BIODIVERSITY OF THE MUSCIDAE (DIPTERA) FROM CHURCHILL, MANITOBA, CANADA, WITH TAXONOMIC ISSUES REVEALED OR RESOLVED BY DNA BARCODING

by

Anaïs Krystel Renaud

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ABSTRACT

A 2007 survey of muscid flies from Churchill, Manitoba, Canada, yielded 155 species. The diversity of this contemporary assemblage was compared to that of an historical (pre-1965) assemblage. Few differences were found between assemblages for material collected by net sweeping and most non-overlapping species between time periods were rare in samples and/or collected by different methods. DNA barcoding was used as a tool to assist with the identification of Muscidae specimens. Performance of the cytochrome *c* oxidase subunit 1 gene (COI) in the discrimination of muscid species was assessed and correspondence levels of 98.6% were established between species limits recovered by DNA barcode clusters and morphology. I conclude that the great majority of species limits currently accepted in the literature are adequate for Muscidae and that DNA barcoding is a useful identification tool for this family.

Key words: insects, low Arctic, species assemblages, collecting techniques, Folmer region.

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CHAPTER 1

GENERAL INTRODUCTION

The last thirty years have been a period of rapid environmental changes (Intergovernmental Panel on Climate Change (IPCC) 2007). The consequences of climate change, pollution, and anthropogenic habitat modifications can be perceived in most ecoregions of the planet (Everett and Fitzharris 1998). In the Arctic, these processes modify the conditions of habitats and affect the resilience of organisms (Arctic Climate Impact Assessment (ACIA) 2004), possibly contributing to changes in species activities. Like most organisms, insects are greatly influenced by biotic and abiotic elements, and are highly susceptible to changes occurring in their environment. However, since little is known about insect species assemblages and their distribution in the north, their response to climate and habitat changes is hard to predict. This is ironic, as insects form by far the most speciose and often most abundant group of terrestrial and freshwater animals in the arctic and worldwide.

The difficulty to access most northern areas and the high cost to conduct research in the north have probably contributed to insects having generally been poorly studied in northern Canada. However, the area of the town of Churchill and its surroundings (within a 25-km radius) on the Manitoban coast of the Hudson Bay has often been sampled by entomologists in the twentieth century, mostly because of its accessibility and infrastructures. True flies (order Diptera), and especially those in the family Muscidae, a group whose relative diversity and abundance increase with latitude when compared to other insects, have frequently been collected in the area. The results from various collecting expeditions in Churchill and other northern localities up to 1965 have been

studied by H. C. Huckett and summarized in his influential 1965 work on the Muscidae from Northern Canada (including Churchill), Alaska and Greenland (Huckett 1965a). In this era of environmental changes, baseline data including large numbers of species such as those compiled by Huckett (1965a, b) are invaluable to monitor insect communities and detect changes over time (White and Kerr 2006).

As muscid flies have not been inventoried in the area of Churchill since 1965, the first objective of this work was to conduct an extensive contemporary inventory of the muscid flies of Churchill using a standardized sampling design and compare the performance of different collecting methods commonly used to collect Diptera (Malaise traps, pan traps and sweep netting). Since muscid flies are abundant, speciose, and historically well documented for the area of Churchill, the second objective was to compare historical and contemporary data sets to determine if the species richness and composition of Muscidae have changed over the last 42 years.

In muscid flies, as in many other insects, species identification can be difficult and species limits are occasionally ambiguous. Since species identification and delimitation is often more accurate when based on multiple sources of character data, the third objective of this work was to investigate the relevance of DNA barcoding as a taxonomic tool to assist in muscid fly identification.

In the following literature review, I shall cover material relevant to the context of this project before addressing key notions pertinent to one or more of the general objectives. Hypotheses and predictions related to the first two general objectives will be presented in Chapter 3 of this thesis; those related to the third will be found in Chapter 4.

CHAPTER 2

LITERATURE REVIEW

Our planet sustains an incredible variety of life forms, and the importance of maintaining biodiversity is largely recognized (Gaston and Spicer 2004; Secretariat of the Convention on Biological Diversity 2005). In spite of the increasing number of initiatives undertaken to preserve natural habitats, global, regional and local biodiversities are changing at an unprecedented rate following habitat fragmentation and climate change (Sax and Gaines 2003; Travis 2003). In the last few decades, the phenomenon has been magnified, especially in some fragile ecoregions such as the Arctic where ecosystems are susceptible to becoming less resilient as they face even minor disturbances (Peters 1992). Little is known about how animals, especially small ones such as insects, are responding to environmental changes in northern areas, in part due to the lack of research infrastructure in the north as well as the high costs associated with traveling to the Arctic.

THE ARCTIC AND SUBARCTIC ECOREGIONS

The Arctic is a vast ecoregion of the Northern Hemisphere encircling the Arctic Ocean. There are several definitions of the Arctic, and areas encompassed in the Arctic vary between physical-geographical (UNEP/GRID-Arendal 2005) and political boundaries (Conservation of Arctic Flora and Fauna (CAFF) 2001). Physical-geographical characteristics used to define the limits of the Arctic include the 10 ºC July isotherm, treeline limit and continuous permafrost, defined as ground that remains continuously frozen (Muller 1945) (Arctic Monitoring Programme (AMAP) 1998). The Arctic is usually divided into three sub-regions: high Arctic (northern islands of the

Arctic Archipelago), middle Arctic (middle Arctic is not considered by all authors) and low Arctic (coastal plains) (Woodward 2003; UNEP/GRID-Arendal 2005) (Figure 2.1). The Subarctic (upland areas) is the region south of the Arctic that also refers to the Taiga. Its southern limit corresponds with the discontinuous permafrost (AMAP 1998).

Hudson Bay Lowlands

In Manitoba, the Hudson Bay Lowlands form a 160 km band of flat coastal plains making the transition between the boreal forest and the arctic tundra (Piercey-Normore 2005). Its recognition as being part of the Arctic or the Subarctic varies among authors. The heterogeneous landscape, a mosaic mainly consisting of uplands, bogs and fens with irregular tree patches (Brook 2001), complicates the position of the boundary. Ponds and lakes are numerous and widely distributed. Plant communities in the Hudson Bay Lowlands are in constant change as a result of isostatic uplift occurring since the Wisconsin glacial retreat (Brook 2001) and the presence of continuous permafrost is a feature of the northern part of the region (Dredge 1992).

Churchill

The town of Churchill (58°46´08N; 94°10´20W) is located on the coastal plains of the Hudson Bay Lowlands, approximately 1500 km north of Winnipeg. The area is at the meeting point of three biomes: boreal forest, tundra and marine, and is thus a transition zone between the boreal forest to the south and the arctic tundra to the north (Brook 2001; Piercey-Normore 2005). Churchill is considered as Subarctic when physical-geographical characteristics such as the vegetation and climate are examined

(CAFF 2001), but is considered low Arctic when physical-geographical and political characteristic are associated to delimit the boundaries of the Arctic and of the Subarctic (UNEP/GRID-Arendal 2005) (Figure 2.1). Here, Churchill will be considered low Arctic to follow the designation of the United Nations Environment Programme (UNEP) (UNEP/GRID-Arendal 2005) even if in reality Churchill would be at the transiant zone between low Arctic and Subarctic. As previously mentioned, the climate and vegetation is not homogeneous and it is practically impossible to label Churchill with one designation.

In 2006, the town of Churchill had a population of 923 inhabitants (Statistics Canada 2006) of which half are Cree, Inuit and Dene. The population has declined since United States Armed Forces left the territory in the 1960s. Easily accessible by train, plane and ship, Churchill is the travel destination for many naturalists and tourists in quest of northern landscapes and wildlife sightseeing and a research station that provides facilities to scientists and students. Since the railroad linked the town to Winnipeg in 1929, Churchill and its area have experienced a large range of development including shipping, military, tourism, research, and the impact of hydroelectric development (Newton *et al*. 2002) (discussed in more detail below).

