species-specific variation beyond a single male morphological character. Genetic analyses showed there was more genetic variation between Palearctic and Nearctic *P. lapponica* specimens than between Nearctic *P. lapponica* and *P. concinna* specimens. The *P. lapponica* species-group is in need of taxonomic revision to resolve the species boundaries among its members.

**Résumé**

Une analyse de la structure génétique des Lycoses néarctiques du Grand Nord appartenant au genre *Pardosa* (Lycosidae) a été effectuée. Les Lycoses sont des espèces communes et abondantes dans le néarctique. Malgré leur importance écologique, peu d'information est disponible sur leur phylogéographie et l'identité taxonomique de certaines espèces n'est pas clairement établie. Dans le contexte de cette étude, nous avons recueilli des Lycoses dans le nord du Canada afin d'étudier l'histoire phylogéographique de *Pardosa glacialis* (Thorell) et d'établir le statut taxonomique des membres néarctiques du groupe d'espèces *Pardosa lapponica*. D'après nos résultats, l'espèce arctique *P. glacialis* aurait autrefois occupé deux importants refuges lors de la glaciation du Wisconsin : la Béringie et un refuge situé dans le Haut-Arctique. Les analyses appuient une expansion de la population de *P. glacialis* en Béringie pendant la glaciation wisconsinienne, une situation vraisemblablement favorisée par une augmentation de l'habitat disponible grâce à un climat adéquat. La dispersion postglaciaire des espèces à partir de ces refuges a créé une zone de contact secondaire, située dans le centre du Haut-Arctique canadien. Par rapport au statut taxonomique, une étude morphométrique de *P. lapponica* (Thorell) et *P. concinna* (Thorell), les deux seules espèces membres du groupe *P. lapponica* dans le Néarctique, n'a dévoilé aucune variation discriminante, mis à part un caractère morphologique chez les mâles. L'analyse des caractères génétiques a démontré qu'il n'y a pas plus de variation entre les spécimens paléarctiques et néarctiques de *P. lapponica* qu'entre les spécimens de *P. lapponica et P. concinna* provenant exclusivement du Néarctique. Une révision taxonomique du groupe d'espèces *P. lapponica* qui réexaminerait les limites interspécifiques entre les membres de ce groupe est nécessaire.

**Thesis format and author contributions**

This thesis is part of a larger program, the Northern Biodiversity Program (NBP), a collaborative effort among research laboratories at McGill University and the University of Toronto. A Natural Sciences and Engineering Research Council of Canada Strategic Project Grant funded this research, allowing arthropod collections at 12 sites by principal investigators and student members.

This thesis is organized in manuscript format, with two original chapters, an introduction (Chapter 1), literature review (Chapter 2), and a summary and conclusions (Chapter 5). The original research chapters of this thesis (Chapter 3 and 4) are written as manuscripts prepared for journal submission. Chapter 3 will be submitted in the future to a peer-reviewed journal. Chapter 4 has been submitted to *Zootaxa*, and formatting for the chapters follows *Zootaxa* guidelines. A comprehensive literature cited list for all chapters is given in Chapter 6.

I participated in the collection of arthropods for the NBP and the species identification of the collected spiders. The laboratory work (e.g. primer selection, DNA extraction) and statistical analyses were performed by me. I prepared the initial drafts of all chapters and completed the final revisions. My co-supervisors, Dr. Chris Buddle and Dr. Terry Wheeler, are co-authors on both manuscripts. They are both principal investigators with the NBP, which provided the funding for the research completed in this thesis. The NBP collection design and field work were overseen by both Dr. Buddle and Dr. Wheeler. As well, they contributed to the conceptualization of both chapters and to the manuscript editing. The additional co-authors on Chapter 3, Dr. Toke T. Høye and Dr. Gergin Blagoev, provided identified specimens of *Pardosa glacialis* from Herschel Island, YT and Greenland.

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## 1. Introduction

The Arctic is a fascinating biome; the high Arctic is a polar desert with a barren, rocky landscape while small shrubs with lichen undergrowth and sphagnum or sedge meadows characterize the southern tundra or sub-Arctic (Downes 1965). The Arctic occupies approximately 25% of Canada's land area (Downes 1965) and, despite its extreme climate, supports thousands of species (Callaghan *et al.* 2004). Its diversity and glacial history make it a model study system for research about biodiversity and biogeography. Understanding the biogeographic history of Arctic species allows predictions regarding how these species will respond to contemporary climatic events – events that are happening quickly at northern Latitudes (Strathdee & Bale 1998).

Species emerged out of glacial refugia to colonize the North American Arctic following the last glacial maximum, 21 kyr BP, during the Wisconsin glaciation (Hofreiter & Stewart 2009). These newly established populations retain genetic signatures of historical population range shifts and isolation that can indicate how these populations responded to climate changes (Hewitt 2004). It is of great interest to understand how species have responded to historical climatic and vicariance events, and how these responses have shaped their current population structure.

The location of Wisconsin glacial refugia, pathways of emergence out of refugia and migration into newly revealed habitat have all received attention in biogeographic studies (Hewitt 1999). Unfortunately, the focus of these studies has been placed extensively on Eurasian vertebrates (e.g. Schmitt & Seitz 2001), plants (e.g. Mráz *et al.* 2007; Naydenov *et al.* 2007; Puşcaş *et al.* 2008) and marine animals (e.g. Hickerson & Ross 2001; Marko 2004). Studies on Canadian species populations are available, but concentrate on plants (e.g. Tremblay & Schoen 1999; purple saxifrage (*Saxifraga oppositifolia*), Abbott *et al.* 2000) and mammals (e.g. Conroy & Cook 2001; Loehr *et al.* 2006). The narrow taxonomic focus of Canadian phylogeographic studies impedes the analysis of generalized biogeographic patterns (Wooding & Ward 1997). A broader sampling of taxa is required before generalized patterns can be seen for Canadian biota.

Arthropods in the North American Arctic have received little attention in regards to their phylogeographic history, despite their ecological importance as predators (e.g. Paetzold & Tockner 2005) and prey (e.g. Bolduc *et al.* 2012). The arthropod abundance and diversity in the Arctic is high (Danks 1992), and many are initial colonizers of newly exposed habitat such as proglacial terrain (Crawford *et al.* 1995; Hodkinson *et al.* 2002). Some of the most important colonizers are spiders (Araneae), due to their involvement in nutrient cycling and capacity for long distance dispersal (Hodkinson *et al.* 2001). Their dispersal ability and swift colonization of newly exposed terrain makes spiders an ideal arthropod group to use in phylogeographic and biogeographic studies as their range shifts should closely mirror biogeographic events.

To my knowledge, there has been only one study on the biogeography of Arctic wolf spiders of the genus *Pardosa* (Koch), with attention on the *Pardosa saltuaria* species-group (Muster *et al.* 2009; Muster & Berendonk 2006) in Eurasia. This study on Lycosidae species, as well as other spider-focused phylogeographic studies from non-Arctic localities, e.g. the trapdoor spider, *Aptostichus simus* (Chamberlin) (Bond *et al.* 2001) or thin-legged wolf spiders in the *Pardosa lapidicina* species-group (Correa-Ramírez *et al.* 2010), were able to shed light on past population dynamics, current population structure and taxonomy (Muster *et al.* 2006). The taxonomy of many spider groups is unclear and identification to species is often difficult. The Lycosidae family, commonly called wolf spiders, is one of the largest spider families, it is abundant and diverse in the Arctic (Bowden & Buddle 2010) and can often be taxonomically challenging, making this family an ideal choice for phylogeographic studies in the Arctic.

In this thesis, I will use genetic variation to examine the population genetic structure of an Arctic wolf spider, *Pardosa glacialis* (Thorell), in order to uncover its phylogeography, as well as to shed light on the two Nearctic members of the taxonomically uncertain *Pardosa lapponica* species-group. I chose the genus *Pardosa*, thin-legged wolf spiders, as they are abundant and found across Canada in every ecoclimatic zone. Focus on this genus, with its widespread species

ranges, allowed maximum use of collection sites, which in turn, allows for better population studies. They are also a difficult group taxonomically (Kronestedt 1975), which makes genetic analysis worthwhile to supplement morphology. All species in *Pardosa* have been placed into species-groups based on similarities in their copulatory structures (Kronestedt 1975). Some species are extremely morphologically similar, to the extent that their species status becomes uncertain. Genetic analysis in combination with morphological analysis should illuminate the relationship between these species.

Chapter 2 of this thesis is a literature review that encompasses the key literature on the late Pleistocene glaciation and its effects on species populations in the North American Arctic. Arctic wolf spiders are presented as model organisms for phylogeographic studies in the Nearctic, as well as a taxon in need of further taxonomic revision.

In Chapter 3, I examine the population genetics and phylogeography of *Pardosa glacialis*, a morphologically diverse species with a Holarctic, tundra-restricted range. As phylogeographic studies on terrestrial, high Arctic arthropods in the Nearctic are absent, *P. glacialis* is an ideal species to study. I focus on the contemporary genetic variation within and among populations in Canada and Greenland, looking for patterns that indicate connectivity and historical or contemporary isolation. Genetic divergence can indicate past fragmentation of a species population, due to vicariance events such as glaciation. A high dispersal capability in species, like that of wolf spiders, is often a sign that they will have occupied multiple refugia (Shafer *et al.* 2010). The distribution of genetic variation across a species range can indicate population dynamics during occupation and subsequent expansion out of glacial refugia.

The glacial history of the Pleistocene has been shown to have led to speciation in some arthropod groups, e.g. montane grasshoppers (*Melanoplus*, Knowles 2000), but whether the same can be said for the closely allied species in *Pardosa* has not been studied. Intraspecific morphological variations in copulatory organs, such as those seen in *P. glacialis*, have in the past been considered to be exceptions in spiders (Huber 2004). Genitalic morphology in

spiders has been assumed to be species-specific; any morphological variation was seen as an indication of speciation. The accuracy of this hypothesis has been called into question, yet it has and continues to be the basis for spider taxonomy since genitalic characters were first proposed to be diagnostic.

In Chapter 4 of this thesis, I focus on, *Pardosa lapponica* (Thorell) and *P. concinna* (Thorell), the two Nearctic members of the taxonomic uncertain *P. lapponica* species-group. The two species have only one known diagnostic character, a morphological variation in the male genitalic character, the terminal apophysis (Dondale & Redner 1986). The low interspecific variation makes species identification difficult. I will examine the relationship between the two species, comparing morphological and genetic variation, to determine if the single character is truly diagnostic of a species break.

**1.1 Summary of objectives**

The overarching goal of my thesis is to study the genetic variation in wolf spider populations in northern Canada to examine population structure and taxonomic status. The climate oscillations during the Pleistocene will have shaped population structure and possibly promoted speciation in the genus *Pardosa*.

In Chapter 3, the objective is to determine the demographic history and population structure of *P. glacialis* in Canada and Greenland. I ask whether this species occupied a single refugium or multiple refugia during the Wisconsin glaciation, and where the refugia were located. Lastly, I assess how this species emerged from occupied refugia and whether populations across the Nearctic range are experiencing gene flow. My two hypotheses are as follows:

$H_1$ – Patterns of genetic diversity will indicate that *P. glacialis* populations have expanded out of multiple ice-free refugia.

$H_2$ – The populations of *P. glacialis* on the Canadian mainland, across the Arctic Archipelago and into Greenland are experiencing contemporary gene flow due to the high dispersal ability of juvenile wolf spiders.

The objective of Chapter 4 is to examine the species boundary between the two species, *P. lapponica* and *P. concinna*. Genitalic characters will be analysed to determine the availability of diagnostic characters beyond the terminal

apophysis. Additionally, the level of genetic divergence between the two species will be measured to determine if there is a genetic indication for speciation that agrees with morphological variation. My hypothesis for this Chapter is as follows: $H_1$ – Variation in the terminal apophysis alone does not indicate a true species division, but rather phenotypic variation in a single species.

## 2. Literature Review

### 2.1 Glacial history of the North American Arctic

The Arctic occupies a vast area of land, covering approximately 2,331,000 $km^2$ in Canada (Downes 1965). Despite its large size, the Arctic is relatively unstudied, but interest is mounting due to predictions that climate change will heavily impact the Arctic (Strathdee & Bale 1998). To predict how the climatic changes will impact the species occupying the Arctic, it is important to known how species responded to past climatic changes.

Fossil evidence and, when available, ancient DNA are used to determine the geographic and temporal location of Arctic species' ranges (review by Hofreiter & Stewart 2009). When fossils are unavailable, phylogeography, relating geography with contemporary genetic variation, provides a method for determining biogeographic histories (Avise 2000). Species histories and population dynamics can be deduced by DNA phylogeography up to 100 kyr BP, but confident conclusions on older events are difficult due to the fluctuating nature of the climate during the Pleistocene (Hewitt 2001).

Glaciation dominated the Quaternary Period, with the most dynamic fluctuations in temperature occurring during the Pleistocene (Hofreiter & Stewart 2009). The climate oscillations during the Pleistocene affected the availability and location of suitable habitat (Hewitt 2004). Expansion and contraction of ice sheets repeatedly isolated populations and influenced multiple range shifts in many North American species (Davis & Shaw 2001). The species that currently occupy Canada's Arctic habitats will have colonized these areas from ice-age refugia following the last glaciation event, the Wisconsin glaciation (Howden 1969).

The Wisconsin glaciation followed an interglacial period that ended *ca* 122 kyr BP (Sirocko *et al.* 2005). A drop in global sea level coincided with the formation of continental glaciers *ca* 118 kyr BP (Sirocko *et al.* 2005). Two ice sheets covered much of North America: the Cordilleran and the Laurentide Ice Sheets (Dawson 1991a). While the Cordilleran Ice Sheet covered the west coast, the Laurentide Ice Sheet extended across the rest of North America (Dawson 1991a). At its maximum, the Laurentide Ice Sheet covered approximately 4,400

km west-east of North America and 3,800 km north-south (Fig. 1 in Fulton & Prest 1987). The growth of these two ice sheets was not continuous, but experienced advances and retreats along edge margins (Dawson 1991a). At their maximum extent during the Late Wisconsin, the two ice sheets merged in the west over what are now Alberta, British Columbia and Yukon (Dawson 1991a).

To the north, the Laurentide Ice Sheet is thought to have been confluent with the Innuitian Ice Sheet, which joined it to the Greenland Ice Sheet, during the last glacial maximum (LGM) (Dyke 1999). The Innuitian Ice Sheet extended over the Queen Elizabeth Islands while the Laurentide Ice Sheet covered the southern islands of the Arctic Archipelago (Dyke 1999). The origin of the Innuitian Ice Sheet is unknown, but England *et al.* (2006) have extensively detailed its advance and retreat during the LGM.

The LGM, for the regional ice sheets, occurred in the Late Wisconsin *ca* 23 – 21 kyr BP, with later advances occurring in the Innuitian Ice Sheet (Dawson 1991a; England *et al.* 2006). During the LGM, the advance of the ice sheets would have caused extinction or displacement of species that previously inhabited these areas (Dyke & Prest 1987; Howden 1969). Species ranges were limited to well-documented ice-free areas that existed to the northwest (Beringia) and south of the ice sheets (e.g. *Dryas integrifolia*, Tremblay & Schoen 1999), and potentially in small ice-free pockets within or along the edges of the ice sheets (Dawson 1991b). Ice-free havens at unexpected longitudes or latitudes for the species that occupy them are termed cryptic refugia (Stewart *et al.* 2010). Evidence for cryptic refugia, nunataks and dry areas on the high Arctic islands, has been mounting in recent years (Loehr *et al.* 2006; Stewart *et al.* 2010).

Brassard (1971) presented strong evidence, based on zoological and botanical studies, supporting the presence of refugia on northern Ellesmere Island, which would have been the most northern refugia in Canada. Additional cryptic refugia in the Canadian Archipelago include ice-free areas on Banks Island and Baffin Island (Dyke & Prest 1987). Cryptic refugia are important to our understanding of species evolution and contemporary genetic diversity (Stewart *et al.* 2010).

## 2.2 Effects of glacial and interglacial periods

Species' ranges during glacial maxima were limited to ice-free refugia. Referring to ice-free areas as refugia in the 'classical' sense would imply range and demographic reduction of species occupying these areas (Bennett & Provan 2008), but this may not be the case for cold adapted species (Dalén *et al.* 2007; Hofreiter & Stewart 2009). For cold-adapted species, range contraction occurs during warming rather than cooling periods as suitable habitat decreases in availability, e.g. the circumpolar arctic fox, *Alopex lagopus* (Dalén *et al.* 2007), and high elevation alpine species (Schmitt *et al.* 2010). The contraction of range and demographic size in response to climate changes is, therefore, species specific and may not occur during the occupation of ice-free areas in glacial periods. The term "refugium", in the context of this thesis, will be used to broadly refer to areas that were ice-free during glaciation and "cryptic refugium" to ice-free areas whose exact locations and limits are unclear, and will not necessarily entail the contraction of species' ranges.