ENVIRONMENTAL CHANGES IN THE NORTH: A FOCUS ON CHURCHILL (MB)

Arctic and subarctic climate systems have worldwide influences, and minor changes can have extended repercussions on global ecology, health and economy. The lands and oceans of northern regions contain a variety of natural and biodiversity resources exploited by many nations. The 20th century was a period of rapid

environmental changes due, at least in part, to anthropogenic activities (IPCC 2007). More than any other ecoregion, the Arctic and Subarctic are particularly vulnerable to contamination, pollution, development and climate change (Everett and Fitzharris 1998). These processes modify the conditions of habitats and affect the resilience of organisms (ACIA 2004).

Over the last fifty years, atmospheric temperatures in the Arctic have risen twice as fast as in other regions of the planet (ACIA 2004; Stendel *et al.* 2008). Increased temperatures were recorded at various circumpolar sites between the 56th and 79th parallels of latitude in Québec, Northwest Territories, Newfoundland and Labrador, Russia and Asia (Jacoby and Darrigo 1989; Darrigo and Jacoby 1993; Overpeck *et al*. 1997; Aanes *et al*. 2002). Following trends reported at these other northern localities, the temperature in Churchill (MB) has also recently changed, with an increase in average annual temperature of 1.78 °C between 1979 and 2007 (Ballantyne 2009, compiled from http://climate.weatheroffice.ec.gc.ca/climateData/canada_e.html).

Environmental impacts of human activities in Churchill

Throughout the 20th century and the beginning of the 21st century, Churchill experienced anthropogenic development and activities that modified the landscape and may have had significant environmental impacts on natural habitats (Newton *et al*. 2002; Edye-Rowntree *et al*. 2006). From 1942 to 1965, the United States Armed Forces were stationed in Fort Churchill and the population peaked at 7000 inhabitants in 1965 (Newton *et al*. 2002). It rapidly decreased afterwards with fewer than 2000 inhabitants remaining in 1971 and no more than 923 in 2006 (not counting seasonal tourists)

(Statistics Canada 2006). The Rocket Research Range (rocket launching complex), an initiative of the Canadian Army, started its activity in 1957 with the construction of an imposing cluster of buildings. In 1959, the site was reopened by the United States Army until 1970 and then used by the Canadian National Research Council until it was abandoned at the beginning of the 1990's. The US Air Force, through military activity and the Rocket Research Range have left behind many unoccupied buildings and dumps that are now contaminating the surrounding wildlife with asbestos fibres and PCB's, among other types of contaminating debris (Newton *et al*. 2002).

In 1976, the Churchill River was diverted to increase the hydroelectric potential of the Nelson River, significantly decreasing the level of the water in the area of Churchill (Edye-Rowntree *et al*. 2006). In 1998, a weir was built approximately 13 kilometres from the mouth of the Churchill River in an effort to return water levels to their pre-diversion state (Edye-Rowntree *et al*. 2006). The diversion of the Churchill River and the subsequent weir have significantly altered water levels and consequently modified the extent of both aquatic and terrestrial habitats.

Churchill has the only deep-sea port of the Canadian Arctic (built in the 1920s) (Greg 2008) and is also one of the only northern localities to be accessible by train, since the inauguration of its railroad in 1929. Passenger and freight trains depart from Winnipeg (MB). Freight trains transport grains from the prairies to be exported to Europe, Russia, South America and Africa from the port in Churchill; and groceries and fuel to communities along the Hudson Bay coast (Newton *et al*. 2002; Greg 2008). Sulphur has also been transported by trains and piled on the dock to be shipped by cargo ships which can contaminate habitats surrounding the dock (T. Galloway (pers. comm.)).

Recently, Canada also started to import merchandise from Russia through Churchill (Greg 2008). According to Kim and McPherson (1993), the North American insect fauna would include more than 2000 immigrant species, but to date no data on introduced species have been compiled for Churchill. Since the area has been receiving agricultural products from the Prairies and, very recently, from Russia, it may be susceptible to alien species introduction, a potential threat to local biodiversity (Wilcove *et al*. 1998; ACIA 2004). Climate change might potentially have a direct influence on the density of traffic at the Churchill sea port as a recent trend towards increasingly thin ice conditions (Falkingham *et al*. 2002; Perovich *et al*. 2010) could eventually result in the opening of the northwestern passage for shipping (Falkingham *et al*. 2002).

Wildlife tourism is a strong and important activity in Churchill, where people mainly go to watch wildlife and the Northern Lights. In 1999, approximately 10,000 tourists visited the area (Newton *et al*. 2002). Among the many activities offered in the region are of polar bear observation for which tourists are transported in tundra vehicles. These vehicles, when driven off the trails, damage the tundra by compacting vegetation and altering soil drainage. Changes in soil configuration and vegetation communities resulting from tundra vehicles have been noted in the Churchill Wildlife Management Area (CWMA) (Newton *et al*. 2002).

INSECTS OF CHURCHILL IN A CONTEXT OF ENVIRONMENTAL CHANGE

Because of its strategic position, Churchill encompasses a variety of habitats that are likely to support a highly diverse insect fauna for a northern region. Churchill's insect fauna, like that of other northern regions, is probably characterized by a few abundant

species and many, but rare, species. However, only a handful of insect taxa have been intensively inventoried and assessed for their diversity patterns in the area. Among the better studied groups are the families Braconidae (Hymenoptera) (Smith *et al*. 2009; Fernández-Triana *et al*. 2011), Culicidae (Diptera) (Haufe and Burgess 1956) and Muscidae (Diptera) (Huckett 1965a).

In cold northern climates, insects have developed physical, behavioural and phenological adaptations in order to survive and exploit their environment. Populations, like communities, are dynamic groups fluctuating along with the condition of their environment. In general, insects, like all animals, are greatly dependent on environmental conditions such as climate, resource availability, integrity of habitat and competition. Climatic factors that influence insect biology are mostly temperature, light (photoperiod), wind, precipitation and humidity (Danks 1981; Bale *et al*. 2002). Among these, temperature usually has the strongest influence on insect physiology, activity, phenology, abundance and distribution (Lewis and Taylor 1965; McAlpine 1979; Danks 1981, 1992; Lee 1991; Roy *et al.* 2001; Bale 2002; Bale *et al*. 2002).

In the actual context of environmental change, it is likely that insects will respond to changes in their surroundings in order to adapt. Responses have already been measured in a number of studies (see below), but causality often remains difficult to establish.

Responses to climate change

Over 500 insect taxa have been studied for their potential response to climate change in the northern hemisphere. Most of those taxa are butterflies from Europe, mostly due to the amount and availability of historical distribution records. Responses of

these insects (mostly Lepidoptera) include: an expansion and/or shift of distribution range (Pollard and Yates 1993; Parmesan 1996, 2006; Hill *et al*. 1999; Parmesan *et al*. 1999; Hickling *et al*. 2006; Wilson *et al*. 2007; Taylor and Westwood 2008; Pöyry *et al*. 2009); an increase of population size (Pollard *et al*. 1995; Roy *et al*. 2001; Goulson *et al*. 2005; Taylor *et al*. 2007); a change in species richness (increase or decrease, including species extirpation) (Parmesan 1996; Rodriguez-Trelles and Rodriguez 1998; Grixti and Packer 2006; Menéndez *et al.* 2006; White and Kerr 2007; Wilson *et al*. 2007); and a shift or extension of period of aestival activities (shift of the active season toward an early spring and/or extension of flight period) (Taylor and Westwood 2008; Westwood and Blair 2010; Høye *et al*. 2007).

In Canada, the actual influence of climate warming on insect diversity and distribution has been examined in relatively few studies (Grixti and Packer 2006; White and Kerr 2007; Taylor and Westwood 2008; Westwood and Blair 2010), and only Fernández-Triana *et al*. (2011) have investigated recent diversity changes in a northern insect community (Churchill).

Responses to habitat modifications

The composition of plant cover influences the composition of insect species in a habitat. Insects interact with vegetation directly, as herbivores or indirectly as decomposers of plant products (saprophages). Plants can also serve as mating, breeding, protection, thermoregulation and predation sites (Danks 1981). Permanent and temporary bodies of water can also be of great importance for aquatic, semi-aquatic and terrestrial species preying on aquatic insects. In Churchill, observations regarding association of

insect species to a habitat or an element of a habitat can be found in McClure (1937, 1943) and Webb (1956).

The modification of natural habitats can have significant impacts, negative or positive, on the diversity and abundance of the local fauna. The intensification of land-use by humans is usually correlated with an increase in habitat degradation and the impacts of habitat degradation on insect diversity and community composition can be severe, occasionally leading to species extirpation (Samways *et al*. 2010). On the other hand, the diversity and abundance of insects can increase in some types of fragmented habitats (Niemelä 1997) and in certain habitats where anthropogenic activities take place such as sanitary landfill sites (Goulson *et al*. 1999).

The nature and intensity of human activities in Churchill have varied over the years. With the army occupation, the research station, the shipping of grains, and tourism, there is no doubt that some habitats are no longer intact in Churchill (see above in Environmental changes in the north: a focus on Churchill (MB)), but whether this has had an impact on its insect communities is still unknown.

MUSCIDAE

The family Muscidae is among the better studied insect families in the northern Nearctic region, including Churchill. Huckett (1965a) compiled all available data on the muscid fauna of northern Canada, Greenland and Alaska, therefore establishing a baseline data set that can be compared to a modern inventory. In addition to the availability of historical data, Muscidae were selected for this study because of their

taxonomic prevalence in northern areas, their diverse ecological habits, and their impacts on human health and economy (Vockeroth 2002).

Muscidae is a large cosmopolitan family of calyptrate Diptera containing more than 5100 species in 170 genera (Kutty *et al*. 2008; Foottit and Adler 2009). The taxonomic limits of the group have been modified several times in the past, with the Anthomyiidae, Scathophagidae and Fanniidae all having been treated as subfamilies of the Muscidae at some point in the past (Huckett 1965a; Huckett and Vockeroth 1987). The Fanniidae was most recently removed from the Muscidae (Roback 1951) and the monophyly of the Muscidae *sensu stricto* is currently well supported by both molecular and morphological data (Kutty *et al*. 2008). For further details on the systematics of the Muscidae, the following influential contributions can be consulted (Verral 1891; Girschner 1894; Pandellé 1898, 1904; Stein 1916, 1919; Karl 1928; Séguy 1937; Roback 1951; Herting 1957; Hennig 1965; Griffiths 1972; Skidmore 1985; Carvalho 1989; McAlpine 1989; Michelsen 1991; Couri and Pont 2000; Savage and Wheeler 2004; Kutty *et al*. 2008).

In the Nearctic Region, 49 genera and over 700 species belong to the Muscidae (Huckett and Vockeroth 1987) and it is considered the second largest family of calyptrate Diptera in Canada after tachinid flies (McAlpine 1979; J.E. O'Hara (pers. comm.)). Based on data compiled by Danks (1981), muscids are the most diverse Diptera family in arctic habitats of Canada and Alaska, where their 147 recorded species represent more than 10% of northern Nearctic insect species. Some genera are especially speciose in the Arctic where *Spilogona* Schnabl, for example, includes more than 40% of the northern

Nearctic muscid species (Danks 1981, 1992; Elberling and Olesen 1999; Larson *et al*. 2001).

Huckett was the major contributor to the taxonomy of Nearctic Muscidae (see Huckett 1932, 1934a, b, 1936, 1954; Huckett and Vockeroth 1987), but other entomologists have also greatly contributed to the taxonomy of Muscidae. Hennig (1955-1964) described and illustrated many species of muscids, mostly with a Palaearctic distribution. Even if Palaearctic species were the focus of Hennig's attention, his work is of great importance for taxonomists working on Muscidae. Other entomologists have also published dichotomous keys to species of various genera of Muscidae (Snow 1891; Malloch 1918, 1919, 1920, 1923, 1935; Collin 1930; Snyder 1940, 1949a, b, 1954; Arntfield 1975; Savage 2003; Michelsen 2006). At the generic level, the key of Huckett and Vockeroth (1987) is the major reference.

Because of their notable importance and ecological richness, reviewing all aspects of muscid biology would be a lengthy job. Therefore, only aspects most relevant to northern habitats will be covered in the following section.

Ecology and natural history

Muscids, like all other Diptera, are holometabolous insects that have four major life stages: egg, larva, pupa and adult. The biology of muscid flies is especially varied (Courtney *et al*. 2009) and spans almost the entire spectrum of ecological habits found in Diptera. Immature muscids can mostly be found on/in decaying organic matter (vegetal and animal, including dung), fungi, plant matter (bark, shoots, stems, roots), live vertebrates, water, and insect/bird/mammal nests (Ferrar 1987). In these various media,

the larva can be saprophagous, coprophagous, phytophagous or carnivorous, depending on the species (Skidmore 1985; Ferrar 1987). Adult muscids can be predaceous, saprophagous, coprophagous, haematophagous (species that feed on blood) or anthophilous (species that visit flowers) (Skidmore 1985) and they are often attracted to patches where breeding media, water, food or heat (including sunlight) are present (Danks 1981). Specimens can be collected on a wide array of substrates, hosts and structures such as breeding media, flowers, aquatic/terrestrial vegetation, excrement, human food, vertebrates (as haematophagous or sweat flies), sunlit tree trunks, foliage or rocks.

Muscids can be found in a wide range of terrestrial and aquatic habitats where they contribute to maintainance of ecosystem function. In the north, they are especially recognized for the following

1. **Decomposition of organic material:** Some arctic muscids, such as those in the genera *Helina* R.-D., *Morellia* R.-D., *Musca* Linnaeus, and *Muscina* R.-D., have generalist saprophagous larvae that feed on decomposing organic matter (in fact, *Musca domestica* Linnaeus is omnivorous, while species of the genus *Muscina* are saprophagous and carnivorous depending on their larval stage). Muscid larvae stimulate decomposition mostly by fragmenting the substrate. The transportation of fungi and microorganisms from one substrate to another can also indirectly help the decomposition process (Savage 2002). Dung is also an important substrate for muscid larvae. Some species, such as those from the genera *Lophosceles* Ringdahl, *Mydaea* R.-D., *Stomoxys* Geoffroy and *Haematobia* Le Peletier and Serville feed on dung or on the microorganisms living in it (Skidmore 1985). Others, such as the predaceous

larvae of *Hydrotaea* R.-D., mostly feed on other insect larvae living in excrement but some *Hydrotaea* species can live on a saphrophagous diet and live prey are not obligatory for their development (Skidmore 1985).

2. **Pollination:** In alpine and arctic regions, muscids are the most active anthophilous flies and among the most significant pollinators after syrphid flies (Diptera: Syrphidae) (Larson *et al*. 2001). Adult Muscidae, such as various species of *Drymeia* Meigen and *Thricops* Rondani, visit arctic flowers to feed on nectar (source of energy) and occasioanlly pollen (source of protein) and to thermoregulate (temperature in flower blossoms can be 4-10 ºC higher than outside (Pont 1993)), and to hunt or copulate (Danks 1981).
3. **Source of food for insectivorous vertebrates and invertebrates:** As insects in general, Diptera are an important source of food for fishes, birds, small insectivorous mammals (Scudder 2009), amphibians and Arthropods such as spiders (Rossi and Godoy 2006) and other Diptera (Vockeroth 2002). No information, however, is available on the importance of muscids specifically as a source of food for other northern animals.
4. **Predation and parasitism:** Many genera in the family Muscidae are predaceous, but among the subfamily Coenosiinae all species are active predators as immature and adult (Werner and Pont 2006). Among these are *Spilogona*, *Coenosia* Meigen, *Limnophora* R.-D. and *Lispe* Latreille that mainly feed on other small and delicate flying insects such as chironomids, culicids, simuliids and other muscids (Skidmore 1985; Keiper *et al*. 2002). Some, such as the species *Coenosia attenuata* Stein, are so effective as predators that they are known as biocontrol agents (Couri and Salas

2010). These predaceous species are usually found near water as most of their prey are semi-aquatic species (Werner and Pont 2003, 2006).