Population genetic structure often reflects past population events. Contraction of species' ranges in response to climatic events can cause fragmentation and isolation of species populations (Hewitt 1996). Isolation during range contraction can lead to population bottlenecks and the loss of genetic variation (Hewitt 1996). The shift of characters, both genetic and morphological, away from the previous standard will occur in fragmented populations, particularly if there were population bottlenecks when the populations were first established (Avise 2000). Over time, these changes may lead to fragmented populations experiencing divergence in morphological and genetic characters, which may lead to speciation (Hewitt 1996).

A study on ancient DNA of the arctic fox found that populations did not track suitable habitat during the Wisconsin glaciation, but that those in the path of glaciers went extinct (Dalén *et al.* 2007). The extinction of populations, rather than movement into unglaciated areas, can result in the complete loss of genetic lineages. Reductions in genetic variation limit a species' adaptive ability, which increases the risk of extinction. Colonization of newly exposed habitat following

glacial retreat would then have been done by populations endemic to areas that remained ice-free (Dalén *et al.* 2007; Stewart *et al.* 2010).

Dispersal of species from refugia followed the retreat of the regional ice sheets, which began *ca* 18 kyr BP for the Laurentide and by 10 kyr BP was underway for all ice sheets (England *et al.* 2006). In addition to non-synchronous melting, climate oscillations occurred even during times of retreat (Dawson 1991b), which caused repeated colonization and replacement events in some species (Ashworth 1996).

As the ice retreated, the newly exposed habitat was colonized, but the pathways of dispersal from refugia differ among species (Hewitt 2004). In addition to the interest in where species survived during glacial times, there is interest in patterns of colonization. Contemporary genetic structure across species' ranges can indicate population origins, demographic histories such as bottlenecks, and potential dispersal barriers. For a single species, determining what refugium they occupied during the Wisconsin glaciation is often based on the geographic location of distinct genetic populations or clades (Avise 2000). High, unique genetic diversity is expected in refugial areas as these populations will be the oldest, while lower diversity will indicate recent colonization (Reiss *et al.* 1999; Schmitt 2007). However, diversity does not decrease during contiguous range expansion, which can result in a widely distributed and highly diverse clade (Schmitt 2007). The origin of the widely distributed clade is attributed to the hypothesized refugium whose range overlaps with that of the clade.

Some species show evidence for the occupation of multiple refugia through the existence of geographically distinct genetic clades. The divergence among clades from different refugia will date back to the initiation of the vicariance or climatic event that isolated them; for temperate species this is often the beginning of the last glaciation (Ashworth 1996). The geographical distribution of different clades will indicate the colonization pathways. Areas of high genetic diversity reminiscent of refugia, but lacking geographically unique variations, are signs of secondary contact among clades (Provan & Bennett 2008). Secondary contact can indicate that there are little to no contemporary dispersal

barriers, e.g. the rock ptarmigan, *Lagopus muta*, appears to have occupied multiple refugia, but is now experiencing gene flow among previously isolated populations (Holder *et al.* 1999). In other cases, secondary contact is limited, indicating dispersal barriers to contemporary gene flow, e.g. the Mackenzie River and Parry Channel have been suggested to disrupt dispersal of the collared lemming, *Dicrostonyx groenlandicus* (Waltari *et al.* 2004).

Occasionally, a species is said to have occupied only one of the major glacial refugia, e.g. the Pacific Northwest, but this can be misleading as there can be "refugia within refugia" and these species may yet display population structure (Shafer *et al.* 2010). The lack of population structure cannot be taken alone to assume a single refugium, but rather requires additional population parameters to be analysed. The arctic fox displayed no genetic structure across its range, but had a high diversity and a population expansion that dated back to the last interglacial period (Dalén *et al.* 2005). Dalén *et al.* (2005) proposed that this species, being cold-adapted, had a larger range during the last glaciation period and was able to disperse effectively, preventing population divergence.

Divergence among populations in separate refugia can be quite extensive and contemporary contact between distinct clades may be limited to suture zones, e.g. two distinct lineages in the flying squirrel, *Glaucomys sabrinus* (Arbogast 2007). In extreme cases, divergence has led to complete speciation with new species displaying high genetic differentiation and monophyly, e.g. speciation in montane grasshoppers, *Melanoplus* (Stål) in the Rocky Mountain region (Knowles 2000). Due to the species specific response to climatic and environmental changes, it is important to study an array of taxa before predictions of general biogeographic patterns during the Wisconsin glaciation can be made for Nearctic biota (Wooding & Ward 1997).

**2.3 Arctic biogeography in North America**

Macpherson (1965) postulated the refugial origins of the Arctic mammals in Canada based on taxonomic variation and found multiple patterns to be present. Discontinuities between high and low Arctic populations supported a high Arctic refugium for some mammals, while others had origins in refugia south of the

Laurentide Ice Sheet (Macpherson 1965). Genetic variation has been used in subsequent studies to look into population histories in greater depth. The phylogeographic history of the collared lemming, for example, shows evidence not only for a single high Arctic refugium, but for multiple refugia on the Arctic islands (Fedorov & Stenseth 2002). However, genetic variation between the high and low Arctic clades is not seen in all of the species Macpherson (1965) hypothesized, like the arctic hare, *Lepus arcticus*, whose high and low Arctic populations share the same genetic clade (Waltari *et al*. 2004). As well, not all species display evidence for the same expansion boundaries; the Mackenzie River is hypothesized to be a boundary between Beringian and Canadian lineages for some taxa (Waltari *et al*. 2004), but appears to have had no effect on others (Reiss *et al.* 1999). Discontinuities between phylogeographic histories highlight the importance of studying multiple taxa.

Unfortunately, most phylogeographic studies in Eurasian and North America are heavily biased towards certain taxa. Extensive reviews summarizing phylogeographic patterns, including locations of known refugia, for North American species have focused on vertebrates and plants (e.g. Shafer *et al*. 2010; Soltis *et al.* 2006). Shafer *et al*. (2010) determined that 60% of the phylogeographic studies for northwestern North America have focused on plants and mammals. Similar biases in taxon focus were seen by Soltis *et al.* (2006) for eastern North America studies. Only 12 of the 396 eastern studies (Soltis *et al.* 2006) and 5 of the 126 western studies (Shafer *et al.* 2010) looked at arthropods.

The Arctic environment supports thousands of arthropod species (Danks 1992), which, along with their abundance and small size, makes them ideal specimens for biogeographic studies. Arthropods account for more than half of the estimated 6000 animal species in the Canadian Arctic ecosystem (Callaghan *et al.* 2004), which makes the underrepresentation of Arctic arthropods in Nearctic phylogeographic studies notable. Of the Nearctic terrestrial arthropods that have been studied in the Arctic, there are two species of beetle (Coleoptera) whose ranges can be considered to include the Arctic: the spruce beetle, *Dendroctonus*

*rufipennis* (Kirby) (Maroja *et al.* 2007) and a sun beetle, *Amara alpina* (Paykull) (Reiss *et al.* 1999).

The phylogeographic studies on the two beetle species revealed discontinuities between the Rocky Mountain and more northern populations (Maroja *et al.* 2007; Reiss *et al.* 1999), indicating gene flow barriers. However, in the north, the Mackenzie River was not considered a dispersal barrier as the Beringian and Canadian populations share genetic clades (Reiss *et al.* 1999). Pleistocene extinctions in carabid beetles were not supported by the fossil record; however, regional extinctions, like those seen in the arctic fox (Dalén *et al.* 2007), were observed for beetles (Ashworth 1996).

Comparisons among the phylogeographic histories of two Arctic-alpine insects and one arachnid from Eurasia revealed that though there was congruence in patterns across the taxa, idiosyncrasies exist within arthropod phylogeography and recognizing these differences is important (Schmitt *et al.* 2010). Divergence in species histories can make some species more at risk than others during ecological change. In addition to beetles and some Lepidoptera, spiders have received attention in Eurasian Arctic biogeographic studies (Schmitt *et al.* 2010), but have yet to be the focus in any northern North American studies. Studies on the biogeographic history of the Nearctic would benefit from phylogeographic studies on spiders due to their importance in the Arctic ecosystem.

Spiders (Araneae) are abundant in the Arctic with some families, Lycosidae and Linyphiidae, increasing in abundance in northern latitudes relative to tropical areas (Bennett 1999). Araneae is the seventh most diverse animal order and their diversity in the Arctic is substantial; over 300 species of spider have been described in the Arctic, on par with the total number of all vertebrate species (Callaghan *et al.* 2004). Spiders are important predators in the ecosystems they inhabit (Paetzold & Tockner 2005), and they are also important prey items (Bennett 1999; Bolduc *et al.* 2012). Their importance in the Arctic ecosystem also extends to their role in colonization.

Spiders are important in the early stages of succession (Hodkinson *et al.* 2002). It has been proposed that spiders play key roles in nutrient recycling in

newly formed ecosystems (Hodkinson *et al.* 2001) and they are often some of the first settlers in unoccupied habitats (Crawford *et al.* 1995). Studies of glacial moraines have revealed spiders to be among the first colonizers (Hodkinson *et al.* 2001), lending support for them being among the first arthropods to extend their range into the Arctic following glacial retreat during the Pleistocene.

**2.4 Aerial dispersal in spiders**

The success of spiders as colonizers is likely due to the high dispersal capacity that some species display (Crawford *et al.* 1995). Dispersal in spiders can happen in one of two ways: cursorial dispersal (short distance movement), or aerial dispersal (long and short-distance movement). Aerial dispersal is accomplished using a silk thread to balloon on air currents (Bonte *et al.* 2003a). Ballooning is common in many spider species though for some species the adults are too large and only the juveniles will balloon (Duffey 1956). Ballooning has been extensively studied in the Lycosidae.

Lycosidae, one of the largest families in Araneae, is currently comprised of 2,393 species, 1/5th of which are in the genus *Pardosa* (Koch) (Platnick 2012). Juveniles of *Pardosa* can disperse by ballooning on silk, covering distances of 100m to over 500m depending on the wind velocity (Bonte & Lens 2007). The high dispersal capability of these spiders has meant most *Pardosa* species have widespread ranges. The large ranges and the abundance of *Pardosa* species have made this genus a target for phylogeographic studies in Eurasia. How populations across such large ranges are related, in both a contemporary and historical context, is of great interest.

Long distance dispersal by ballooning may be sufficient to prevent vicariance events from defining the biogeographic history of wolf spiders, even among populations on different continents (Vink & Paterson 2003). However, Lambeets *et al.* (2010) found that for *Pardosa agricola* (Thorell) a river was sufficient to lower gene flow between populations on opposite sides, thereby causing the populations to differentiate. It is likely then that glacial events would have had an impact on the population structure of *Pardosa* species.

Wolf spiders, like other passive dispersers, cannot control where they land, which means many will arrive in unsuitable environments (Foelix 1996). The potential benefits of leaving their current habitat must outweigh the potential risks before dispersal will occur (Bonte *et al.* 2006). Aerial dispersal in spiders has been found to be influenced by environmental factors (e.g. wind, temperature and humidity, Richter 1970), and habitat structure (Bonte *et al.* 2006). Environmental factors affect the propensity of pre-dispersal behavior or "tip-toeing" in juvenile wolf spiders, either increasing or decreasing it (Duffey 1956; Richter 1970; Bonte *et al.* 2003b). The movement among different populations is then influenced by the environment. Fragmented landscapes can decrease the distance of aerial dispersal in spiders, thus decreasing the size of metapopulations and increasing the risk of isolation (Ramirez & Haakonsen 1999; Richter 1970).

The dispersal behaviour of a single species within local populations may vary due to differences in habitat structure, affecting the population dynamics at a metapopulation and geographical scale (Hanski 1991). Metapopulations are held together by immigration and emigration among local populations (Hanski 1991). The size of a metapopulation is, therefore, dependent on interpopulation dispersal and long distance dispersal is needed to maintain genetic cohesion. Intraspecific variations in dispersal tendencies will have an influence on population connectivity that is reflected in population structure (Bonte *et al.* 2006).

The genetic variation between populations can indicate their level of connectivity. Some populations are directly connected while others are connected by stepping-stone populations (Hanski 1991), meaning gene flow occurs via movement of individuals through adjacent populations (Manel *et al.* 2003). Low variation between populations implies a high level of gene flow due to interpopulation dispersal, whereas more isolated populations will have high genetic variation between populations (Bonte *et al.* 2003a). If a population becomes isolated, genetic variation may be lost, potentially leading to extinction (Hanski 1991); in turn, the population may become genetically divergent, leading to speciation (Hansson 1991). The genetic variation observed in spider populations can be used, not only to determine recent patterns in gene flow, but

also to determine phylogeographic histories and identify species and speciation events (Bond & Stockman 2008; Muster *et al.* 2009).

**2.5 Spider taxonomy**

Identification of Lycosidae species is often difficult due to morphological conservatism, a common problem for many spider groups (Bond *et al.* 2001), and reliance upon characters that are relative and the extent of whose variation is unknown (Chamberlin 1908).As with all spiders, taxonomic focus in Lycosidae has been placed on the genitalia (Chamberlin 1908). However, the characters used to delimitate species vary and have been poorly studied.

The initial use, and subsequent widespread adoption, of copulatory organs as major diagnostic characters in spiders (1850-1900) saw the proliferation of newly delimited species (Huber 2004). The emphasis placed on copulatory characters in spider taxonomy and phylogenetics has increased over the years (Huber 2004), yet there has been little consensus on what characters should receive priority and to what degree variation must be discontinuous to make drawing a species boundary acceptable. The lack of consensus can be attributed to the long held belief that copulatory organs evolved by a lock-&-key mechanism, whereby selective pressure ensures little intraspecific variation and conspicuous interspecific variation, and could, therefore, indicate species limits (Shapiro & Porter 1989). However, actual evaluation of intraspecific variation has been rare, especially across a geographical range, and many species descriptions are based on small sample sizes from only one location (Huber 2004). There are 43,244 recognised species and subspecies of spider worldwide (Platnick 2012), 46% of which are described based on only one sex (Robinson *et al.* 2009).

Despite the extensive reliance on copulatory characters, evidence against the lock-&-key hypothesis explaining variation has mounted (reviewed by Eberhard & Huber 2010) and intraspecific genitalic variation in spiders is likely higher than once assumed. Studies have shown that morphological variation in arthropod genitalia can occur due to varying conditions during development (Arnqvist & Thornhill 1998) and throughout adulthood (Shapiro & Porter 1989). Known cases of cryptic species and intraspecific variation in spiders have

previously been dismissed as exceptions (Huber 2004), but may not be as rare as once believed.

Bennett (2006) found female genitalia can vary in appearance over an individual's life cycle, variation that could be mistaken for different species. Changes in the shape of the spermathecae in female genitalia following copulation have been observed in the Nephilidae species *Nephila clavipes* (Linnaeus) (Higgins 1989). The sclerotization following insemination is suspected to contribute to the high intraspecific shape variation observed in the spermathecae of this species (Higgins 1989). Species identification based on the spermathecae, a common character, might then be less accurate than once thought. Without knowledge of what characters display intraspecific variation, current taxonomic designations may be based on variation that has incorrectly been assumed to be species-specific. Character choice for species identification should be done with careful consideration and acknowledgement of intraspecific variation.

**2.6 The genus *Pardosa***

Many species in *Pardosa* were described based on limited material or damaged specimens; often only a single male or female was used in the first description of a species (Platnick 2012). Some species have yet to have both sexes described despite being named over 100 years ago (Platnick 2012). Arguably, this lends itself to inaccuracies, especially considering the extensive ranges of *Pardosa* species that could allow for unappreciated intraspecific variation.

Species identification in *Pardosa* is based almost exclusively on characters of the copulatory organs (Dondale & Redner 1990). In males, the copulatory organs are formed by the modification of the tarsus and pretarsus of the palps to form pedipalps (Dondale & Redner 1990). Unlike closely related families (e.g. Pisauridae), the femur, patella and tibia of the pedipalps in Lycosidae are not modified to possess a process or apophysis (Chamberlin 1908). The genital bulb, housed within the cymbium, is composed of multiple structures (Vogel 2004) including an intromittent organ called the embolus (Dondale & Redner 1990). On the female abdomen, the epigynum, a sclerotized plate, marks the openings to the ovaries (Dondale & Redner 1990). There are structures used for taxonomic

purposes both on the external and internal portion of the epigynum (Vogel 2004), though the female characters are used less often than male characters for *Pardosa* species.

There are 46 *Pardosa* species in Canada and Alaska, which have been divided into 13 informal species-groups (Dondale & Redner 1990). Some of these species-groups contain members that are difficult to identify due to similarities with other species in genitalic morphology. As there is no agreement on which structures are significant in species delimitation for Lycosidae, species identification can be difficult and occasionally relies on a single character. Kronestedt (2007) identified the embolus as a key character, while Correa-Ramírez *et al.* (2010) found variation to be more extensive and correlated to genetic variation in female characters. When Zyuzin (1985) reviewed the taxonomic structure of the Palearctic species in the genus *Pardosa*, the terminal apophysis (TA) was mentioned as a key character because, for multiple species, it is the sole character available.

The argument for the importance of the TA in *Pardosa* species delimitation is circular since the hypothesis that its shape is species-specific has not been tested. It is possible that reliance on variation in the TA is unfounded and assignment of species boundaries should rely on a combination of variation in other male structures, such as the embolus and median apophysis. The unknown extent of intraspecific variation in the TA raises the question of whether the species boundaries based only on variation in the TA are, in fact, accurate or if the species are over-split.

The genus *Pardosa* would benefit from review of the species boundaries that are based on limited morphological variations, using a combination of morphological and genetic analyses. Genetically distinct haplotypes in spiders have been found to be associated with morphometric variation over broad geographic areas (Bond & Stockman 2008; Crews & Hedin 2006). The few studies on *Pardosa* species that have employed both morphological and genetic characters (Chang *et al.* 2007; Correa-Ramírez *et al.* 2010) found the combination of characters to be effective in species delimitation. Together, genetic variation

and morphometric data can provide strong evidence for recent speciation events, including those that occurred during the Pleistocene (Knowles 2000).

**2.7 The barcode region, COI**

The cytochrome *c* oxidase subunit 1 (COI) sequence is a short, standardized marker that provides species level identification in many cases (Casiraghi *et al.* 2010; Hebert & Gregory 2005). This region has been shown to be effective for discriminating animal species (e.g. spiders, Barrett & Hebert 2005; Lepidoptera, Hajibabaei *et al.* 2006; birds, Kerr *et al.* 2007; fish, Ward *et al.* 2005) and highlighting groups that require taxonomic revision (e.g. Bond *et al.* 2001). Arguments have been raised against the COI or barcode region due to failures to produce monophyletic trees (review of phylogenetic studies by Funk & Omland 2003) or consistent barcode gaps among the tested species (e.g. Robinson *et al.* 2009). However, species designations are hypotheses and not inflexible entities; review of anomalous results should be undertaken before the barcode region is dismissed as ineffective for species delimitation (Kerr *et al.* 2007).

Taxonomy that is unsupported by the barcode region should be reviewed to determine if the taxonomic status is incorrect or the result of recent speciation (McKay & Zink 2010). Instances of nonconformity indicating cryptic species (i.e. multiple distinct genetic clusters found in a single species) are often corroborated by differences in other traits such as behaviour, pheromones and/or geographic location (e.g. the western black widow *Latrodectus hesperus* (Chamberlin & Ivie), Barrett & Hebert 2005). Alternatively, non species-specific barcodes may be observed among previously delimited species. While nonspecific barcodes may be the result of introgression or incomplete lineage sorting reflecting recent speciation (Avise 2000), they may also be the result of incorrect taxonomy (Hebert & Gregory 2005). Polyphyly or paraphyly may not, therefore, be a sign that the barcode region is ineffective, but rather a signal as to where further taxonomic study is necessary (Hebert & Gregory 2005). Studies examining the reliability of the barcode region that do not employ a critical eye when selecting their study species or thoroughly review observed inconsistencies may be

misleading in regards to the reliability of COI as a species-specific marker (Funk & Omland 2003; Robinson *et al.* 2009).

A review of other characters and the geographic location of anomalous barcode results should aid in the evaluation of species status. Paraphyly has been shown to often be the result of inaccurate taxonomy and/or inclusion of taxonomically difficult or unresolved species (McKay & Zink 2010), but is occasionally the result of recent speciation. In cases of recent speciation, introgressive hybridization is usually signaled by genetic overlap in the boundary area between morphologically distinct species or at sites where the populations are sympatric (Avise 2000). Speciation may occur faster than mutations accumulate in neutral genes, which can cause low genetic divergence and incomplete lineage sorting between new species (Funk & Omland 2003). However, a study on dytiscid beetles (Coleoptera) identified fixed nucleotide differences in the barcode region that agreed with prior taxonomic species limits despite the lack of a barcode gap (Hendrich *et al.* 2010). Therefore, even when the barcode region does not show a species-specific gap, it is not necessarily ineffective in species delimitation.

**2.8 Summary**

Northern Nearctic species are of great biogeographical interest due to the glacial history of the Arctic. In many taxa, evidence has been found to support multiple refugia and subsequent dispersal from these ice-free areas following the retreat of the Wisconsin ice sheets (Brochmann *et al.* 2003). Multiple studies have found evidence for cryptic refugia in the central Canadian high Arctic (Fedorov & Stenseth 2002), as well as sites of secondary contact , where dispersal pathways from refugia have brought previously isolated populations together (e.g. *Daphnia* (Müller), Weider and Hobæk 2003). Common widespread genetic haplotypes observed in the populations of Canadian Arctic wolf spiders will indicate dispersal pathways out of glacial refugia that can inform us of their phylogeographic histories.

The molecular barcode region, the cytochrome *c* oxidase I (COI) gene, has been shown to be effective in studying phylogeography and species level

taxonomy in the genus *Pardosa* (Chang *et al.* 2007; Correa-Ramírez *et al.* 2010). For spiders, genitalic characters are often the only diagnostic morphological character (Huber 2004); however, mistakes in species delimitation can occur when genitalic characters are used alone (Costa-Schmidt and Araújo 2010; Huber *et al.* 2005). Agreement between genetic and morphometric variation can help elucidate taxonomic status in *Pardosa* (Bond and Stockman 2008; Crews and Hedin 2006).

**Chapter 3.** Phylogeography of the Arctic wolf spider, *Pardosa glacialis* (Araneae: Lycosidae)

KATHRIN A. SIM[1], TERRY A. WHEELER[1], CHRISOPHER M. BUDDLE[1], TOKE T. HØYE[2] & GERGIN BLAGOEV[3]

[1]*Department of Natural Resource Sciences, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, CANADA* (kathrin.sim@mail.mcgill.ca,christopher.buddle@mcgill.ca,terry.wheeler@mcgill.ca)

[2]*Department of Wildlife Ecology and Biodiversity, National Environmental Research Institute, Aarhus University, Grenåvej 14, DK-8410 Rønde, Denmark*

[3]*Biodiversity Institute of Ontario, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada*

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### 3.1 Abstract

The glacial origin of contemporary populations is largely unknown for many Nearctic Arctic taxa, especially arthropods. This paper studies the phylogeography of a high Arctic Nearctic spider, *Pardosa glacialis* (Lycosidae). Nine sites across Canada and Greenland were sampled, and 143 specimens were analysed using one nuclear ribosomal (765bp ITS) and two mitochondrial gene fragments (585bp COI; 789bp ND1). The nuclear DNA displayed low genetic variation supporting a single species, while mitochondrial DNA showed support for two major lineages originating in separate glacial refugia, Beringia and the eastern high Arctic. The time estimate for gene divergence supports a late Pleistocene separation of the two lineages. Demographic expansion in Beringia, unlike in the high Arctic, precedes the last glacial maximum, suggesting an increase in suitable habitat in the Beringian refugium, but limited availability in the high Arctic during glaciation. Secondary contact in population on the Arctic islands shows contemporary contact occurring between the two lineages, but local unique haplotypes suggest that cryptic glacial refugia may have existed on the islands.

**Keywords**: Pleistocene, Nearctic, population structure, range expansion, genetic variation, cold-adapted biota

### 3.2 Introduction

Glaciation and climate oscillations during the Pleistocene and Holocene have shaped the biogeography of North American species. For example, Nearctic species were confined to refugia during past glacial advances, the most recent being the Wisconsin glaciation reaching a maximum at 21 kyr BP (Hofreiter & Stewart 2009). The retreat of the Laurentide Ice Sheet began around 14 kyr BP, exposing habitat that was colonized by Arctic species dispersing out of glacial refugia. Multiple glacial refugia have been hypothesized as possible source populations for the species now occupying the Arctic, but what role each refugium played in the recolonization of the Arctic is still unclear (Hewitt 2004).

Understanding the biogeography of the Arctic is vital to predicting the response of Arctic fauna to environmental change (Dalén *et al.* 2005), but general patterns can only be interpreted when multiple taxa are examined through phylogeographic studies (Wooding & Ward 1997). Congruent patterns will lend support to the locations of cryptic refugia and help elucidate patterns of dispersal (Waltari *et al.* 2004). The incongruent patterns that have emerged through phylogeographic studies indicate that occupation of and dispersal from refugia is species dependant. It is important to broaden taxonomic focus to understand factors that influenced dispersal out of occupied refugia (Waltari *et al.* 2004).

Biogeographic studies of terrestrial Arctic biota in North America have focused primarily on plants (e.g. Abbott *et al.* 2000), birds (e.g. Jones *et al.* 2005) and mammals (e.g. Fedorov & Stenseth 2002). Few studies have focused on other groups such as terrestrial invertebrates (Schafer *et al.* 2010), despite their high diversity in the Arctic and important ecological roles in northern systems (Danks 1992). Studies focusing on a broader selection of taxa, such as invertebrates, are required to clarify the overarching patterns in the expansion of Arctic biota from glacial refugia.

Following the retreat of the Laurentide Ice Sheet in North America, spiders (Araneae) were likely some of the initial animal colonizers of Arctic Canada, since they are known as excellent dispersers, including in the Arctic (Hodkinson *et al.* 2002). Spiders are capable of long-distance dispersal by

ballooning on silk threads (Bell *et al.* 2005), which allows members of families, such as Lycosidae (wolf spiders) to quickly colonize newly deglaciated terrain (Gobbi *et al.* 2006). Despite their importance in colonization, the biogeographic history of Lycosidae in the Nearctic has received little attention (Murphy *et al.* 2006).

The Arctic wolf spider, *Pardosa glacialis* (Thorell) is a model species to study biogeography in the Canadian high Arctic. *Pardosa glacialis* is restricted to the tundra zone in Canada and Greenland, although it can be abundant in such habitats (Dondale & Redner 1990). The dispersal ability of *P. glacialis* means genetic evidence for population contraction and expansion should track glaciation events closely. Thus, a phylogeographic analysis of the population structure of *P. glacialis* may uncover early dispersal pathways out of refugia and shed light on contemporary demographic processes of Arctic species.

The objective of this study is to use multiple genetic markers to determine: (i) if there is evidence of population structure across the range of *P. glacialis* in Arctic Canada and Greenland; (ii) if the population structure is due to contemporary fragmentation following occupation of a single refugium or historical fragmentation in multiple refugia; and (iii) the demographic history of *P. glacialis* since its dispersal from refugia postglacially. We hypothesize that a genetic structure will be present across this species' range due to fragmentation of populations during the Wisconsin glaciation, but their dispersal ability will have brought the fragmented populations into secondary contact postglacially.

## 3.3 Methods

**3.3.1 Specimens.** Adult *P. glacialis* were collected in the summers of 2010, 2011 and 2012 (Appendix 3.1). Specimens were collected from six sites across northern Canada (Fig. 1, Table 1) and stored in 95% ethanol at -20˚C. Additional specimens from Herschel Island (YT), Zackenberg (GL) and Hochstetter (GL) were borrowed to extend the sampled range (Fig. 1, Table 1).

**3.3.2 Gene amplification.** A single leg, the left fourth leg when possible, was used for DNA extraction. The left third leg was also used for the Herschel

Island specimens collected in 2007 as genomic DNA yield is lower in older specimens due to DNA degradation. The genomic DNA was extracted using the DNeasy Tissue Kit™ (Qiagen) and stored in a -80˚C freezer.

Three gene regions were selected for analysis. Two mitochondrial DNA (mtDNA) genes: cytochrome *c* oxidase subunit 1 (COI) and NADH dehydrogenase subunit 1 (ND1); and one nuclear ribosomal RNA (rRNA) region: the internal transcribed spacer (ITS) region spanning a partial section of ITS1, 5.8S rRNA, and a partial section of the ITS2 region (Ji *et al.* 2003). Because mutation rates in mtDNA tend to be higher than in nuclear rRNA (Mitton 1994), mtDNA should have a higher sensitivity to changes in gene flow and nuclear rRNA should reflect deeper demographic events, such as speciation.

The COI region was amplified using forward primer LC01490 (5'- GGTCAACAAATCATAAAGATATTGG -3') (Folmer *et al.* 1994) and reverse primer Chelicerate Reverse2 (5'- GGATGGCCAAAAAATCAAAATAAATG -3') (Barrett & Hebert 2005). The PCR amplification was performed in 25µL reactions; approximate concentrations of reactants were 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, 0.25 µL BSA, and 0.05U/µL Taq polymerase. The amount of genomic DNA used ranged from 2-3µL depending on the age of the specimen. The reaction conditions were 95˚C for 5 min; followed by 30 amplification cycles of 95˚C for 30 s, 52˚C for 40 s, 72˚C for 1 min 30 s; and a final step for 10 min at 72˚C in an Eppendorf thermocycler.

The ND1 region was amplified using the forward primer TL1-N-12718 (5'- TGCATTAGAATTAGAATCTA -3') (Hedin 1997) and a newly designed reverse primer KSFN1 (5'- GGATATGTTCCTCGTACTCA -3'). The PCR amplification was performed in 25µL reactions; approximate concentrations of reactants were 1X PCR buffer, 1 mM $MgCl_2$, 0.16 mM dNTPs, 0.4 µM of each primer, 0.2 µL BSA, and 0.04U/µL Taq polymerase. The quantity of genomic DNA used ranged from 3-5 µL. Conditions of the PCR runs were 94˚C for 5 min; followed by 40 amplification cycles of 94˚C for 40 s, 52˚C for 60 s, 72˚C for 1 min 30 s; and a final step for 5 min at 72˚C.

The ITS region was amplified using the forward primer CAS18sF1 (5'-TACACACCGCCCTCGCTACTA -3') and the reverse primer CAS28sB1d (5'-TTCTTTTCCTCCGCTTATTTATATGCTTAA -3') (Ji *et al.* 2003). A reaction volume of 25µL was used with the following concentrations: 1X PCR buffer, 1 mM $MgCl_2$, 0.1 mM dNTPs, 0.075 µM of each primer, 0.2 µL BSA, and 0.04U/µL Taq polymerase. The quantity of genomic DNA added ranged from 2-4µL. Reaction conditions were 94˚C for 4 min; followed by 25 amplification cycles of 94˚C for 30 s, 62˚C for 40 s, 72˚C for 1 min 10 s; and a final step for 4 min at 72˚C.

Amplicons were run through 1.5% agarose gels that were stained in ethidium bromide for 15 min, following electrophoresis. Successful PCR products were sequenced with both the forward and reverse primers. Samples were submitted to the McGill University and Genome Quebec Innovation Centre's Sanger sequencing service. The COI sequences of specimens from Herschel Island were obtained from Genbank (www.ncbi.nlm.nih.gov/Genbank/index.html) (Appendix 3.2).

**3.3.2 Genetic analyses.** Sequences were viewed and modified using FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com) and aligned in Jalview (Waterhouse *et al.* 2009) using ClustalWS with default parameters (McWilliam *et al.* 2007). Each of the three gene regions was cut to remove the primer regions and remove overhangs so all sequences were the same length. The sequences were translated to amino acids (using the invertebrate mtDNA code) to determine that no stop codons were present and that the sequences were in the appropriate reading frame (Bikandi *et al.* 2004).

The ITS sequences of heterozygous individuals were dealt with following Sorenson and DaCosta's general guidelines for handling heterozygous sequences (see fig. 1, 2011). In cases where two or more polymorphisms were present in a sequence and their phase could not be determined, IUPAC ambiguity codes were used to indicate the polymorphisms. The ambiguities were treated as missing data and underwent pairwise deletion during analysis.

The jMODELTEST (Posada 2008) software was used to determine the best nucleotide substitution model for COI and ND1 individually and combined, as well as ITS (Appendix 3.3). The Tamura-Nei model (TrN; Tamura & Nei 1993) was the best fit. Gamma correction (+G= 0.05) was included during analysis for the combined mtDNA region. Invariable sites (+I= 0.49) and a gamma correction (+G= 0.05) were included in analyses for the ITS region whenever possible.

Pairwise distances within and between populations, for the combined mtDNA and ITS, were computed in MEGA 5.0 (Tamura *et al.* 2011) using 2,000 bootstrap replicates. Tajima's D statistic (Tajima 1989) to test for neutrality was also calculated in MEGA for the combined mtDNA and ITS dataset.

The GENELAND software version 4.0.3 (Guillot *et al.* 2005), run in R (2011, http://www.R-project.org/), was implemented to test population structure with no *a priori* grouping, based on genetic variation and geographic location. This software program was chosen because the spatial coordinates are incorporated and treated as parameters (Guillot *et al.* 2005). GENELAND uses a Markov chain Monte Carlo (MCMC) algorithm to determine the number of sub-populations within the sampled population based on posterior probability.

The two mtDNA regions were treated as separate loci and the program was run for 5,000,000 steps and thinning was set at 200. The correlated allele frequency model was used assuming a shared ancestral history as a single species. A meta-population max (K) was fixed to five with a minimum of one. A burn-in of 5,000 was used following each run. Five independent runs were done to check for consistency in predicted meta-population number. The model with the highest mean logarithm of posterior probability value was selected as the best model for current meta-population structure. The posterior probability of population membership was computed for each collection site.

A haplotype network, using a 95% parsimonious connection limit, was constructed using TCS 1.21 (Clement *et al.* 2000). Nested clade analysis, which tests associations between haplotypes and geography, was performed with the GEODIS program (Posada *et al.* 2000) on the haplotypes following nesting.