Some species can also be offensive to mammals. Haematophagous species, for example, can be irritating when they incessantly try to feed on the blood of their host. Prior to the present study, no haematophagous muscid fly species had been recorded in Churchill.

Medical and economic importance

Muscids, like many other flies, are mostly renowned for their impacts (often negative) on human and animal health as well as their role as important agricultural pests. In the north, muscids are not of great concern for human health and economy. However, with the evidence for climate change, the north could experience a change in insect communities and see the establishment of some vectors of microorganisms pathogenic for humans, including *Stomoxys calcitrans* Linnaeus, *Haematobia irritans* Linnaeus and *Musca domestica*.

Dispersal

Little information is available on the dispersal capacity of muscid flies. Most studies were done in the 1950's-1960's, and only a few pest species, such as the house fly and the stable fly were investigated (Quarterman *et al*. 1954; Schoof and Siverly 1954a, b; Hanec 1956).

In urban and agricultural environments, house flies appear to remain within 1.0-1.5 km from their breeding site, but travel distances of 16 to 32 km have also been

recorded (Stafford 2008). The availability of food, shelter and breeding site(s) are important determinants of their dispersal (Schoof and Siverly 1954b). Wind is another major determinant in the dispersal of flies as it influences the distance and the direction of their flight (Quarterman *et al*. 1954). In northern environments, dispersal of flies is most likely influenced by climate variables, such as temperature and wind, and the scattering of food in the environment (Danks 1981).

Additional information

For the sake of brevity, the detailed ecological requirements of northern Muscidae recorded by Huckett (1965a) from Churchill were summarized in Appendix A. For each genus, the following information is given: adult and larval habitat, feeding habits and special behaviours of adults and immature, thermal preference and/or tolerance, and medical, veterinary, economical and ecological roles.

Additional details on the ecology of the family can be found in McLintock and Depner (1954), McAlpine (1965, 1979), Zumpt (1973), Burger and Anderson (1974), Vockeroth (1979), Danks (1981), Levesque and Burger (1982), Skidmore (1985), Ferrar (1987), Morris and Cloutier (1987), Drummond *et al*. (1989), Campbell and Thomas (1992), Pont (1993), Totland (1993), Thomas and Jespersen (1994), Elberling and Olesen (1999), Larson *et al*. (2001), Gilioli *et al*. (2005), and Zych (2007).

INVENTORIES OF NORTHERN INSECTS

Insects have been collected in various arctic localities in Canada and Alaska since the beginning of the nineteenth century. Specimens of many taxa, including Muscidae,

have been collected by the following groups of people: 1) explorers (Richardson 1825-1827; Ross 1829-1833; Handbury 1899-1902; Johannsen 1913-1918, 1927; Freuchen 1921-1924; Ramsmussen 1921-1924; Porsild 1926-1948; Bryant 1929-1930), 2) missionaries (Hübner 1924; Boisduval 1929; Reverend Perrett 1930-1940; Reverend Turner 1930-1940), 3) individuals involved in Arctic mapping investigations, entomological studies, and survey projects such as: the United States Polar Expedition of 1881-1883 (Greely 1888), the Canadian Arctic Expedition of 1913-1918 (Malloch 1919), the Fifth Thule Expedition of 1921-1924 (Henriksen 1937), the Geological Survey of Canada (started in 1842) (Vodden 1992), the Canadian Northern Insect Survey of 1947-1957 (Freeman 1959), the Insects of Keewatin and Mackenzie Project of 2000 (Currie *et al*. 2000) and the Insects of the Arctic Project also of 2000 (Giberson 2005), and 4) scientists conducting taxonomic or ecological studies (for example: Brown 1937; McClure 1937, 1943; Shelford and Twomey 1941; Freeman and Twinn 1948; Weber 1950, 1954; Hicks 1955; Webb 1956; Huckett 1964, 1965a; Danks and Byers 1972; Hodkinson 1978; Maclean and Hodkinson 1980; Danks 1981; Harper and Harper 1981; McCafferty 1985; Currie and Adler 2000). For summaries of relevant expeditions, see Freeman (1956) and Riegert (1985).

Historical records of the Muscidae of Churchill

An extensive review of northern Nearctic Muscidae was completed more than 45 years ago by H. C. Huckett (1965a). Huckett's work was based on material collected in the north and material deposited in North American collections by various collectors. The major collections attended by Huckett were the Canadian National Collection of Insects,

Arachnids and Nematodes (CNC) (Ottawa) and the United States National Museum of Natural History (USNM) (Washington), but others were also examined. Churchill was also sporadically visited by entomologists after 1965 and their specimens have mostly been deposited in various North American collections.

Current diversity of the Muscidae of Churchill

Since the work of Huckett (1965a), no one has monitored changes in muscid assemblages in Churchill. In part, as a result of recent environmental changes in the area such as temperature increase (Ballantyne 2009) and anthropogenic habitat disturbances (Newton *et al*. 2002; Edye-Rowntree *et al*. 2006), the Diptera fauna of Churchill may have significantly changed. In order to investigate this possibility, a contemporary inventory of the Muscidae of Churchill is necessary to compare past and present patterns of diversity and to generate new baseline data for future monitoring.

SAMPLING MUSCIDAE

Before performing an inventory, sampling methods need to be chosen carefully as they influence the species composition and abundance in the catch (Ausden and Drake 2006). Important considerations for collecting methods used in northern areas include: stability in wind and storms, transportability to remote areas, non-offensiveness to non-target wildlife, and more importantly, efficiency to collect the target group(s). Flies can be actively collected with a sweep net or passively with stationary traps. Passive traps may have an attractant, such as bait or pheromones (Martin 1977). There is a strict

interdiction to bait in the area of Churchill because of the danger of attracting polar bears, and therefore methods employing bait will not be further discussed.

The sweep net

The sweep net is composed of a mesh bag supported by a rigid frame and a handle. The shape and dimensions of the frame, the dimensions and mesh size of the bag and the length of the handle vary depending on the needs and the comfort of the collector (Samways *et al*. 2010). For high-flying insects, a smaller net (opening of 38 cm) (less resistance to air) with a long handle is preferable, while a short handle is more suited to catch very active insects (Martin 1977). The sweep net is used to collect flying insects or insects resting in low vegetation or on surfaces, for example rocks or walls of buildings (Martin 1977). Its major advantage is the ability to be selective towards the target group(s) and the collector can sort its catch directly in the field to keep only the desired specimen(s) (Samways *et al*. 2010). However, unlike the Malaise or pan trap, it cannot be used to collect continuously, and obviously the effort of the collector is required in permanence. At Churchill, before 1965, the sweep net was the only collecting method used to collect adults of flying insects in northern areas (Danks 1981; D.M. Wood (pers. comm.)).

The pan trap

The pan trap is an open container placed on the ground and filled with a mixture of water and dish soap (to reduce surface tension) in which insects sink, and to which a preservative such as propylene glycol may be added, (Samways *et al*. 2010). It is used to

catch flying insects and differently coloured traps (yellow, blue and white) may attract different insect taxa (Leong and Thorp 1999; Campbell and Hanula 2007). For example, most Diptera are more attracted to white or yellow pans (Disney *et al*. 1982), while white and blue pans attract bees (Patenaude 2007). Even if the degree of attraction to a colour varies, yellow is still the most commonly used colour in faunistic studies for most taxa (Leong and Thorp 1999).

The Malaise trap

The Malaise trap, a tent-like structure, is another passive sampling method designed to collect flying insects. It was first designed to collect tabanids, muscoids and stinging vespids (Breeland and Pickard 1965). The first Malaise trap was square and had four open sides to collect insects flying from all directions (Malaise 1937). Today, besides the original Malaise trap design, there are many different designs, such as the Townes style (rectangular, light-weight design with two open sides), which collects passively at any time and under variable weather conditions, and can be installed in many places, even windy and hard to access areas (Breeland and Pickard 1965; Townes 1972). The Townes style trap was designed to decrease the weight of the trap for shipping purposes (Townes 1972). A Malaise trap can be used to collect a large number of specimens of resident species, species from closely adjacent habitat and species dispersing from distant site(s) (Danks 1993; Campbell and Hanula 2007). The placement of the trap and its orientation are very important to maximize efficiency. The exact placement is even more important with the Townes style as it has only two open sides, whereas the original model catches insects from any direction. Therefore, when using the

Townes model, the trap should be placed in flight corridors, such as paths, roads, edges of woods and bushes (Townes 1972). The front end of the trap should face the light and the back end face the vegetation (Townes 1972). Malaise traps have been widely used to determine insect abundance and diversity as they can collect large numbers of specimens and species (Campbell and Hanula 2007).

No sampling methods are unbiaised (Danks 1981). For example, the Malaise trap selectively captures flying insects in accordance with the specific height of the trap, and is occasionally biased in sex ratio (J. Savage (pers. comm.)), especially in taxa where females are more active fliers than males. Males of these species can often be more successfully collected using a sweep net. Pan traps, on the other hand, tend to collect low flying insects attracted to a certain colour. Therefore, Disney *et al*. (1982) suggested the simultaneous use of a variety of collecting methods in order to obtain a more complete list of the species in an area. Considering their ecological diversity, this approach may be especially relevant for muscid flies.

SPECIMEN PREPARATION

Once collected, muscid flies need attentive preparation before they can be identified. Specimens need to be dried, pinned directly or glued to paper points, and labelled. As is often the case in Diptera, Muscidae can rarely be identified to species while still in ethanol. Chaetotaxy and wing veination are important features to identify muscid flies and specimens need to be dried to distinguish patterns of body hairs and wing veins. Larger specimens can be pinned and air dried, while it is recommended to dry smaller specimens chemically before they can be pointed (Brown 1993). Labelling

also needs to be done following standard preparation guidelines, but most important is to ensure that labels are clearly written and permament. The information written should be as exact as possible in order to allow the site to be found again later. See Wheeler *et al*. (2001) for preparation guidelines and the minimum data required on labels.

SPECIMEN IDENTIFICATION

In a faunistic study, the next step following specimen collection and preparation is specimen identification. Specimen identification can be done at different taxonomic levels, from class to species depending on the objectives of the study. To determine the taxonomic composition of an area, the lowest taxonomic rank should be preferred since identification limited to higher ranks may have little ecological significance, especially in highly diverse taxa such as muscids (Danks 1993; Underwood and Chapman 1999; Samways *et al*. 2010). Species level determination may be essential, for example when species of the same genus have different ecological requirements. High taxonomic resolution may be especially relevant when patterns of responses to environmental changes are investigated (Samways *et al*. 2010).

Specimen identification to species ensures the repeatability of a study in the context of long-term monitoring only when voucher specimens, representatives of each species collected during a study in ecology, are deposited in a recognized natural history collection. Specimens of species listed in published works are then available for consultation and their identity can be verified by others. Thus, voucher specimens should be properly labelled with species names, the identity of the person who identified them, and details of collection locality and method. Moreover, every study in ecology or

faunistic surveys using insects should list the name of the institution storing the voucher specimens related to the study. Voucher specimens in collections have been used to: 1) validate the identification of collected specimens (Resh 1976), 2) confirm the presence of some species in an area and map species distribution (McCorquodale 2001), and 3) assess faunal changes of insects over time (Favret and DeWalt 2002; Liebherr and Song 2002).

Morphology

Insect specimens can be identified using a variety of character sources, the most commonly used being morphology and DNA. Until relatively recently, specimen identification was almost entirely based on morphological characters. Morphology-based identification involves specimens sorted by species or recognizable morphological forms such as morphospecies. Unfortunately, accurate identification to the species level using even the best identification keys often requires a high level of expertise and time that restricts the practicability of faunal studies on insects. Morphospecies identification, on the other hand, typically takes less time to perform and does not require the input of an expert taxonomist (Samways *et al*. 2010). However, this approach is prone to an over- or underestimates of species richness and can only be used if the identifications were done by the same person. An additional disadvantage of morphology-based identification is that errors are more likely when the studied taxa exhibit high within species variability and/or sexual dimorphism, and when cryptic species are present (Hebert *et al*. 2003a).

DNA barcoding

In an attempt to alleviate the problems related to morphology-based identification, DNA barcoding, a molecular method that is used to perform rapid taxon recognition through a short DNA sequence (Hebert *et al*. 2003a), has gained much popularity over the last decade. DNA barcoding is a standardized method proposed by Hebert *et al*. (2003a) that uses the "Folmer region", a 658 base-pair region found at the 5' end of the mitochondrial gene, cytochrome *c* oxidase subunit I (COI) (Ratnasingham and Hebert 2007), to identify known species and discover new ones. COI is mainly used because it is present in all animals and because of its low variability within species, combined with differentiation between species (Hebert *et al*. 2003a, b). DNA barcoding relies on the assumption that genetic interspecific variations exceed intraspecific ones.

For insects, the removal of tissue samples, usually of one or more leg(s), is required to generate sequences from a standardized automated protocol performed at the Canadian Center for DNA Barcoding (CCDB) (Ivanova *et al*. 2006). The protocol includes DNA extraction (Ivanova *et al*. 2006), PCR amplification (Hajibabaei *et al*. 2005; Ivanova and Grainger 2007) and cycle-sequencing (Hajibabaei *et al*. 2005; Ivanova and Grainger 2007). Once sequences have been, obtained they are deposited on the Barcode of Life Data System (BOLD) web site (www.boldsystems.org) where information on specimens, such as collection data, taxonomy and pictures, is available. Online tools are also available on Bold to analyse distances between sequences, build trees, browse specimen taxonomy, and examine accumulation curves.

However, before reference libraries can be used to identify specimens, it is essential that taxonomists with expertise in the study group validate species-level identifications in order to generate reference sequences linked to a species name that will enable future identification of specimens sharing an identical (haplotype) or similar sequence. The reference libraries developed by these expert taxonomists are made available online to the public for comparison with other specimen sequences.

DNA barcoding is a valuable addition to taxonomy in biodiversity studies as it can: 1) facilitate rapid species identifications, 2) accelerate the discovery of new species, 3) improve the quality of taxonomic information, and 4) make taxonomic information available for non-specialists (Miller 2007). DNA sequencing does not replace taxonomic work using morphology, but should rather be used as a complementary tool (Hajibabaei *et al*. 2007). DNA barcoding has been used successfully in a number of studies to: determine the number of species of insects in a sample (Hebert *et al*. 2003a, b; Zhou *et al*. 2010), reveal the presence of cryptic species (Hebert *et al*. 2004a; Smith *et al*. 2006; Zhou *et al*. 2010), associate sexes in dimorphic taxa (Janzen *et al*. 2005), associate immatures to adults (Janzen *et al*. 2005; Zhou *et al*. 2010), associate parasitoids with their host species (Smith *et al*. 2008), and establish or confirm existing species limits (Sheffield *et al*. 2009).

Despite the general enthusiasm for DNA barcoding, limits have been recognized as the method has gained in popularity. COI has occasionally failed to delimit species boundaries and separate individuals of different species (Ballard 2000; Monaghan *et al*. 2006; Whitworth *et al*. 2007; Magnacca and Brown 2010). It is possible to use DNA barcoding to determine the number of species in an assemblage (Ratnasingham and

Hebert 2007). But in order to determine the identity of these species, DNA-based species recognition must usually be assisted by morphology-based identification.

A phenetic tree, on which taxa or individuals are grouped together based on their degree of similarity, can be constructed using different distance analyses. Among these, Neighbor-joining (NJ) analysis calculates the number of differences between all pairs of sequences of a multiple alignment producing a distance matrix from which is built the phenetic tree. To calculate distances, the selected analysis must be implemented with a substitution model predicting the proportion of nucleotide change expected in a sequence. The Kimura 2-parameter model, like other existing models, makes assumptions about the substitution process. In contrast to the One parameter or the Jukes-Cantor models, the Kimura 2-parameter model considers that transitions (purine (A + G) substituted by a purine or a pyrimidine (C + T) substituted by a pyrimidine) and tranversions (purine substituted by a pyrimidine or the opposite) occur at different rates (Hall 2004). Although pairwise and Jukes-Cantor models are also available when performing analysis with BOLD (http://www.boldsystems.org), the Kimura model is the most popular, possibly because of its accuracy to discriminate between species even under low genetic distances (Nei and Kumar 2000).