GEODIS was run using 1,000,000 permutations on haplotype clades nested following Templeton *et al.* (1987). GEODIS calculates both nested contingency analysis, where geographic locations are treated as categories, as well as nested clade analysis, which determines the distance among individuals that share the same haplotype (Dc) and the distance among individuals with different haplotypes within the same clade (Dn).

The inference key by Templeton (2004) was used to interpret any significant distances observed. A supplementary test, to evaluate evidence for secondary contact, was performed following Templeton (2001). The test assesses the average pairwise distances among the geographic centres of the haplotypes and step clades found at each site. A low distance is expected in cases of isolation by distance due to population divergence decreasing the number of haplotypes found spanning large ranges. At sites of secondary contact, distances should be large and potentially increase as clade level increases (Templeton 2001).

The program MDIV (Nielsen & Wakeley 2001) was used to estimate the divergence time between meta-populations with no secondary contact based on the mtDNA sequences. Common ancestry among populations from separate glacial refugia should predate the last glacial maximum (Holder *et al.* 1999), due to glacial advances fragmenting populations. The HKY finite sites model, which allows for the possibility of multiple mutations at a single locus, was assumed during runs with 1,000 random seeds. Parameters for each run were set at: 5,000,000 MCMC steps, a 500,000 burn-in time, a $M_{max}$ and $T_{max}$ of 10 and theta set to the default, 11.29.

The posterior probabilities of four parameters are estimated by MDIV for mtDNA: theta ($\theta=2N_{ef}\mu$), ongoing migration ($M=N_{ef}m$), recent divergence ($T=t/(N_{ef}*gen)$), and the time to the most recent common ancestor ($T_{MRCA}$) (Appendix 3.4). We assumed a mutation rate ($\mu$) of $4.12\times10^{-5}$ per site per year (Schenekar & Weiss 2011), based on the average substitution rate (1.51% $Myr^{-}$) between the COI and ND1 rates estimated for the Dysderidae spider genus *Parachtes* (Alicata) (Bidegaray-Batista & Arnedo 2001), and a 2-year life cycle (Høye *et al.* 2009). Though calculated for a different family, estimated mtDNA

substitution rates specific for a spider genus will be more appropriate than a general arthropod rate.

The demographic history can be inferred from the genetic diversity. Population diversity, at the haplotype and nucleotide level, was calculated using ARLEQUIN 3.5 (Excoffier & Lischer 2010). Genetic diversity is seldom lost during range expansion, but it tends to be lost following colonization and stepping stone dispersal (Schmitt 2007). High nuDNA nucleotide diversity will indicate that populations are old enough to have accumulated new mutations following time spent in refugia or the founder effects and bottlenecks that usually accompany colonization. High haplotype diversity can be present in refugia while areas of secondary contact may have high haplotype diversity, but this is usually due to admixture by haplotypes from different areas (Provan & Bennett 2008).

Mismatched distribution analysis (MDA) was performed in Arlequin to further analyze past demographic expansion as well as spatial expansion based on mtDNA (Rogers & Harpending 1992). The distribution of the number of observed pairwise differences between mtDNA sequence pairs was compared to that estimated based on the null hypothesis of demographic or spatial expansion. The sum of squared deviations (SSD) was used to determine the fit of the observed frequency distribution to that of the estimated (Schneider & Excoffier 1999). The MDA provides moment estimates of mutational time, $\tau$ (Rogers & Harpending 1992), which was used in Schenekar and Weiss's (2011) calculator to estimate time since expansion (Appendix 3.4).

### 3.4 Results

The COI sequences (total=143) were cut to fragments 585bp long and the ND1 sequences (total=135) to a length of 789bp. The total number of combined COI+ND1 (1374bp) sequences is given in Table 1. The ITS region was cut to a length of 765bp; the total number of sequences analysed is given in Table 1. Tajima's D values for the combined mtDNA sequences (-2.072960, C.I. -1.774~2.084) and ITS (-0.825066, C.I. -1.781~2.073) revealed them to be neutral meaning they can be used to infer population history.

There are 13 haplotypes identified from COI (pairwise distance 0.1–0.4%) and 28 from ND1 (pairwise distance 0.1–1.2%). The combined mtDNA dataset has 34 unique haplotypes (Table 1) and the average pairwise distance between populations is 0.1–0.3%. The lowest pairwise distances are between the Canadian (CA) mainland populations (Churchill, Dempster Highway, Herschel Island and Kugluktuk) and between the EI-GL populations (Ellesmere Island, Hochstetter and Zackenburg) (Appendix 3.5), which, along with the lack of shared haplotypes between the CA mainland and EI-GL groups, suggests population structure. Unlike the concatenated mtDNA, the most common of the 23 ITS haplotypes (Table 1) is shared among all populations and the average pairwise distances between populations (0.1–0.2%) suggests no divergence among the populations (Appendix 3.5).

Four geographically structured meta-populations were supported by GENELAND (Table 2). Two meta-populations were formed by the CA mainland and EI-GL groups, reflecting the low pairwise distance within each group. The Axel Heiberg and Iqaluit populations were each assigned to their own meta-population (Table 2). The combined mtDNA haplotypes clustered geographically in the parsimony network as well (Fig. 2). The haplotypes in the CA mainland group are more closely related to each other than to the haplotypes in the EI-GL group, while those from Iqaluit and Axel Heiberg are scattered across the network (Fig. 2). The most frequent ITS haplotype is found among all populations with no geographical clustering of haplotypes (Fig. 3).

The NCA of the mtDNA network showed a correlation between genetic variation and geographic location (Table 3). Clades 2-1 and 3-1, comprised principally of haplotypes found only in CA mainland populations, show evidence of contiguous range expansion. The NCA of the entire cladogram alone did not discriminate between historical fragmentation with secondary contact and contemporary fragmentation following long distance colonization (Table 3). The supplementary test for secondary contact revealed the highest pairwise location distance for haplotypes at Iqaluit (Fig. 4). Increasing the unit of analysis from haplotype to the step clades reveals a drop in the pairwise location distances at all

sites with the exception of Axel Heiberg, Ellesmere Island and Iqaluit, where the distances increase (Fig. 4). The large pairwise distance at the three sites indicates secondary contact among previously fragmented populations.

Theta was estimated in MDIV to be 3.07 and the $T_{MRCA}$ was 1.963 when the CA mainland and EI-GL populations were compared. The time since gene divergence between the populations was estimated to be 145,228 yr BP (Table 4). Axel Heiberg and Iqaluit have the highest combined mtDNA haplotype (*h*) and nucleotide ($\pi$) diversity, though all populations are highly diverse (Table 5). However, the *h* and $\pi$ of ITS are highest in the CA mainland populations and Greenland (Table 5).

The MDA null hypothesis of demographic and spatial expansion was not rejected for any of the four meta-populations based on combined mtDNA, though the fit was weak for Axel Heiberg and Iqaluit (Fig. 5). The populations at Axel Heiberg and Iqaluit display a central mode, indicating that these populations are approaching equilibrium following past population and spatial expansion. There is a left shift in the mode for the CA mainland group and the EI-GL group (Fig. 5), indicating a recent demographic expansion (Rogers & Harpending 1992). The mode shifts for spatial expansion reflected those seen in the demographic expansion for every meta-population. Estimates of the time since expansion (Table 4) show that population size increased in the CA mainland populations prior to the last glacial maximum, but spatial expansion was relatively recent. The demographic and spatial expansions in the EI-GL populations are closer in age than in the CA mainland populations, and follow the end of the last glacial maximum (Table 4).

### 3.5 Discussion

The objective of this study was to determine if population structure exists across the range of *P. glacialis* in Canada and Greenland, and to explore the historical or contemporary processes that may be responsible. Our results show strong support for four meta-populations with two main phylogeographic groups, one from the Canadian mainland sites and one from EI-GL sites. The major phylogeographic

groups have a divergence time that predates the last glacial maximum. Therefore, the population structure appears to originate from isolation in two refugia: Beringia and a northeastern refugium, such as Pearyland, or high Arctic Canada (Brassard 1971; Holder *et al.* 1999).

**3.5.1 Population structure.** It has been suggested that vicariance events are unlikely to define wolf spider biogeography, as they might other spiders, due to the high dispersal capability of wolf spiders (Vink & Paterson 2003). As dispersal by ballooning is believed to be sufficient to allow intercontinental species ranges in wolf spiders, fragmentation due to glaciation may create weak population structure that is quickly erased following deglaciation. However, dispersal behaviour in wolf spiders is influenced by the structure of the landscape; fragmented landscapes tend to reduce pre-ballooning behaviour (Bonte *et al.* 2003b; Lambeets *et al.* 2010). Reduced dispersal during glaciation or due to the archipelagic structure of the Canadian Arctic may have produced strong population structure across the range of *P. glacialis*.

Support for a contemporary population structure comes from GENELAND. Every collection locality has a clear population assignment, indicating geographically structured meta-populations. There is geographic structuring of the mtDNA haplotypes in the parsimony network that reflects the CA mainland and EI-GL populations as major phylogenetic groups that experience little gene flow. The Axel Heiberg and Iqaluit haplotypes are spread across the network, suggesting that these metapopulations interact with both the CA mainland and EI-GL groups. The shared haplotypes among the meta-populations supports past or ongoing gene flow. The clear population structure across the range of *P. glacialis* may be the result of contemporary processes restricting gene flow or a signature of multiple glacial refugia.

**3.5.2 Demographic history in refugia.** There are two alternative hypotheses to explain the postglacial history of *P. glacialis*: (i) a single refugium was occupied during the Wisconsin glaciation, providing the source populations for colonization of the entire Nearctic, followed by subsequent fragmentation; or (ii) multiple refugia were occupied and expansion out of all refugia colonized the

Nearctic, leaving distinct lineage patterns (Hewitt 2004). The mtDNA structure alone may not indicate historical isolation in separate refugia, but could be the result of fragmentation following long distance colonization from a single refugium. Isolation of island populations, such as those on the Canadian Arctic archipelago, is not uncommon; divergence, even to the point of speciation, in relation to island colonization has been observed in spiders (Hormiga *et al.* 2003). However, the divergence times between populations pre-date the last glacial maximum. In addition, isolation by distance should result in localized mutations with small average pairwise location distances and not in the large distances observed at Iqaluit, Axel Heiberg and Ellesmere Island. The evidence for secondary contact on the Arctic islands supports multiple refugia.

Though the lack of divergence in ITS could be seen as support for a single refugium, the slower divergence rate relative to mtDNA (Avise 2000) means there may not have been sufficient time for ITS to show evidence of isolation. Nuclear DNA has often been observed to be ineffective in studies of intraspecific genetic variation in spiders (Agnarsson 2010) and our ITS results likely indicate only a single species status rather than support for a single refugium.

If glaciation isolated the *P. glacialis* population into two separate refugia, secondary contact should be present due to dispersal out of those refugia (Templeton 2001). The NCA and the pairwise location distance do suggest historical fragmentation followed by secondary contact between the main phylogeographic groups. The gene divergence time estimate for the CA mainland and EI-GL populations, 145 kyr BP, is around the end of the last interglacial period. Gene divergence can occur before populations are truly isolated (Loehr *et al.* 2006), placing the divergence of these populations in the correct time frame to have been isolated by the Wisconsin glaciation (Sirocko *et al.* 2005). The two main phylogeographic groups correspond to the well-supported Beringia refugium and to a putative high Arctic refugium, such as Pearyland.

The pattern of colonization of the North American Arctic from Beringia in the northwest and cryptic refugia in the high Arctic has been documented in multiple taxa (Holder *et al.* 1999; Waltari *et al.* 2004), as has the hypothesis for

multiple local refugia in the high Arctic (Brassard 1971). The high mtDNA haplotype variation, including unique haplotypes, and differentiation of the Axel Heiberg and Iqaluit populations indicate that small, cryptic refugia may have contributed to the colonization of the Arctic. Fedorov and Stenseth (2002) found a similar pattern of distinct phylogeographic clades in the Arctic collared lemming, *Dicrostonyx groenlandicus*, populations that supported multiple local refugia on the Arctic islands. Additional sampling from the Canadian Arctic islands, especially in the west where cryptic refugia are hypothesized to have existed (Fedorov & Stenseth 2002), would allow a better insight into the number of refugia that contributed to the colonization of the Arctic by *P. glacialis*.

**3.5.3. Dispersal out of refugia.** The last objective was to study the demographic events following deglaciation that may have contributed to the population structure. The relatively high haplotype diversity and low nucleotide diversity suggests recent population expansion of *P. glacialis*, which corresponds to dispersal from glacial refugia. The Beringian and high Arctic refugial populations are supported as the oldest by the higher nuDNA diversity in the CA mainland and EI-GL populations. However, the estimated expansion times suggest populations in the two refugia were not affected by glaciation in the same way.

The CA mainland populations are indicated by NCA and high nuDNA diversity to have been colonized by contiguous range expansion. The hypothesized range limit of Beringia overlaps with the sampled range of the CA mainland group, which supports colonization from Beringia. Recent expansion is indicated by the MDA; however, the timing of the demographic expansion precedes the last glacial maximum. The Beringian population, therefore, is unlikely to have been constricted in size; instead, the cooler climate during glaciation likely increased the availability of suitable habitat for *P. glacialis* in Beringia, allowing this refugial population to expand.

The expansion of the Beringian populations into the CA mainland populations is estimated to have followed the retreat of the Laurentide Ice Sheet. Ancestral origins for Canadian populations in Beringia agrees with the findings of

Reiss *et al.* (1999) for the beetle *Amara alpina* (Paykull) (Carabidae), but not with Fedorov and Stenseth's (2002) results for collared lemmings. The fragmentation of the Beringian and Canadian mainland populations of collared lemmings coincides with the location of the Mackenzie River, which has been hypothesized to act as a dispersal barrier (Fedorov & Stenseth 2002; Waltari *et al.* 2004). As neither *A. alpina* (Reiss *et al.* 1999) nor *P. glacialis* appears to have been influenced by the Mackenzie River, it likely has had less influence over arthropods capable of aerial dispersal.

The NCA and loss of diversity from Greenland populations to Ellesmere Island suggests non-contiguous expansion from a refugium in Greenland. Both demographic and spatial expansions from the Greenland refugium appear to coincide with the beginning of glacial retreat. The demographic expansion is much later than in the Beringian population, suggesting that, despite the cooler climate, the high Arctic population was limited to a smaller range than is available today.

The low nuDNA diversity at Axel Heiberg and Iqaluit indicate that these populations are young relative to the Greenland and CA mainland populations and likely the product of colonization. The secondary contact on the Arctic islands shows that gene flow is occurring, but the lack of shared haplotypes between the CA mainland and Greenland populations suggests these two lineages are still experiencing low gene flow. This may be due to reduced gene flow among island populations or barriers, such as the Parry Channel (Waltari *et al.* 2004). Sampling of additional populations on more Arctic islands would allow for more insight into contemporary gene flow.

### 3.6 Conclusion

*Pardosa glacialis* across its range in Canada and Greenland shows significant structure that likely originates from the occupation of multiple glacial refugia during the Wisconsin glaciation. At least two major refugia were occupied, Beringia and a high Arctic site such as Pearyland, though there is evidence for the presence of small, cryptic refugia. The existence of cryptic refugia on the western Arctic islands, such as Banks Island, has been hypothesized by previous studies

(Fedorov & Stenseth 2002). Distinct genetic clades on the Arctic islands seen in collared lemmings (Fedorov & Stenseth 2002) reflect those seen in *P. glacialis*, suggesting similar gene flow barriers may exist. Further collection from the Arctic islands may shed light on past cryptic refugia and current population dynamics that are shaping the population structure of *P. glacialis*.

### 3.7 Acknowledgements

We thank Doug Currie and the other members of the Northern Biodiversity Program (NBP) for their efforts in the field during collection and in the lab during sorting and identification of specimens. Thanks to Guillaume Lamarche-Gagnon, and the Whyte lab at McGill, for setting-up traps for spider collection on Axel Heiberg. Thanks to Dr. Marcia Waterway for allowing access to her laboratory where the genetic procedures were performed. Thanks to Dr. R. Nielsen for advice on the use of MDIV and to Dr. S. Weiss for advice on substitution rates. Permits for the collection of NBP specimens were granted by Aurora Research Institute (NT), Environment Yukon Parks Branch, the Yukon Scientists and Explorer's Act, and the Nunavut Wildlife Act. Support for this research came from Natural Sciences and Engineering Research Council of Canada Strategic Project Grant, as well as a W. Garfield Weston Award for Northern Research in partnership with Association of Canadian Universities for Northern Studies.

**Table 3.1.** Collection site localities, geographic coordinates, number of individuals sequenced for the concatenated mtDNA and ITS regions, and total haplotypes (designation given in network) for concatenated mtDNA and ITS from the nine populations of *Pardosa glacialis*.

| **Population** | **Lat N/Long W** | **No. of ind.** | | **No. of haplotypes** | |
|---|---|---|---|---|---|
| **Site** | | **mtDNA** | **ITS** | **mtDNA** | **ITS** |
| **EI-GL** | | | | | |
| Hazen Camp, NU (El) | 81.832/-71.441 | 18 | 10 | 5 (1 – 5) | 4 (1 – 4) |
| Hochstetter, GL (Ho) | 75.326/-19.688 | 20 | 9 | 8 (2,16 – 22) | 5 (1,3,6,17,18) |
| Zackenberg, GL (Za) | 74.500/-20.767 | 19 | 8 | 7 (2,23 – 28) | 4 (1,3,6,18) |
| **Axel Heiberg** | | | | | |
| McGill Arctic Research Station, NU (Ax) | 79.559/-90.811 | 10 | 8 | 6 (6,30 – 34) | 3 (1,3,23) |
| **Iqaluit** | | | | | |
| Iqaluit, NU (Iq) | 63.765/-68.530 | 15 | 8 | 8 (1,2,6 – 11) | 5 (1,3,5 – 7) |
| **CA mainland** | | | | | |
| Churchill, MB (Ch) | 58.730/-93.795 | 7 | 3 | 3 (1,11,13) | 5 (1,9,12,15,16) |
| Dempster Hwy, YT (Dm) | 66.997/-136.215 | 10 | 7 | 2 (1,29) | 6 (1,3,19 – 22) |
| Kugluktuk, NU (Ku) | 67.798/-115.266 | 19 | 10 | 2 (1,12) | 9 (1,3,8 – 14) |
| Herschel Island, YT (He) | 69.579/-139.076 | 13 | 0 | 3 (1,14,15) | - |
| **Total** | | 131 | 63 | 34 | 22 |

**Table 3.2.** Posterior probability of population membership to each of the four predicted meta-populations for each collection locality. Mean posterior density of the selected model was 755.98. The highest posterior probability for each site is in bold, indicating population assignment.

| Collection site | EI-GL | Axel Heiberg | Iqaluit | CA mainland |
|---|---|---|---|---|
| **El** | **0.50522** | 0.13904 | 0.18208 | 0.17366 |
| **Ho** | **0.50458** | 0.13940 | 0.18254 | 0.17347 |
| **Za** | **0.50495** | 0.13931 | 0.18236 | 0.17338 |
| **Ax** | 0.16203 | **0.51548** | 0.17054 | 0.15195 |
| **Iq** | 0.20315 | 0.13153 | **0.47353** | 0.19179 |
| **Ch** | 0.13024 | 0.20837 | 0.18428 | **0.47710** |
| **Dm** | 0.12878 | 0.21057 | 0.17650 | **0.48415** |
| **He** | 0.12878 | 0.21057 | 0.17650 | **0.48415** |
| **Ku** | 0.12878 | 0.21057 | 0.17650 | **0.48415** |

**Table 3.3.** Inferences from the nested contingency analysis (NCA) performed on the parsimony network from the combined mtDNA haplotypes of *Pardosa glacialis* specimens, following the key by Templeton (2004). Only inferences made on clades that displayed a significant relationship between genetic variation and geographic location are shown. Perm. stat. – permutation statistic, P. – probability.

| Clade | Perm. stat. | P. | Chain of inference | Inference |
|---|---|---|---|---|
| **1-1** | 109.3 | 0.056 | 1-2-11-17 No | Inconclusive outcome |
| **2-1** | 20.1 | 0.012* | 1-2-11-12 No | Contiguous range expansion |
| **3-1** | 33.1 | 0.000* | 1-2-11-12 No | Contiguous range expansion |
| **Entire cladogram** | 109.2 | 0.000* | 1-2-11-12-13 YES 21a)YES b)YES c)No Insufficient evidence | Recent or historical fragmentation |

* Significant at the 5% level with Dunn-Sidak correction= 0.025

**Table 3.4.** Time estimates (MDIV and MDA) of significant population events based on mtDNA for the two metapopulations that show support for expansion out of refugia. Populations with secondary contact, Iqaluit and Axel Heiberg, were not analysed as shared haplotypes disrupt estimates. Shown is the estimate of gene divergence (Gene d.) time between the EI-GL and CA mainland populations based on the MDIV $T_{MRCA}$. The time in years since both demographic and spatial expansion for each metapopulation is presented based on estimates of $\tau$. The 95% confidence intervals are in parentheses. A 2 year generation time was used and all estimates are in yr BP. D.SSD – sum of square deviation of MDA for demographic expansion; S.SSD – spatial expansion

| Metapopulation | Gene d. | D. SSD $\tau$ | D. expansion | S. SSD $\tau$ | S. expansion |
|---|---|---|---|---|---|
| **EI-GL** | 146,196.3 | 3.000 | 21,231.6 (0–25,015) | 0.64 | 15,424 (2,940–25,063) |
| **CA mainland** | | 0.881 | 72,298.2 (4,217–42,173) | 0.271 | 6,531 (1,349–13,664) |

**Table 3.5.** Haplotype diversity (*h*) and nucleotide diversity (π) based on combined mtDNA and ITS for the 9 populations of *Pardosa glacialis*. Diversity calculated in Arlequin, the TrN model +G was used for calculations of nucleotide diversity.

| **Population** | **Haplotype diversity** | | **Nucleotide diversity** | |
|---|---|---|---|---|
| **Site** | **Concat. mtDNA** | **ITS** | **Concat. mtDNA** | **ITS** |
| **EI-GL** | | | | |
| **El** | **0.4052** ± 0.1428 | **0.5000** ± 0.1222 | **0.000405** ± 0.000388 | **0.000773** ± 0.000720 |
| **Ho** | **0.6474** ± 0.1201 | **0.6601** ± 0.1020 | **0.000648** ± 0.000527 | **0.000291** ± 0.000404 |
| **Za** | **0.5439** ± 0.1364 | **0.7917** ± 0.0451 | **0.000605** ± 0.000504 | **0.000557** ± 0.000595 |
| **Axel Heiberg** | | | | |
| **Ax** | **0.8889** ± 0.0754 | **0.5667** ± 0.1090 | **0.002827** ± 0.001752 | **0.000325** ± 0.000433 |
| **Iqaluit** | | | | |
| **Iq** | **0.9143** ± 0.0425 | **0.6000** ± 0.1267 | **0.001780** ± 0.001144 | **0.000690** ± 0.000679 |
| **CA mainland** | | | | |
| **Ch** | **0.5238** ± 0.2086 | **1.0000** ± 0.0962 | **0.000416** ± 0.000435 | **0.002327** ± 0.001795 |
| **Dm** | **0.2000** ± 0.1541 | **0.7473** ± 0.1114 | **0.000146** ± 0.000222 | **0.000703** ± 0.000695 |
| **Ku** | **0.1053** ± 0.0920 | **0.9216** ± 0.0391 | **0.000077** ± 0.000149 | **0.001057** ± 0.000892 |
| **He** | **0.2949** ± 0.1558 | NA | **0.000224** ± 0.000277 | NA |

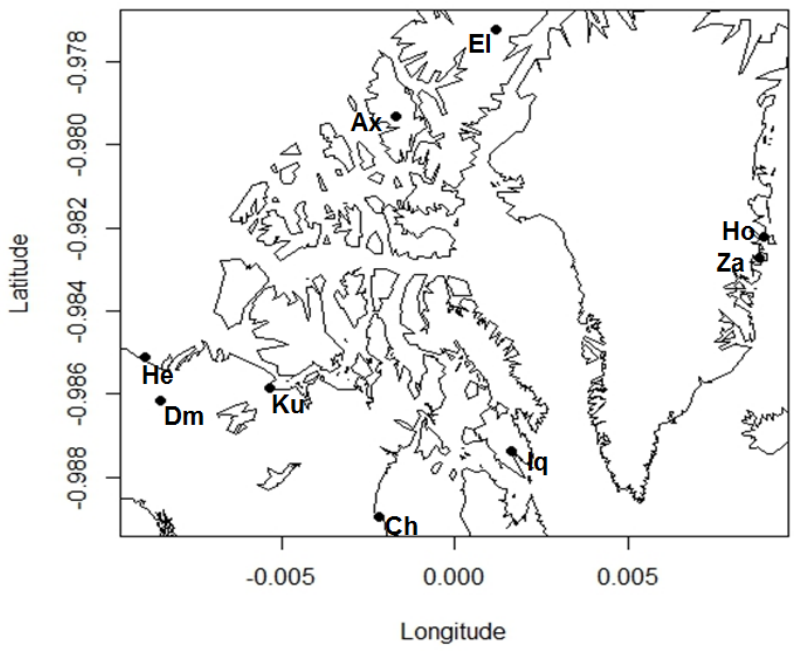


**Fig. 3.1.** Map of northern Canada and Greenland showing the collection localities of *Pardosa glacialis* specimens used in this study. Lambert coordinates were used to account for the Earth's curvature when geographic positions were mapped.

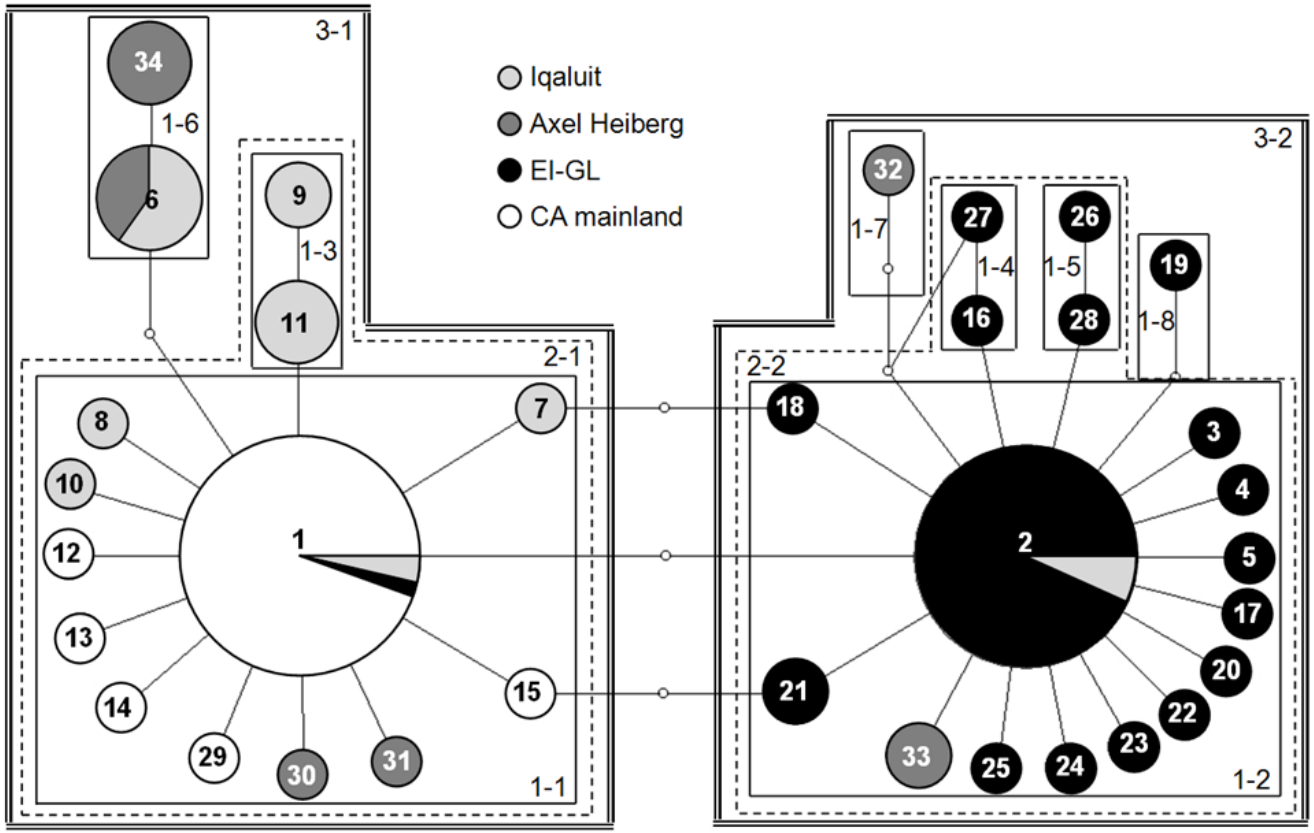


**Fig. 3.2.** Statistical parsimony network of the combined COI and ND1 mitochondrial DNA gene regions from *Pardosa glacialis* specimens. Haplotypes are represented by circles with 1 bp change represented by the connecting lines. Nodes on the connecting lines indicate intermediate mutational steps. Loops in the haplotype network indicate ambiguities where the mutational pathway is unclear. Frequency of the haplotype corresponds to the size of the circle. Haplotypes from the CA mainland localities cluster on the left (white) while those from the EI-GL localities are clustered on the right. NCA 0-step clades are depicted by the singe lines, 1-step clades by the dashed lines, 2-step clades by the double lines and the final 3-step clade includes all haplotypes in the network.

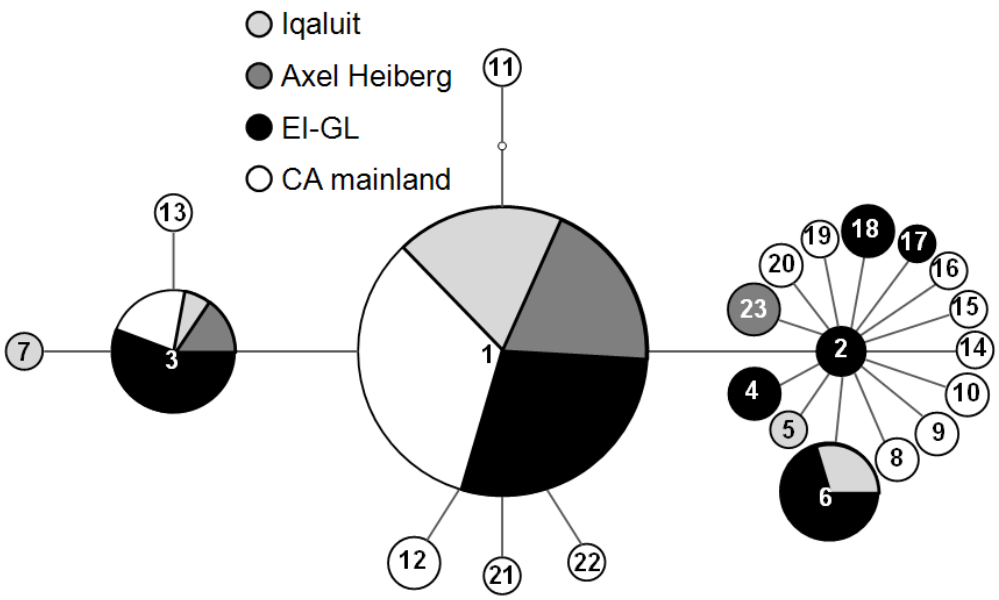


**Fig. 3.3.** Statistical parsimony haplotype network depicting the variation in the nuclear ribosomal ITS gene region of *Pardosa glacialis* specimens. Haplotypes are represented by circles with 1 bp change represented by the connecting lines. Nodes on the connecting lines indicate mutational steps that were not found but are assumed to be or have been present in the population. Frequency of the haplotype corresponds to the size of the circle. Haplotypes do not cluster in relation to collection site location.

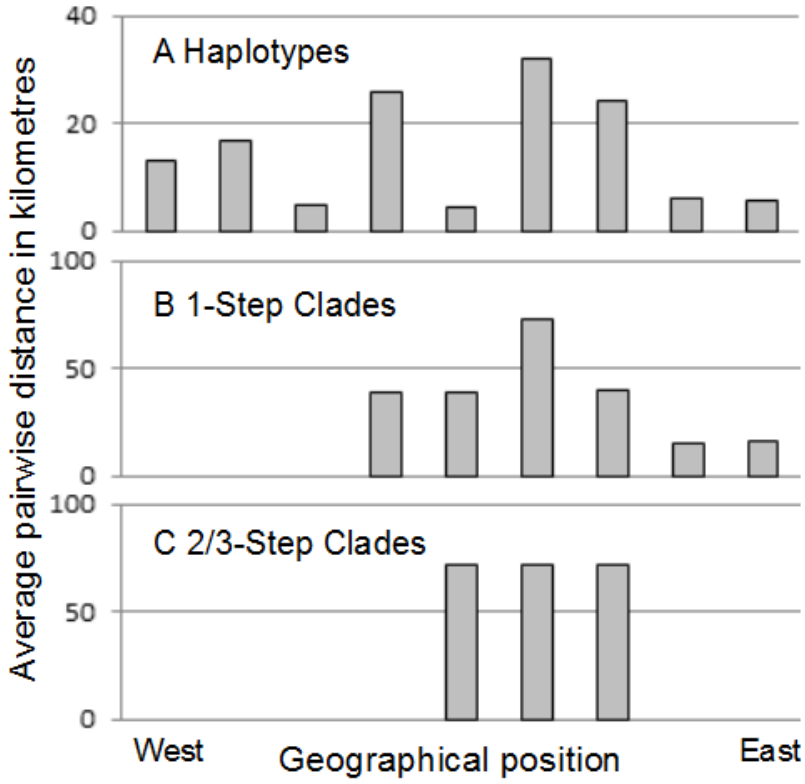


**Fig. 3.4.** Average pairwise distances among geographical centres of mtDNA haplotypes and clades found at each of the nine *Pardosa glacialis* collection localities (ordered by longitude). Panel A displays average distances among geographical centres of haplotypes, B the comparable distances for 1-step clades, and C the distances for both 2-step and 3-step clade as the distances were comparable.