MEASURING DIVERSITY

Once specimens are identified, the diversity of the studied assemblage(s) can be assessed. The variability of living organisms in time and space can be considered a definition of biodiversity (Pielou 1975; Lévêque and Mounolou 2003; Gaston and Spicer 2004; Secretariat of the Convention on Biological Diversity 2005), and this variability is

usually measured to characterize or compare assemblages (Mac Nally *et al*. 2004; Magurran 2004). The different aspects of regional diversity (*gamma* diversity) include *alpha* and *beta* diversities. *Alpha* diversity represents the diversity of local assemblages or communities, such as that of one habitat or ecosystem, while *beta* diversity refers to variations in diversity between assemblages or communities (Mac Nally *et al*. 2004; Magurran 2004; Maclaurin and Sterelny 2008).

Components of diversity include species composition, species richness and abundance, where the composition is the list of species found in the assemblage, the species richness is the number of species in the assemblage and the abundance is the individual frequencies of each species. Once *alpha* diversity has been measured at various localities and/or different points in time, *beta* diversity can be used to measure species turnover between assemblages (Maclaurin and Sterelny 2008).

Comparing species diversity can be done by comparing presence-absence of species in an assemblage or by using quantitative data. A simple way to compare species composition between assemblages is to determine shared and unique species between assemblages, as was done by Grixti and Packer (2006) in their comparison of past and recent bee communities at a site in Ontario. It is also possible to use similarity or distance indices to compare assemblages such as the Jaccard and the Sorenson indices (Magurran 2004). These can be used with presence-absence data, but also when frequencies are available. Similarly, cluster analyses use an index to join together the most similar or distant (depending on the index used) assemblages on a dendrogram (Magurran 2004). Ordinations can also be used to assess the relationship between assemblages. The

Principal Component Analysis (PCA) is one of the most used ordination methods in ecology.

Comparing raw species richness between two or more assemblages may lead to incorrect conclusions as samples may differ in species richness because of differences in sample size, collecting methods, sampling effort, local climatic conditions, sample locations and habitat richness (Gotelli and Coldwell 2001). If abundance data are available, standardized species richness can be obtained with individual-based rarefaction curves or accumulation curves from which the number of species in an area can be estimated (Magurran 2004). Rarefaction curves are also used to compare assemblages at a common low abundance level (Magurran 2004). It is a useful method for the study of historical changes, as sample size and sampling effort usually vary between datasets or are unknown because of collection management artifacts (Grixti and Packer 2006). Moreover, accumulation curves can help to determine if the collecting effort was adequate to sample most species in a given area. Curves that have reached an asymptote indicate that most of the species expected for the area were collected.

As an alternative to rarefaction and accumulation curves that can be used to estimate species richness at different levels of specimen abundance in a sample, total species richness can be extrapolated using estimators such as the abundance-base coverage estimator (ACE), Chao 1 and 2, Jacknife 1 and 2, Boostrap and Michel-Menten (Magurran 2004). Of those, only the first two can be calculated when no replicates are available for a data set. The ACE is a non-parametric estimator of species richness based on the number of species with fewer than 10 individuals (Chazdon *et al*. 1998). The ACE seems to extrapolate species richness more accurately than other estimators such as the

Chao 1 index, calculated only based on the ratio of singletons to doubletons in a sample (Chazdon *et* al. 1998; Magurran 2004). Unfortunately, the value of the Chao 1 index tends to be overestimated as the frequency of singletons increases (Magurran 2004).

Different values of biodiversity provide meaningful information about the state of the environment, and thus arrays of measures have been developed to fit each situation. Different opinions on the value of each measure are found in the literature and tend to be confusing. This is why only measures relevant to this study were presented here. Extensive reviews of diversity measures are provided by Pielou (1975), Southwood (1978), Gotelli and Coldwell (2001), Magurran (2004) and Maclaurin and Sterelny (2008).

APPLICATIONS AND LIMITATIONS OF MUSEUM AND LITERATURE DATA FOR COMPARATIVE PURPOSES

Various sampling initiatives in northern habitats have led to the accumulation of insect material that represents a non-negligible source of information about northern insect diversity and distribution (Samways *et al*. 2010). Published literature and museum collections are the best sources of historical species records, via voucher specimens in collections, as they provide data accumulated over a long period (Ponder 1999) and provide a historical perspective to complement modern faunistic studies (Ponder *et al*. 2001).

Unfortunately, data from collections must be considered with care as some artifacts of collectors and collection management may lead to misinterpretations. Among problems related to the use of collection data, Ponder *et al*. (2001) identified spatial and temporal gaps, biased sampling effort, lack of access or availability (no electronic

database), unpublished data, and the lack of abundance data (presence only). Although specimen abundance and sampling effort can be incorrectly represented in published monographs and museum collections, data from museum collections are still the most valuable information available about historical species richness and distribution (Coddington *et al*. 1991; Ponder 1999).

CONCLUSION

The potential vulnerability of insects to environmental changes was demonstrated in this literature review. While it has been confirmed that some taxa such as butterflies have already responded to climate warming and habitat disturbances, the ecological response of most other taxa such as muscid flies remains unknown. The muscid fauna of Churchill was last surveyed by Huckett in 1965 and since then many environmental changes have been observed at this locality. The historical data available has been used here to assess historical changes in the biodiversity of muscid flies and possibly, since these insects have significant ecological diversity, the results may be representative of trends in other groups of arctic flying insects.