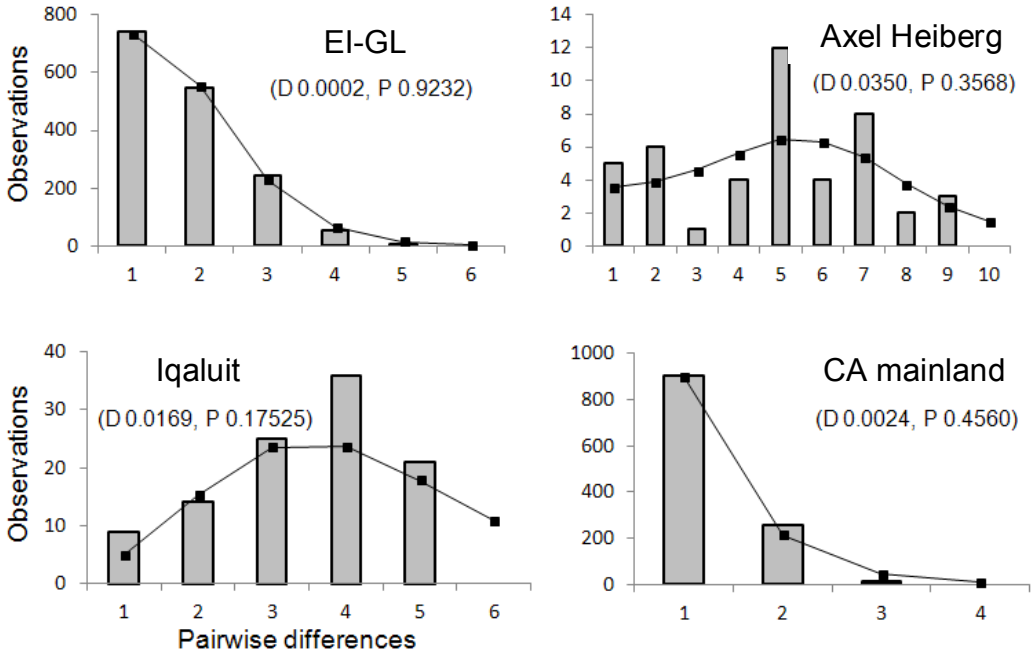


**Fig. 3.5.** Mismatch distribution analysis for demographic expansion in the combined mitochondrial DNA dataset for the four proposed meta-population groups within the species range of *Pardosa glacialis*. Expected pairwise substitutions under a population expansion model (black line with markers) are compared to the observed (grey boxes). All four mtDNA meta-populations fail to reject the MDA null hypothesis of demographic expansion as there were no significant sums of square deviations (D). The null hypothesis was also not rejected for spatial expansion. The mode is shifted to the left in CA mainland and EI-GL populations, indicating a recent demographic expansion. The same patterns are seen for the spatial expansion in the mtDNA with no significant sum of square deviations.

**Appendix 3.1.**

General collection: The Northern Biodiversity Program (NBP) sampled 12 sites across Canada to study the biodiversity and ecology of arthropods in northern Canada. Three ecoclimatic zones in Canada were chosen as the focus for sampling efforts: the northern boreal, sub-Arctic and the high-Arctic. Within each ecoclimatic zone, four sites were selected for collection: six were sampled in the summer of 2010 (Iqaluit, Schefferville, Goose Bay, Churchill, Moosonee and Hazen Camp on Ellesmere Island) and six in 2011 (Norman Wells, along the Dempster Highway, Cambridge Bay, Banks Island, Yellowknife and Kugluktuk). Sampling at each site took place over a two week span, aimed for the peak arthropod activity in each ecoclimatic zone. The dates are from approximately 1 June - 20 June in the boreal, 21 June - 10 July in the southern arctic and 11 July - 31 July in the northern arctic.

Collection methods for the 12 standardized sites consisted of using pitfall and yellow pan traps. The design had eighteen traps (nine pan and nine pitfall) set in a wet habitat and eighteen set in a mesic or dry habitat. Ideally, these two habitats were located in close proximity. The design was replicated three times at each site, with replicates spaced at least 250 m apart when possible. A 50:50 solution of propylene glycol and water was used in both pitfall and pan traps. The traps were serviced every three to four days, three times over the two week study period. The arthropods collected were transferred to 95% ethanol for storage and shipped to McGill University for sorting and identification.

Additional notes on *P. glacialis* collection: Two additional sites, the border between the Yukon and the Northwest Territories on the Dempster Highway and a site on Axel Heiberg were sampled in the summer of 2012. Collection on the Dempster in 2012 was done by hand, while yellow pan traps were used on Axel Heiberg. Spiders from all sites were identified using morphological keys. When possible, 20 specimens (10 male and 10 female) of *P. glacialis* were chosen at random from each site where they were found. Only 7 specimens of *P. glacialis* were collected from Churchill and only 10 specimens

from the sites sampled in the summer of 2012 were used due to their late addition and low genetic diversity observed within other sites.

**Appendix 3.2.**

The accession numbers for the COI sequences from Herschel Island specimens that were taken from Genbank.

| ID | Accession |
|---|---|
| WOLF-A02 - WOLF-A05 | HM384663 - 384666 |
| WOLF-A10 | HM384670 |
| WOLF-B03 | HM384675 |
| WOLF-B11 | HM384683 |
| WOLF-C08 | HM384691 |
| WOLF-C09 | HM384692 |
| WOLF-D05 | HM384696 |
| WOLF-D08 | HM384698 |
| WOLF-E09 | HM384708 |
| WOLF-E11 | HM384710 |
| WOLF-F05 | HM384715 |
| WOLF-F08 | HM384718 |
| WOLF-F10 | HM384720 |
| WOLF-F11 | HM384721 |
| WOLF-G09 | HM384726 |
| WOLF-H02 | HM384729 |
| WOLF-H09 | HM384734 |

**Appendix 3.3.**

The jMODELTEST (Posada 2008) software was used with each mtDNA gene region individually and combined, as well as with the nuclear rRNA ITS region to determine the best nucleotide substitution model based on an optimized maximum likelihood tree and an AICc analysis. Five substitution schemes were tested, with base frequencies, invariable sites (+I) and gamma correction (+G; 5 discrete categories of rate variation among sites) included as possible parameters. The settings chosen tested 40 possible substitution models. The best-fit model was the Hasegawa, Kishino and Yano (HKY) model for all gene regions (with +G for concatenated mtDNA and +GI for the ITS region). The HKY model allows base pair frequencies to be unequal, and also assumes transitions and transversions

may different rates (Hasegawa *et al.* 1985). However, this model is not available for use in every analysis and so the next best model was used for these analyses.

The best substitution model capable of being implemented during all analyses was the Tamura and Nei (TrN; Tamura & Nei 1993) unequal frequency model. The TrN model allows for the same assumptions as the HKY model with the additional assumption that the rate of the different transitions can be different. Gamma correction (+G= 0.05) was included during analysis for the combined mtDNA region. Invariable sites (+I= 0.49) and a gamma correction (+G= 0.05) were included in analyses for the ITS region whenever possible.

**Appendix 3.4.**

A detailed description of MDIV calculations using mtDNA is given by Buehler and Baker (2005). The MDIV program was run with a variety of random seeds and estimated maximums to determine when the length of the MCMC chain was sufficient to give consistent results. Bidegaray-Batista and Arnedo (2001) estimated the substitution rate for multiple mtDNA regions of the genus *Parachtes*. The ND1 region had a substitution rate of 1.77% $Myr^{-1}$, the COI a rate of 1.25% $Myr^{-1}$. The average between the two rates (1.51% $Myr^{-1}$) was used as the parameters were calculated based on the concatenated sequence.

Following the calculation method by Schenekar and Weiss (2011), which takes into account generation time, the mutation rate (μ) was 3.02 $Myr^{-1}$. The length of the sequence (1374bp) was then taken into account to calculate the final rate of $4.15\times10^{-5}$. Calculation of $T_{MRCA}$ between EI-GL and CA mainland metapopulations:

$N_{ef}$ = 3.07(theta) / ( 2 * $4.15\times10^{-5}$(mu) )

$T_{MRCA}$ = 1.962935 * 36992.58702($N_{ef}$) * 2(generations)

The MDA values of τ (both demographic and spatial) for metapopulations were used, with 95% confidence intervals, in the equation:

t = τ / ( 2 * u ),

where t is the time since expansion in generations, and u the cumulative probability of substitution. The time since expansion in years was then calculated in the equation:

Time = t * g,

where g is the generation time. Schenekar and Weiss (2011) provided an on-line spreadsheet, which was used for all calculations of time since expansion in years.

**Appendix 3.5.**

The genetic pairwise distance between collection sites of *Pardosa glacialis* computed in MEGA 5.0 with the Tamura-Nei model +G (0.05). Below the diagonal are the mean pairwise distances based on the combined COI+ND1 mtDNA sequences. Above the diagonal are the mean pairwise distances based on the nuclear rRNA ITS sequences.

| **Site** | **El** | **Ho** | **Za** | **Ax** | **Iq** | **Ch** | **Dm** | **He** | **Ku** |
|---|---|---|---|---|---|---|---|---|---|
| **El** | | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | - | 0.001 |
| **Ho** | 0.001 | | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | - | 0.001 |
| **Za** | 0.001 | 0.001 | | 0.001 | 0.001 | 0.002 | 0.001 | - | 0.001 |
| **Ax** | 0.003 | 0.003 | 0.003 | | 0.001 | 0.001 | 0.001 | - | 0.001 |
| **Iq** | 0.002 | 0.003 | 0.002 | 0.003 | | 0.002 | 0.001 | - | 0.001 |
| **Ch** | 0.002 | 0.002 | 0.002 | 0.002 | 0.001 | | 0.002 | - | 0.002 |
| **Dm** | 0.002 | 0.002 | 0.002 | 0.002 | 0.001 | 0.000 | | - | 0.001 |
| **He** | 0.002 | 0.002 | 0.002 | 0.002 | 0.001 | 0.000 | 0.000 | | - |
| **Ku** | 0.002 | 0.002 | 0.002 | 0.002 | 0.001 | 0.000 | 0.000 | 0.000 | |

### 3.11 Connecting statement

Chapter 3 has shown that the mtDNA and ITS support a single species status of *P. glacialis* across its range in Canada and Greenland. *Pardosa glacialis* is well known to have high intraspecific variation in the female genitalia or epigynes (Dondale and Redner 1990), a fact that is considered unusual for this genus. Despite the high morphological variation within the genitalia of *P. glacialis*, the current taxonomic boundaries between other *Pardosa* species leave little room for intraspecific variation. The epigynes of many *Pardosa* species are difficult or impossible to distinguish and species status may rest on only single character variations of the male pedipalp. Species-groups in *Pardosa* that rely on single characters are, for the most part, taxonomically uncertain and would benefit from molecular analysis. Molecular analyses in combination with morphometric analyses on *P. lapponica* (Thorell) and *P. concinna* (Thorell) of the *P. lapponica* species-group will help elucidate the relationship between these two species.

**Chapter 4.** Morphological and mtDNA examination of the species boundary between *Pardosa concinna* and *P. lapponica* (Araneae: Lycosidae) in the northern extent of their Nearctic range

KATHRIN A. SIM, CHRISTOPHER M. BUDDLE & TERRY A. WHEELER

*Department of Natural Resource Sciences, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, CANADA (kathrin.sim@mail.mcgill.ca,christopher.buddle@mcgill.ca,terry.wheeler@mcgill.ca)*

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**4.1 Abstract**

The Holarctic *Pardosa lapponica* (Thorell) and the Nearctic *P. concinna* (Thorell) are the only North American members of the *Pardosa lapponica* species-group. The morphological similarity between the two species raises the question of whether or not they are separate species. To examine the boundary between *P. lapponica* and *P. concinna*, morphological and genetic variation within and between the species was analysed. Copulatory organ characters of Nearctic specimens were analysed to determine if additional diagnostic characters exist, while the mtDNA COI region was sequenced to look for species-specific variation. Morphometric analysis of copulatory characters in females (e.g. length of median septum) and males (e.g. embolus length) of both species did not reveal any diagnostic characters beyond the terminal apophysis. No species-specific genetic patterns were found between the two species. The interspecific similarities in morphology and low genetic divergence between Nearctic specimens of *P. lapponica* and *P. concinna* contrasts with the high genetic divergence between Palearctic and Nearctic specimens of *P. lapponica*. The results suggest that a taxonomic revision is necessary for members of the *P. lapponica* species-group.
**Key words:** morphometrics, COI, DNA barcode, haplotype

## 4.2 Introduction

The genus *Pardosa* (Koch) (Lycosidae) contains 46 species in North America, which have been arranged into 13 informal species-groups (Dondale & Redner 1990). Assignment to a species-group is based on morphological similarities in both noncopulatory and copulatory characters (Dondale & Redner 1990). Unfortunately, the morphological conservatism that allows species to be placed in these informal groups can also make identification difficult. Indeed, some of the species-groups are taxonomically challenging, containing members that can only be distinguished based on a few characters, often from only one sex.

One such challenging species-group is the *Pardosa lapponica* species-group, which contains seven species (Zyuzin 1985). It is represented in North America by two species: the Holarctic *Pardosa lapponica* (Thorell 1872) and Nearctic *P. concinna* (Thorell 1877). *Pardosa concinna* was described by Thorell (1877) based on a single female specimen from Colorado, USA. Bishop (1949) described the first female specimen of what is now recognised as *P. lapponica,* as *P. harperi* (Bishop). Dondale and Redner (1986) synonymized *P. harperi* with *P. lapponica* originally from Lapland.

Dondale and Redner (1986) did not synonymise the Nearctic *P. concinna* with *P. lapponica*, despite high morphological similarity. The separate species status was retained due to variation in the terminal apophysis (TA) of the male pedipalp (Dondale & Redner 1986). While the length of the median septum in females was suggestive of species, the TA was found by Dondale and Redner (1986) to be the only truly diagnostic character (Dondale & Redner 1986). The terminal apophysis is often a key character for species delimitation in this genus (Zyuzin 1985), but variation in this character may not be species-specific as was once assumed.

Intraspecific variation in spider genitalia has not been well studied, owing to a long standing assumption of fast-paced genitalic coevolution (Huber 2004). Influenced by the lock-&-key hypothesis, many species were split based on any morphological variation observed in the genitalia as the variation was assumed to represent species boundaries. Variation within a single species, due to ontogenetic

changes or phenotypic plasticity, was dismissed in the past (Huber 2004), and is only recently being recognised (Bennett 2006). This bias against intraspecific variation may have led to over-splitting of species.

The objective of this study is to examine the species boundaries between *P. lapponica* and *P. concinna* across the northern extent of their range in North America using morphological and genetic analyses. The mitochondrial gene cytochrome *c* oxidase subunit I (COI) was used in tandem with morphological characters to assess the species limits and to determine the extent of phenotypic variation and gene flow between the two described species.

## 4.3 Methods

**4.3.1 Specimens and Identification.** Adult specimens used in genetic analyses were collected from seven sites across northern Canada in the summers of 2010 and 2011 (Table 1) and were stored in 95% ethanol (Appendix 4.1). These specimens are deposited in the Lyman Entomological Museum, McGill University, Montreal QC (LEM). Additional specimens for genetic study were obtained from the University of Alaska Fairbanks entomology collection (UAF) and from the private collection of D. J. Buckle (DJB) (Table 1). Descriptions and measurements of morphology are based on the above specimens, as well as specimens in the Canadian National Collection, Ottawa (CNC) (Table 1). No type specimens were analysed, but the terminal apophysis of collected males were compared to those in males of *P. concinna* from Colorado in CNC, and males of *P. lapponica* in CNC that had been compared to a Lapland type by Dondale and Redner (1986).

Terminology used for the external female epigynes follows Kronestedt (1975), the internal female structures follow Dondale and Redner (1990), and the male pedipalp follows Vogel (2004). Identification to species was done by examination of the male terminal apophysis and no specimens were used from sites where males were unavailable. The epigynes were dissected in order to view the internal structures and, when necessary, were cleared using lactic acid. The pedipalps were dissected to separate the tegulum and palea.

**4.3.2 Morphological analyses.** Specimens in ethanol were examined using a Nikon SMZ1500 microscope and measurements (in mm) made using an ocular micrometer in a 10X ocular. Body size measurements were made at 1X objective while a 6X objective was used when taking measurements of copulatory organs. The following measurements were taken: carapace width and length of males and females; width (face on) and length of the whole cymbium; length of the embolus; and width and length of the septum. The spination on the tibia and metatarsus of the first leg was examined on five male and five female specimens of each species and compared. Copulatory characters used in the morphological analysis (with hypothesized differences in brackets) were: paleal protrusion (absent in *P. lapponica*, present in *P. concinna*); conductor (protruding in *P. lapponica*, below terminal apophysis in *P. concinna*); shape of the septal pockets (smooth in *P. lapponica*, irregular in *P. concinna*); shape of the spermathecae (curved in *P. lapponica*, straight in *P. concinna*); and shape of the copulatory tubes (straight in *P. lapponica*, curved in *P. concinna*).

**4.3.3 Genetic analyses**. Specimens were stored in a -20°C freezer prior to DNA extraction. For specimens collected after 2009, the left fourth leg was removed and used for DNA. The left third and fourth leg were used when specimens were older because DNA degradation reduces the genomic DNA yield during extraction. Genomic DNA was extracted using DNeasy Tissue Kit™ (Qiagen) following the protocol provided for animal tissue. Extracted DNA was stored in a -80°C freezer.

A 612 base pair (bp) fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene (the DNA barcode) was targeted and amplified using the forward primer LC01490 (5'- GGTCAACAAATCATAAAGATATTGG -3') (Folmer *et al*. 1994) and reverse primer Chelicerate Reverse2 (5'- GGATGGCCAAAAAATCAAAATAAATG -3') (Barrett & Hebert 2005). Amplification was performed in 25µL reactions. Approximate concentrations of reactants were 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, 0.25 µL BSA, and 0.05U/µL Taq polymerase. The amount of genomic DNA used ranged from 2µL to 3µL. The reaction conditions were 95˚C for 5 min;

followed by 30 amplification cycles of 95˚C for 30 s, 52˚C for 40 s, 72˚C for 1 min 30 s; and a final step for 10 min at 72˚C in an Eppendorf thermocycler. The PCR amplicons were visualized on 1.5% agarose gels, which were stained in ethidium bromide and illuminated with UV light.

Successful PCR amplification products were submitted to the McGill University and Genome Quebec Innovation Centre's Sanger sequencing service. Forward and reverse sequences were obtained for every specimen included in genetic analysis. Mitochondrial COI sequences were taken from the Barcode of Life Data System (BOLD) for: *Pardosa lapponica* from Russia, *P. caucasica* (Ovtsharenko), *P. koponeni*, *P. moesta* (Banks), *P. adustella* (Roewer), *P. furcifera* (Thorell), *P. podhorskii* (Kulczynski) and *P. falcifera* (P.-Cambridge) (Appendix 4.2) (Ratnasingham & Hebert 2007).

**4.3.4 Sequence analyses.** The COI mtDNA sequences, 41 in total, were viewed and modified using FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com). Sequences were aligned with those from BOLD using ClustalWS in Jalview (Waterhouse *et al.* 2009) then cut to a 609 bp fragment. Initial species grouping of the sequences was based on the current species divisions for the Nearctic *P. lapponica* group (i.e. *P. lapponica* and *P. concinna*), with Palearctic *P. lapponica* specimens placed in their own group.

The software jMODELTEST (Posada 2008) was used to determine the best nucleotide substitution model based on an optimized maximum likelihood tree and an AICc analysis. The best-fit model was the Tamura-Nei (TrN) model, which takes into account different rates of substitution for transitions and transversions (Tamura & Nei 1993), with a gamma correction of 0.15.

The TrN model was used to compute pairwise distances within and between species in MEGA 5.0 (Tamura *et al.* 2011) using 2,000 bootstrap replicates. An intraspecific distance threshold of 2% (Barrett & Hebert 2005) was used to determine if the *a priori* species groups contained potentially oversplit or lumped species. Fixed nucleotide substitutions among species sequences were detected by eye.

**4.3.5 Haplotype network.** A haplotype network was constructed for the *P. lapponica* species-group sequences from the Nearctic and Palearctic using TCS 1.21 software (Clement *et al*. 2000) to identify population-level genetic relationships and diversity. The network is based on the most parsimonious connections with a 95% connection limit. Ambiguous relationships in the network form loops and missing mutational steps between haplotypes are indicated by nodes.

## 4.4 Results

We examined 122 specimens from 12 localities from central Alaska to southern Labrador (Table 1). Both species are sexually dimorphic in size, with females larger (Fig. 1). Description of the type specimens by Thorell (1872) indicates *P. lapponica* from Lapland is larger than those in the Nearctic (Female type: cephalothorax length 4 mm, width 2.2 mm; Nearctic: max. length 2.9 mm, max. width 2.1 mm; Male type: length 3.25 mm; Nearctic: max. length 2.8 mm). The size of the *P. concinna* type reported by Thorell (1877) is close to the size of those collected and examined here (Female type: length 3.2 mm, width 2.33 mm; Nearctic: length 2.9 mm, width 2.2 mm).

The average size of both males and females of *Pardosa concinna* is larger than in *P. lapponica*, but the ranges overlap (Fig. 1). The sizes of the copulatory organs and their characters follow the same pattern as body size; although, the overlap between the septum lengths is narrow (Fig. 2). The spination on the tibia and metatarsus of the first leg does not vary between the species. A single spine, the proximal front spine on the metatarsus, varies between male and female specimens; the spine is missing or small in females, but present in all males.

**4.4.1 Female morphology.** The characters examined are variable and overlap between the two species. The overlap in character shape was most extensive in specimens collected from Kugluktuk and Churchill. In most female *P. lapponica* specimens the lips of the epigynes converge to form a low, flat hood, shortening the visible length of the septal ridge (Fig. 3a). In some specimens, however, the hood is high, exposing more of the septal ridge (Fig. 3c).

The lips of the epigynes in most *P. concinna* specimens converge to an elevated hood, exposing most of the septal ridge (Fig. 3d). In some cases the hood is malformed and the entire septal ridge is exposed (Fig. 3e); this was seen in some *P. lapponica* specimens from Churchill as well (Fig. 3c). The septal pocket in most *P. lapponica* specimens is smoothly curved (Fig. 3a), while in most *P. concinna* specimens there is a bulge and the pocket is irregular (Fig. 3e). This difference is not consistent between species (Figs 3b, 3d).

The shape of the spermathecae and the copulatory tubes was not found to be diagnostic. Neither the copulatory tubes (Figs 3d, 3e) nor spermathecae (Fig. 3f) were consistently straight or curved in either species. The exact curvature of the spermathecae varied, as did the length in both straight and curved pairs of spermathecae. The reservoirs of the spermathecae varied from slightly inflated to bulbous and nodules were inconsistently present in both species.

**4.4.2 Male morphology.** In male specimens of *P. concinna*, the palea often has a protrusion near the tip (Fig. 4a). This protrusion is absent in some *P. concinna* specimens and most *P. lapponica* specimens (Figs 4b, 4c). The conductor is more consistent than other characters, curving up in both species, but further in *P. lapponica* specimens (Fig. 4c). In *P. lapponica* specimens, the conductor protrudes past the lower limit of the terminal apophysis, whereas it ends before this point in most *P. concinna* specimens (Figs 4a, 4b).

The embolus length overlaps between the two species, with *P. concinna* specimens larger on average (Fig. 2). The shape is thin and blade-like within and between species (Figs 4b, 4c). The terminal apophysis was larger in *P. concinna* and bulbous or finger-like in shape (Figs 4a, 4b) and pointed in *P. lapponica* (Fig. 4c).

**4.4.3 Genetic variation.** The 609 bp COI dataset, including only the sequences from Nearctic *P. lapponica* and *P. concinna*, has 17 polymorphic sites and a total of 15 haplotypes. Six haplotypes were obtained from single specimens and two are shared between the two recognised species (Fig. 5). No fixed species-specific nucleotide substitutions were observed in the dataset before and after the shared haplotypes were removed.

There are multiple fixed substitutions between the Nearctic *P. lapponica* group specimens and those in the Palearctic (Table 2). Mean divergence between the Palearctic and Nearctic *P. lapponica* specimens, as well as between the Palearctic *P. lapponica* and *P. concinna* specimens, is greater than 2% (Table 2). The mean interspecific sequence distance between Nearctic *P. lapponica* and *P. concinna* is below 2% (Table 2). Between other *Pardosa* species for whom sequence divergence is below 2%, there are fixed nucleotide differences that are species-specific (Table 2).

The haplotype network for the Nearctic members of the *P. lapponica* species-group has two central haplotypes (6 and 8) from which the other 13 haplotypes radiate (Fig. 5). Haplotypes shared between the two recognised species were recovered from males and females of both species (1: mf *P. lapponica* and mf *P. concinna*, 2: m *P. lapponica* and m *P. concinna*). The haplotypes of the two species do not aggregate into distinctive groups, but are intermixed (Fig. 5). The haplotypes from the Palearctic *P. lapponica* specimens cluster together and are separate from the Nearctic specimens.

**4.5 Discussion**

The objective of this research was to examine the species boundary between the two Nearctic species of the *Pardosa lapponica* species-group. The morphological evidence suggests high intraspecific variation and no interspecific morphometric gaps, with the exception of the terminal apophysis. Genetic analyses also failed to reveal interspecific variation in the COI sequence that would support the status of Nearctic *P. lapponica* and *P. concinna* as two separate species. The results indicate phenotypic variations in the TA in this species-group are unlikely to be indicators of speciation.

All morphological characters, except the terminal apophysis, revealed a high degree of intraspecific variation. The variation and sharing of character shape among the examined characters precluded the identification of reliable diagnostic characters for female and male specimens alike. In accordance with Dondale and

Redner (1986), only the shape of the terminal apophysis supports the current morphological species limits.

The maximum pairwise divergence between the two species (1.2%) is lower than intraspecific divergence calculated for this gene region in other spider species (e.g. 2.06% for the *Pardosa lapidicina* species-group, Correa-Ramírez *et al.* 2010), as well as below Barrett and Hebert's (2005) estimated barcode gap of 2% for spiders. The lack of a barcode gap suggests the Nearctic *P. lapponica* group may constitute a single species.

Robinson *et al.* (2009) cast some doubt on the application of the barcode region to spider delimitation: they found a barcode gap in less than 90% of the monophyletic morphospecies used. Some of the species included, however, are taxonomically uncertain, such as *Pardosa groenlandica* (Thorell), and this could skew the results (Robinson *et al.* 2009). Robinson *et al.* (2009) proposed potential explanations for the irregular barcodes found, but the anomalous results were not investigated beyond speculation. It would be premature to conclude that the barcode gap is lacking in many spider groups based on the unresolved results of Robinson *et al.* (2009) alone.

An additional challenge to the use of the COI barcode region in spiders can come from BOLD, which often shows shared haplotypes and a lack of barcode gap among species (Ratnasingham & Hebert 2007). However, Slowik and Blagoev (2012) identified errors that can occur in the BOLD database, as the results do not undergo review prior to being uploaded. Slowik and Blagoev (2012) demonstrated that the barcode region can be effective when appropriately critiqued. When records of *Pardosa* species on the BOLD system were reviewed, we found similar errors as Slowik and Blagoev (2012), such as the inclusion of juvenile specimens (e.g. juvenile *P. podhorskii* causing paraphyly with *P. furcifera*) and potentially mislabeled specimens (e.g. one specimen from Russia labeled as *P. falcifera*).

The removal of errors did not result in a barcode gap among all species, which means the divergence among sequences should not be the only statistic reported. In cases where no barcode gap exists, but separate species are suspected,

fixed nucleotide substitutions have been used to show diagnostic differences between species (Hendrich *et al.* 2010). Interspecific variation between the Australian Dytiscidae (Coleoptera) species *Neobidessodes samkrisi* (Hendrich & Balke) and *N. flavosignatus* (Zimmermann) was as low as 0.85% (Hendrich *et al.* 2010). Despite the low interspecific variation, there were five fixed nucleotide substitutions that Hendrich *et al.* (2010) used as diagnostic characters.

Examination of the barcodes of *Pardosa* species for fixed nucleotide differences appears to agree with what was found in the beetles. Species-specific substitutions were present among morphologically distinct species that did not conform to the 2% barcode gap (Table 2). The Palearctic *P. lapponica* specimens appeared to be divergent from both the *P. concinna* and Nearctric *P. lapponica* specimens in both pairwise distance and fixed substitutions (Table 2). Neither a barcode gap nor fixed substitutions were observed between the Nearctic *P. lapponica* and *P. concinna* specimens.

The lack of divergence between the Nearctic specimens is evident in the close relationship among haplotypes from the two species, as well as the lack of species-specific clustering in the haplotype network that would be expected if the species were diversifying separately (Řezáč *et al.* 2008; Walker & Avise 1998). Shared haplotypes can be a sign of introgression between separate species, but the pattern of haplotype distribution does not support multiple species experiencing introgression. The shared haplotypes are found at multiple localities that are not in a suture zone between the ranges of the two species (Avise 2000) and are not in contrast with pronounced species-specific clustering (Lu *et al.* 2001).

The genetic divergence between Palearctic and Nearctic *P. lapponica* specimens, as well as the potential size difference, calls into question the synonymization of the Nearctic *P. harperi* with the Palearctic *P. lapponica*. Considering the low morphological and genetic divergence between the Nearctic "*P. lapponica*" and *P. concinna* specimens, there may be justification for synonymizing *P. harperi* with *P. concinna*, although genetic study of specimens from the type locality of both species is recommended. The *P. lapponica* species-group would benefit from taxonomic revision using morphology and molecular

characters of all species. There may be undocumented morphological variations between the Palearctic and Nearctic "*P. lapponica*" specimens that would further support a species split. Examination of *P. concinna* specimens from the southern parts of its Nearctic range would determine whether these populations are conspecific with those in northern North America.

**4.6 Acknowledgements**

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**Table 4.1.** Coordinates, number and haplotypes of *Pardosa lapponica* and *P. concinna* specimens used in the study li. Collection localities are followed, in parentheses, by standard province/state abbreviations, site code, and repository for specimens. The geographic midpoint is given for localities where specimens were collected from multiple replicates. Number of specimens from each locality used in COI analysis and morphological analyses (female and male) are shown. The haplotypes found in each location follow the COI sample size in parentheses and correspond to haplotype numbers in Fig. 3.

| **Locality** | **Coordinates (latitude N, longitude W)** | **Specimens** | | |
|---|---|---|---|---|
| | | **COI** | **F** | **M** |
| ***Pardosa lapponica*** | | | | |
| Dempster Hwy km 82, YT (Tm, LEM) | 64.596, -138.312 | 5 (1,2) | 10 | 10 |
| Toolik Field Station, AK (To, UAF) | 68.629, -149.598 | 4 (6,11,12,13) | 3 | 5 |
| Kugluktuk, NU (Ku, LEM) | 67.817, -115.233 | 5 (3,4,5,6) | 11 | 10 |
| Churchill, MB (Ch, LEM) | 58.731, -93.804 | 5 (3) | 10 | 6 |
| ***Pardosa concinna*** | | | | |
| Norman Wells, NT (Nw, LEM) | 65.305, -126.709 | 7 (1,7) | 7 | 8 |
| Yellowknife, NT (Yk, LEM) | 65.513, -113.392 | 4 (1,7,8,10) | 8 | 8 |
| Whitehorse, YT (Wh, LEM) | 60.716, -135.055 | 3 (9) | 5 | 4 |
| Goose Bay, NL (Gb, LEM) | 53.320, -60.297 | 2 (7,8) | 1 | 1 |
| Willmore WP, AB (Wi, LEM) | 58.731, -93.804 | 2 (7) | 0 | 2 |
| Fairbanks NP, AK (Fa, UAF) | 63.987, -145.316 | 4 (1,2,14,15) | 0 | 6 |
| North Battleford, SK (SK, DJB) | 52.767, -108.283 | 0 | 3 | 3 |
| **TOTAL** | | 41 | 58 | 63 |

**Table 4.2**. Estimates of average evolutionary divergence over sequence pairs within and between species. The mean pairwise divergence is shown below the diagonal, with the range in parentheses. Intraspecific divergences are on the diagonal and above are the fixed nucleotide substitution. Analyses were conducted using the Tamura-Nei model with 0.15 gamma correction (Tamura & Nei 1993). Codon positions 1st+2nd+3rd+Noncoding were included. There were a total of 609 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.* 2011).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1. *P. caucasica* | 0.3 (0.2–0.5) | 9 | >10 | >10 | >10 | >10 | >10 |
| 2. *P. koponeni* | 2.1 (1.6–2.5) | 0.1 (0.0–0.2) | >10 | >10 | >10 | >10 | >10 |
| 3. Nearctic *P. lapponica* | 13.6 (11.9–15.6) | 13.6 (12.5–15.1) | 0.4 (0.0–0.9) | 0 | 6 | >10 | >10 |
| 4. Nearctic *P. concinna* | 12.8 (10.9–15.5) | 13.5 (12.4–14.9) | 0.7 (0.0–1.2) | 0.7 (0.0–1.4) | 6 | >10 | >10 |
| 5. Palearctic *P. lapponica* | 8.6 (6.6–11.4) | 9.7 (8.2–12.5) | 3.0 (1.9–4.1) | 3.1 (2.0–3.9) | 0.6 (0.0–1.2) | >10 | >10 |
| 6. *P. moesta* | 12.8 (11.3–14.6) | 12.7 (11.4–14.0) | 15.3 (13.6–16.8) | 14.5 (12.4–16.8) | 10.7 (9.0–12.1) | 0.6 (0.0–1.5) | 5 |
| 7. *P. adustella* | 10.7 (8.8–11.9) | 9.3 (8.4–10.2) | 10.5 (9.1–11.8) | 10.1 (8.2–12.0) | 8.8 (6.9–10.1) | 2.6 (1.4–3.3 | 0.4 (0.0–0.9) |