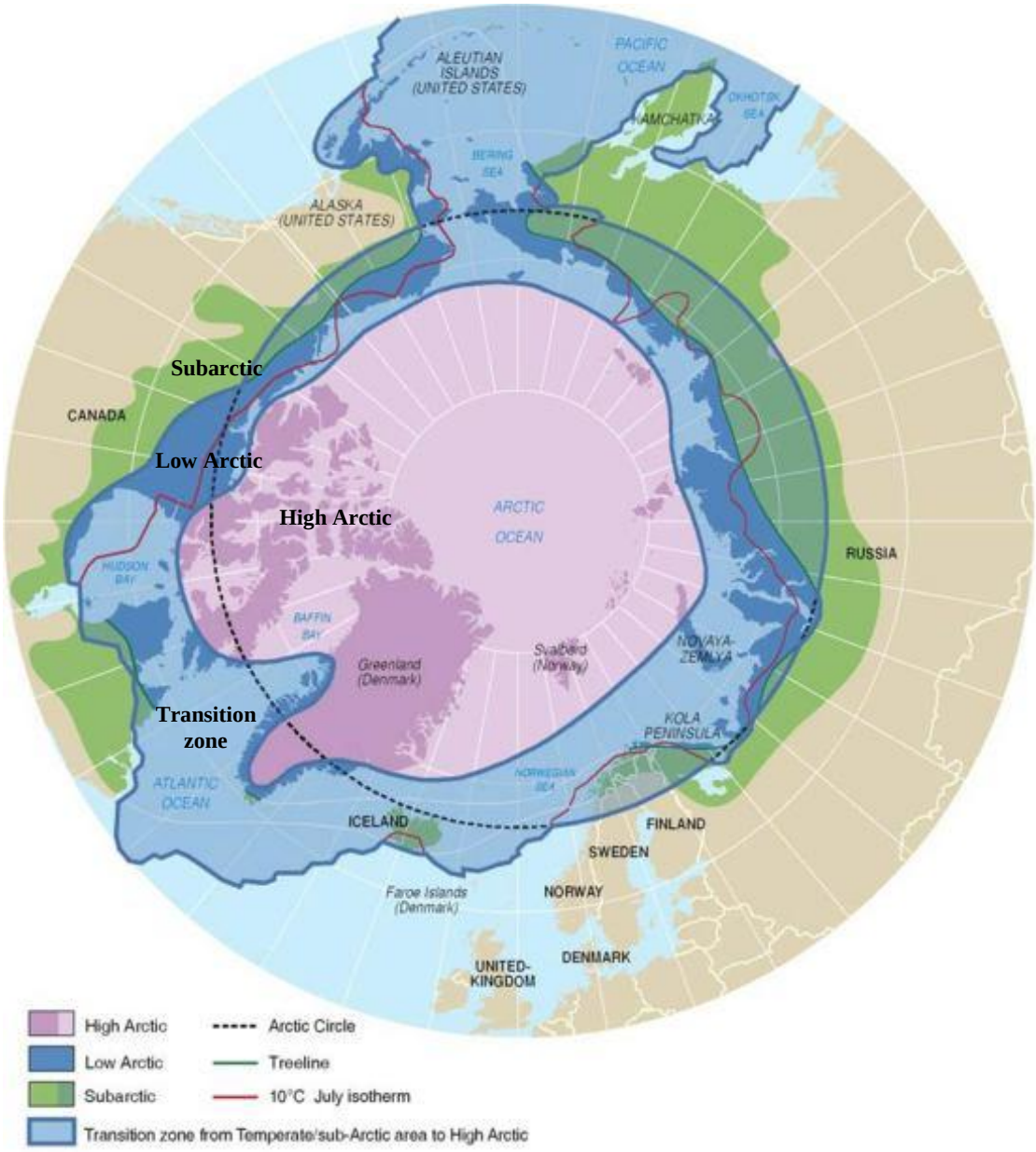


Figure 2.1. Geographic boundaries of the Arctic (UNEP/GRID-Arendal 2005). Several physical-geographical characteristics were used to delimit the extent of the Arctic.

CHAPTER 3

MUSCIDAE (DIPTERA) DIVERSITY IN CHURCHILL, MANITOBA, CANADA, BETWEEN TWO TIME PERIODS: EVIDENCE FOR LIMITED CHANGES SINCE 1965

ABSTRACT

A 2007 survey of muscid flies from Churchill, Manitoba, Canada, yielded 155 species belonging to 24 genera. Some diversity components of this contemporary assemblage such as species richness and composition were compared to those of a historical (pre-1965) assemblage, and the contribution of three collecting methods used in the 2007 survey protocol was evaluated. Malaise traps yielded more species (n = 134) than pan traps (n = 77) or net sweeping (n = 86) and species composition did not differ significantly between Malaise trap and pan trap catches. The results suggest that Malaise traps and net sweeping are sufficient methods to survey northern Muscidae. No significant differences were found in estimated species richness between time periods for material collected by net sweeping, and when all specimens from the contemporary survey were pooled (irrespective of collecting method) 87% of species collected pre-1965 were collected again in 2007. While 15 species were unique to pre-1965 and 54 to 2007, Malaise traps collected 65% of all new 2007 records and most non-overlapping species between time periods were rare in samples. Therefore, most differences in species composition between assemblages appear to be attributable to collecting method and/or rarity in samples. However, the high proportion of aquatic and semi-aquatic species of *Spilogona* Schnabl unique to the pre-1965 assemblage may suggest that the recent decrease in water cover for the area may have influenced the species composition of this genus in Churchill.

Keywords: insects, muscid flies, temporal changes, collecting techniques, low Arctic.

INTRODUCTION

Since its first assessment report, the Intergovernmental Panel on Climate Change (IPCC) has predicted that at current rates of change, climate warming and habitat disturbances would have significant biological impacts on plants and animals (IPCC 1990). In spite of greater environmental awareness from governments, the level of anthropogenic activities and rate of climate change have continued to increase over the last twenty years (IPCC 2007) and ecosystems have been observed to change in response to these new environmental conditions (Thuiller 2007). While there is a growing body of literature linking climatic changes to alterations in insect diversity, phenology and distribution range in temperate zones (Parmesan 1996, 2006; Parmesan *et al*. 1999; Hickling *et al*. 2006; Menéndez *et al.* 2006; White and Kerr 2007; Westwood and Blair 2010), the impacts of climate changes on northern insect communities are relatively poorly documented. Emerging trends, however, are alarming; recent rates of northern range margin shifts for Finnish butterflies are unprecedentedly high (Pöyry *et al*. 2009) while the median date of a variety of phenological changes including arthropod emergence peaks has advanced on average by 14.5 days in northern Greenland between 1996 and 2005 (Høye *et al.* 2007). In the northern Nearctic region, dramatic shifts in community composition for chironomids (Axford *et al.* 2009) and microgastrine wasps (Fernández-Triana *et al.* 2011) over the last decades have been linked to recent climate warming.

The monitoring of arctic insect communities has had a short and sporadic history, especially in the Nearctic region. Consequently, material housed in museum collections represents an invaluable historical resource to document changes in biodiversity (Shaffer

et al. 1998; White and Kerr 2006; Newbold 2010). The Canadian Northern Insect Survey (1947-1961) was an initiative aiming at the discovery and documentation of arctic species in the Nearctic region (see Freeman (1956) and Danks (1981) for details), and has generated the bulk of northern Nearctic specimens housed in museum collections for that time period. Some localities (and taxa) were sampled more heavily during the northern survey, and there were many collecting expeditions to Churchill, Manitoba. The family Muscidae, one of the most species rich and abundant families of terrestrial insects in the Nearctic region (Vockeroth 1979; Danks 1981), was commonly collected during that period. Specimens were subsequently curated mostly by H.C. Huckett, not only for Churchill, but for the whole of the northern Nearctic region, an endeavour that culminated in his 1965 monograph on the Muscidae of Northern Canada, Alaska and Greenland (Huckett 1965a). The work of Huckett (1965a) and the large number of muscids found in collections form an extensive set of historical baseline data on the distribution of northern Nearctic Muscidae that can now be compared to the results of modern day surveys.

The Muscidae is a cosmopolitan family that spans almost the full spectrum of ecological habits known for Diptera (Courtney *et al.* 2009). In arctic and alpine habitats, however, the majority are saprophagous or predaceous in the immature stages and commonly found in humic soils, various damp or aquatic habitats, dung or carrion (Skidmore 1985; Ferrar 1987) where they may play an important role in accelerating the decomposition rate of organic refuse in cold environments (Vockeroth 1979; Savage 2002). Adults, such as those of *Thricops* Rondani and *Drymeia* Meigen, are anthophilous, feeding on pollen and/or nectar (Pont 1993; Elberling and Olesen 1999) while members

of the subfamily Coenosiinae, such as *Spilogona* Schnabl, *Limnophora* R.-D. and *Coenosia* Meigen, are predaceous but occasionally visit flowers (Skidmore 1985; Larson *et al.* 2001).

Close to 3000 historical specimens representing more than 100 species of Muscidae are available for the area of Churchill, a locality found in northern Manitoba along the southern boundary of the Arctic biome at the transition of boreal forest, tundra and the ocean. Since 1929, Churchill and its surrounding area has been modified by military and hydroelectric development as well as an increase in freight transport and ecotourism which have modified terrestrial and aquatic habitats to various extents (Newton *et al.* 2002; Edye-Rowntree *et al.* 2006). Regional climate has also recently changed in Churchill where the mean annual temperature has risen by 1.78 Celsius degrees (^{o}C) from 1970 to 2007 (Ballantyne 2009, compiled from http://climate.weatheroffice.ec.gc.ca/climateData/canada_e.html). Climate change can have the effect of shifting ecological zones: one Celsius degree of temperature change can move ecological zones by more than 160 km (Thuiller 2007). In North America, an average isothermal shift of 105 km has been reported for 1900-1994 (Karl *et al.* 1996), a shift most likely to have intensified recently with the last decade being the warmest on record (Hansen *et al.* 2010). Ballantyne (2009) suggested climate warming as a possible cause for the significant increase in tree and shrub cover and decrease in water and low vegetative cover measured in Churchill over the last 30 years.