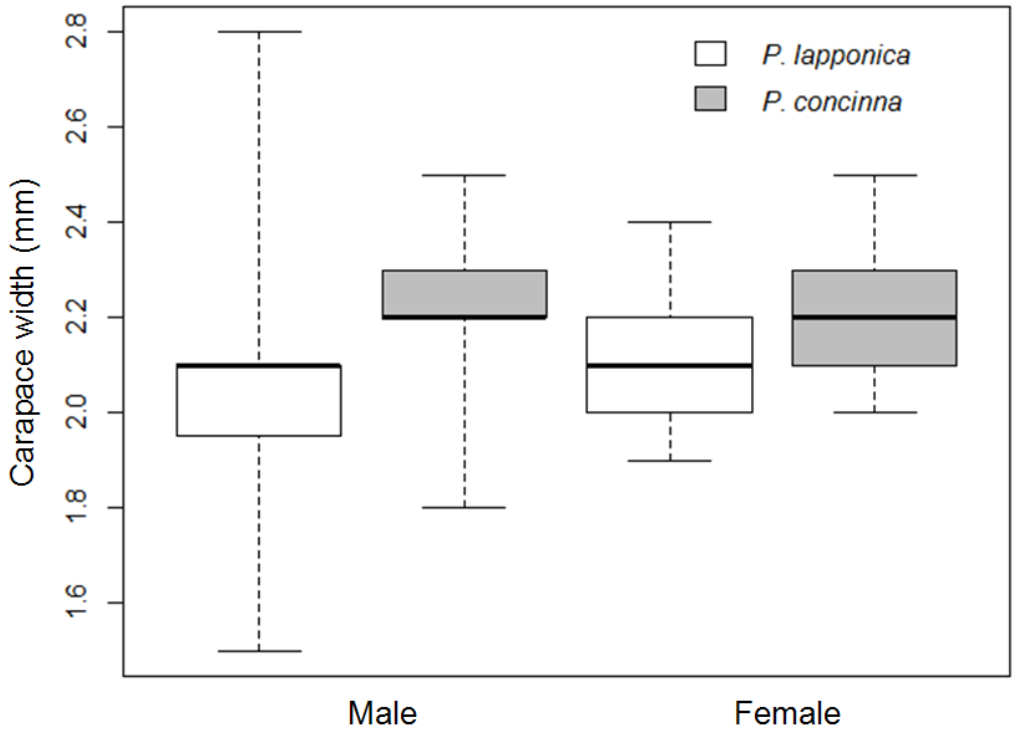


**Figure 4.1**. Box plots (heavy line = median; box = quartiles; dashed line = range) of carapace width in males and females of *Pardosa lapponica* and *P. concinna*. The range of body size overlaps between the two species, with the mean body size significantly larger in *P. concinna* (grey boxes).

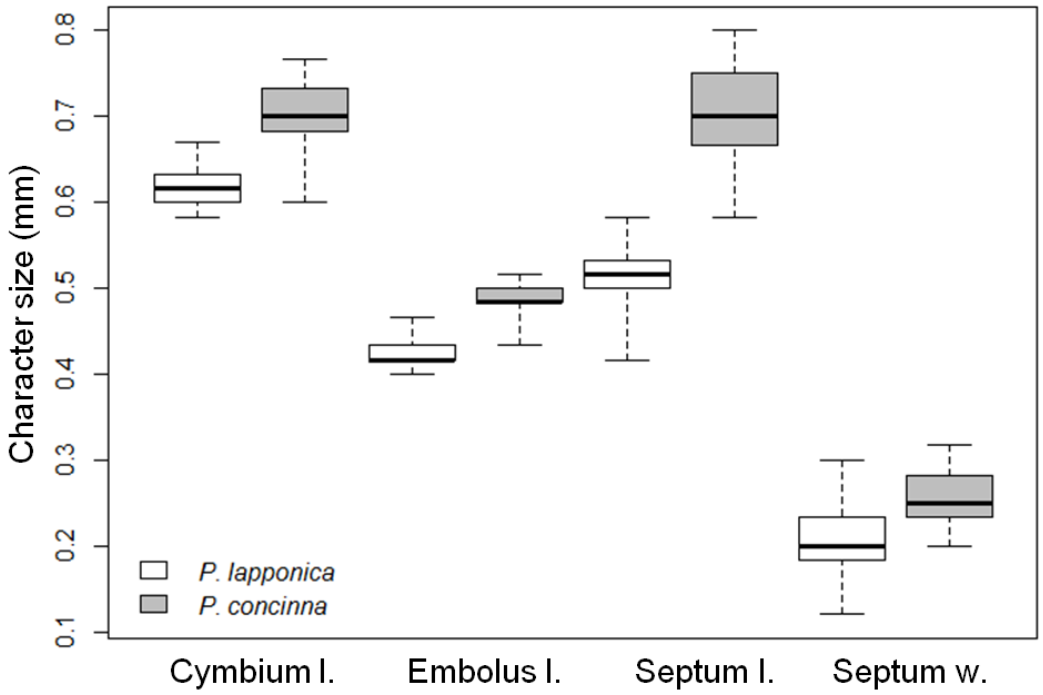


**Figure 4.2**. Box plots (heavy line = median; box = quartiles; dashed line = range) of genitalic characters of *Pardosa lapponica* and *P. concinna*. The mean size of all characters is significantly larger in *P. concinna* (grey boxes), but the size range overlaps between the two species. l. – length; M. – median; w. – width.

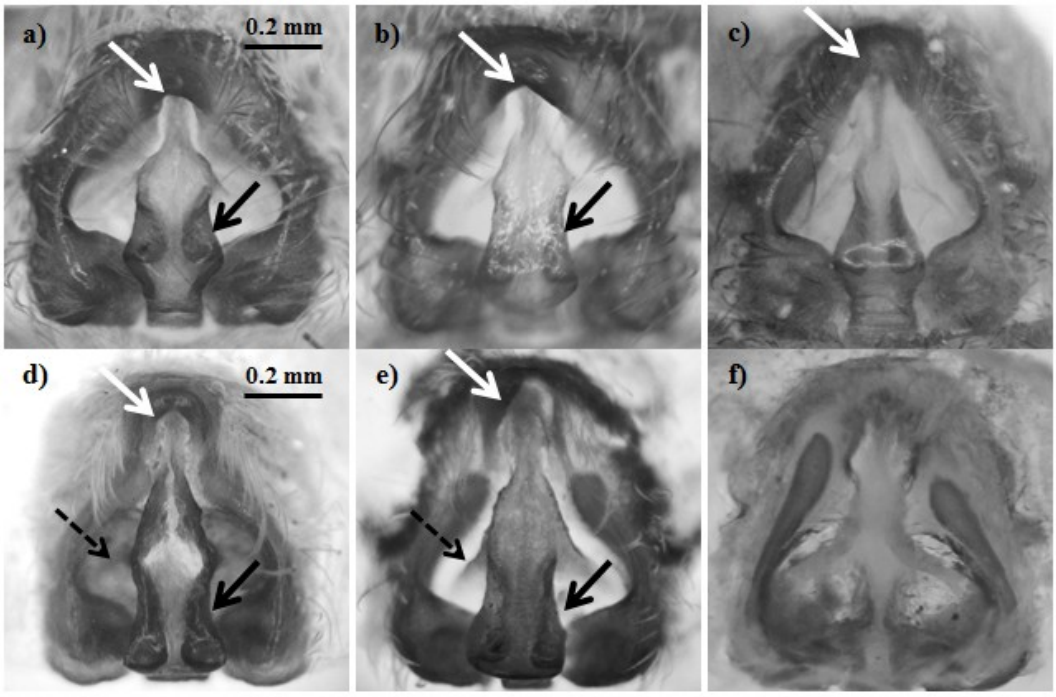


**Figure 4.3**. Variation within the epigynes of female *Pardosa lapponica* and *P. concinna* specimens: a) *P. lapponica* specimen from Tm, hood low and short septal ridge; b) *P. lapponica* from Tm, hood low and septal pocket irregular; c) *P. lapponica* from Ch, hood malformed and septal ridge completely exposed; d) *P. concinna* from SK, hood high and septal pocket smooth; e) *P. concinna* from Yk, hood malformed with exposed septal ridge; and f) *P. lapponica* from Ch, straight and curved spermathecae. White arrows indicate the hood. The black arrows indicate the septal pockets. The dashed arrows indicate the copulatory tubes, curved in d) and straight in e).

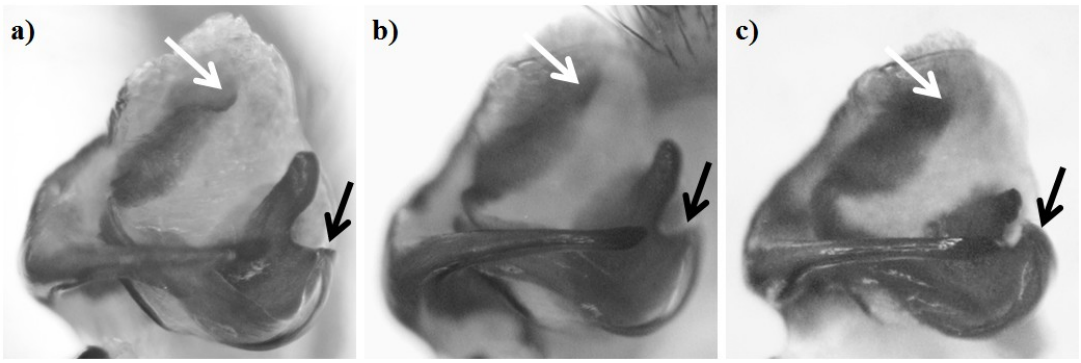


**Figure 4.4**. Apical division of the male pedipalp from: a) *Pardosa concinna* from Yk; b) *P. concinna* from Yk; and c) *P. lapponica* from Tm. The white arrow indicates the position of the protrusion on the palea, present in a) and absent in b) and c). Black arrows indicate the conductor, protruding past the lower limit of the terminal apophysis in c). The conserved shape of the embolus can be seen in b) and c). Interspecific variation in the terminal apophysis can be seen between a), b) and c).

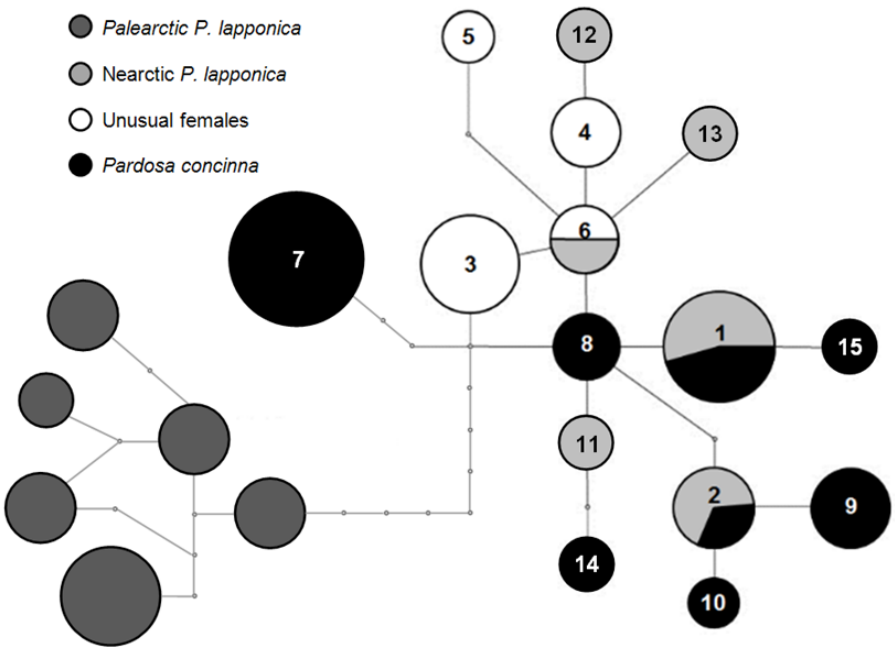


**Figure 4.5**. Statistical parsimony haplotype network of variation in the COI gene region of the *Pardosa lapponica* species-group. Haplotypes are represented by circles with 1 bp change represented by the connecting lines. Nodes on connecting lines indicate intermediate mutational steps that were not found. Loops in the network indicate ambiguities where the mutational path is unclear. The size of the circle corresponds to the frequency of the haplotype. Haplotype numbers found in each locality are listed in Table 1.

**Appendix 4.1.**

Collection of arthropods for the NBP follows that laid out in the general collection methods in Appendix 3.1. Initial identification of specimens was done prior to comparison to those at the CNC. Following examination of *P. lapponica* group specimens at the CNC, specimens were re-identified based solely on the TA and range. When possible, 5 specimens from each site were selected and sequenced.

**Appendix 4.2.**

Specimen codes for the BOLD sequences.

| Species | Database ID |
|---|---|
| *P. lapponica* | SPIRU048-10 |
| | SPIRU049-10 |
| | SPIRU051-10 - 054-10 |
| | SPIRU076-10 |
| | SPIRU077-10 |
| | SPIRU1664-12 |
| | SPIRU1650-12 |
| | SPIRU2200-12 |
| | SPIRU2202-12 |
| | SPIRU2203-12 |
| | SPIRU2238-12 |
| | SPIRU2239-12 |
| *P. caucasica* | SPIEU1341-11 – 1344-11 |
| *P. koponeni* | SPRMA1008-12 –1010-12 |
| | SPRMA1012-12 |
| *P. moesta* | SPICA715-10 |
| | SPICA722-10 |
| | SPICA737-10 |
| | SPICA740-10 |
| | SPICA746-10 |
| | SPICH074-09 |
| | SPICH1169-09 – 1171-09 |
| | SPICH191-09 – 194-09 |
| *P. adustella* | SPIRU2215-12 – 2223-12 |
| *P. podhorskii* | TWSC074-07 |
| | TWSC085-07 |
| *P. furcifera* | TWSC073-07 |
| | TWSC076-07 |
| | TWSC086-07 |
| | TWSC172-08 |
| *P. falcifera* | SPIRU1559-12 |

## 5. Summary, conclusions and future directions

Phylogeographic studies on Arctic arthropods are limited despite their ecological importance. The lack of focus on arthropod phylogeography means we have very limited knowledge on how arthropods may react to future climate change. This is problematic because arthropods may be among the taxa to respond most strongly and rapidly to climate change. Placing more focus on taxa within Arthropoda will allow general biogeographic patterns to be predicted for the Arctic.

Wolf spiders are ecologically important as predators and prey, and they have a high capacity for dispersal, which makes them a model study taxon for arctic biogeography. The arthropod collections made by the Northern Biodiversity Program (NBP) were geographically widespread and had an abundance of wolf spiders, allowing a unique opportunity to perform the first phylogeographic study on a Nearctic Arctic wolf spider, *Pardosa glacialis*. A genetic analysis on this species, using three gene regions, allowed us insight into the glacial history and contemporary population structure of *P. glacialis*.

Our results showed that *P. glacialis* inhabited multiple glacial refugia during the last glacial maximum (LGM). Populations within the major two refugia were isolated from each other, allowing genetic divergence to occur. Despite the population fragmentation, speciation did not occur and these populations are now experiencing secondary contact in sites across central high Arctic Canada. As this species appeared to have a range increase during glacial periods, indicating the cold climate favoured the preferred habitat of this *P. glacialis*, warming in the Arctic may be detrimental to this species.

Molecular analyses can provide insight not only into the phylogeography of wolf spiders, but can also aid in taxonomic revisions. *Pardosa* is a taxonomically challenging genus. The high degree of morphological conservation in this genus makes species delimitation a challenge. Many species in this genus have been based on variation in only a few morphological characters and are, therefore, contentious. However, the inclusion of genetic characters in taxonomic studies can provide additional support for species boundaries.

Chapter 4 explored the limited variation between the two species of the *P. lapponica* species-group that occur in the Nearctic. Results from a morphometric and genetic analysis suggested that Nearctic *P. lapponica* and *P. concinna* are not separate species. In addition, genetic divergence between the Palearctic and Nearctic *P. lapponica* specimens indicated that multiple species are currently lumped into *P. lapponica*. The assumption that many wolf spiders have intercontinental ranges may be incorrect and requires genetic and morphological review. This species-group, like many others in *Pardosa*, would benefit from a comprehensive taxonomic revision that uses genetic characters.

Future directions to be pursued in molecular phylogeography and taxonomy studies of the genus *Pardosa* include:

- The collection of specimens from the western islands of the Canadian Arctic Archipelago as multiple cryptic refugia on these islands have been proposed. Genetic analyses of specimens from these areas will determine if island refugia played a part in shaping the population structure of *P. glacialis* and other *Pardosa* species.
- Phylogeographic studies focusing on other *Pardosa* species from the high Arctic and boreal zones to determine if their glacial histories are similar, allowing inference on the biogeographic history of wolf spiders in the northern Nearctic.
- The continued inclusion of genetic characters alongside morphological characters in revisions of taxonomically difficult *Pardosa* species-groups, especially those that use single, morphological character variations.

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