A few northern monitoring initiatives based on standardized sampling programs that include arthropods have been developed over the last two decades in Europe (Høye *et al.* 2007; Schmidt *et al.* 2010) and since 2005 in North America (Buddle 2009;

Cannings 2009). No such data set has been determined to species level for muscid flies or any other calyptrate Diptera in the Nearctic region. Consequently, we know little of the impacts of environmental changes on one of the dominant taxa of terrestrial northern insects and even less about the influence of sampling techniques commonly used on the fauna collected. For muscid flies, as in most organisms, sampling methods undoubtedly affect not only the abundance of species in a sample, but their composition as well (Longino *et al.* 2002; Ellison *et al.* 2007).

Objectives

The impacts of climatic changes on insect distribution have been predicted to be most severe along boundaries of the Arctic biome (Danks 1981). Therefore Churchill, with its strategic location, confirmed warming trend, and large number of historical specimens stored in collections, appears to be a locality of choice to study northern insect assemblages and monitor possible changes in their community composition over time. I conducted the first survey of the muscid flies from Churchill using an explicit and repeatable sampling design and used the opportunity to: 1) contrast patterns of species richness and composition of muscid flies in Churchill between two time periods (pre-1965 and 2007), and 2) evaluate the contribution of three different methods commonly used to collect Diptera (Malaise trap, pan trap and net sweeping).

MATERIALS & METHODS

Two primary data sets (pre-1965 and 2007) were compiled in order to compare and contrast various aspects of past and recent muscid biodiversity in the Churchill area. Since these data sets were assembled from different sources, they will be described separately.

2007 data set

Study area and sampling design

From 19 June to 25 August 2007, adult muscid flies were sampled in the region of Churchill, Manitoba, in an area of approximately 100 km^2 ranging from the mouth of the Churchill River and the Hudson Bay coast to the Twin Lakes area and referred to as "Churchill" throughout this work (Figure 3.1). Vegetation in the sampling area was a mosaic consisting mostly of tree communities juxtaposed to tundra patches in a landscape with a high proportion of wetlands, bogs and fens (Brook 2001). In order to sample from a set of representative habitats from the regional landscape, nine permanent and 26 occasional sites (each visited four times or less) were selected (Figure 3.1, Appendix B). Permanent sites were chosen based on their accessibility, level of disturbance, and ecological and geographical characteristics, while occasional sites were chosen based on citations in published records, distinction from permanent sites and presence of peculiarities (atypical plant composition, accumulation of organic material such as garbage or grain piles).

Occasional sites were sampled only by sweep netting with a collapsible insect net (handle 43.2 cm, bag diameter 38.1 cm, mesh size 24 stitches x 20 rows per 2.54 cm). All

permanent sites were sampled continuously and serviced bi-weekly using a Townes style Malaise trap (1.83 x 2.44 x 1.83 m) surrounded by four yellow pan traps ("Carry-out" brand, 5 cm high with an outer diameter of 15.2 cm) positioned at cardinal compass points, 2 m from the edges of the Malaise trap. Yellow was selected for pan trap colour as it catches more muscoid specimens than white traps (Disney *et al*. 1982) and has been used in a number of recent studies on higher fly diversity where Muscidae were well represented (Grégoire Taillefer and Wheeler 2010; Savage *et al.* 2011). Collection heads on the Malaise traps were loaded with either dichlorvos insecticide cubes (Vapona®), 70 or 95% ethanol and killing agents were alternated at each trap service. Pan traps were half filled with 2/3 water, 1/3 propylene glycol (food quality) and three drops of dishwashing soap. At each site visit, 60 net sweeps were taken without interruption around the Malaise trap, keeping a distance of at least 4 m from the trap and insects were immediately transferred to a killing jar loaded with either ethyl acetate or sodium cyanide.

All specimens collected at occasional sites were processed but due to the large number of samples and specimens collected at permanent sites, subsampling was necessary, as is often the case with Diptera surveys (Marshall *et al.* 1994). For all permanent sites and for every week of the sampling period, material collected on the first biweekly visit (four day period) was processed for identification while specimens collected on the second visit (three day period) were kept in storage. Large specimens were pinned fresh or from alcohol, while small specimens were chemically dried using hexamethyldisilazane (HMDS) (Brown 1993) before being pointed to prevent them from shrivelling. Voucher specimens were deposited in the Bishop`s University Insect Collection, Sherbrooke, Québec, Canada (BUIC), the J.B. Wallis/R.E Roughley Museum

of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada (JBWM), and the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada (CNC).

Pre-1965 data set

Historical data were assembled through the combination of published records and material housed in collections. Huckett (1965a) provided the most extensive list of published records for the area and was supplemented with Huckett (1965b), Webb (1956) and Arntfield (1975). While museum data are of great value to study the distribution of species, their accuracy can be problematic (Graham *et al*. 2004; Newbold 2010). Therefore, to confirm the identity of all published records, to investigate dubious or problematic records and localities, as well as to curate undetermined specimens and establish the abundance of species found in collections, 28 North American and three European collections were contacted to enquire about holdings of Muscidae from Churchill (Appendix C). A total of 2947 specimens were found in collections and since all but 66 of these were collected from 1929 to 1964, a total of 2881 specimens (including over 500 undetermined specimens) was retained and pooled together in the pre-1965 data set. Collecting dates ranged from 20 May to 5 September with 90% of the specimens collected within the sampling period of the 2007 inventory. According to label data and D.M. Wood (pers. comm.), all specimens in this data set were collected by sweep net.

Taxonomic identification

Specimens from both primary data sets were identified using the following identification keys: Arntfield (1975), Collin (1930), Hennig (1955-1964), Huckett (1932, 1934a, b, 1936, 1954, 1965a), Huckett and Vockeroth (1987), Malloch (1918, 1919, 1920, 1923), Michelsen (2006), Savage (2003), Snow (1891), and Snyder (1949a, b, 1954). To ensure up to date taxonomic nomenclature, the following catalogues, revisions and type studies were also consulted: Huckett (1965b), Pont (1984, 1986, 2011), and Michelsen (2006). Species identity was then verified through comparison with determined material housed in the CNC, the Bishop's University Insect Collection, Sherbrooke, Québec (BUIC), the American Museum of Natural History, New York, NY, USA (FAMNH) and the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

Identification of female muscid flies can occasionally be problematic. Therefore, in order to allow for the inclusion of female specimens in all analyses requiring abundance data, six pairs of species had to be merged into the following complexes: *Coenosia tarsata* Huckett/*Coenosia verralli* Collin, *Limnophora nigripes* (R.-D.)/*Limnophora rotundata* Collin, *Phaonia consobrina* (Zetterstedt)/*Phaonia rugia* (Walker), *Schoenomyza dorsalis* Loew/*Schoenomyza litorella* (Fallén), *Spilogona atrisquamula* Hennig/*Spilogona pusilla* Huckett, and *Thricops septentrionalis* (Stein)/*Thricops spiniger* (Stein).

As an additional means to improve the quality of specimen identification, DNA barcodes from 976 specimens collected between 2005 and 2008 at Churchill (Manitoba), Gaspésie (Québec) and Sweden were generated to test/resolve a number of taxonomic

issues and refine morphological species limits used for all other 2007 and pre-1965 specimens. Details of the DNA barcoding study can be found in Chapter 4.

Data analyses

A variety of factors can influence the composition and abundance of taxa captured at a site, such as sampling effort, collecting techniques, weather, natural or anthropogenic changes in habitats or microhabitats sampled, yearly variations in abundance, and timing of survey (Marshall *et al.* 1994; Shaffer *et al.* 1998; Longino *et al.* 2002). In the present study, sites selected for the 2007 survey included all those listed in the literature, collection dates between surveys overlapped extensively and net sweeping was used in both time periods. While I received assurance from collection managers that no trimming took place for their holdings of Muscidae from Churchill, it is possible that series of conspecific specimens were purged by previous collectors interested primarily in species richness, thereby skewing the abundance of species found in collections. It is, however, unrealistic to assume that none of the assumptions related to repeatability will be violated between surveys conducted many decades apart and while care should be taken during survey planning and data analysis to minimize potential biases, results from comparative studies of faunal assemblages from different time periods should always be interpreted in light of those potential biases.

Rarefaction curves were generated to: a) determine if any of these data sets have reached an asymptote indicating that the collecting effort was adequate to sample the area, and b) compare species richness of different data sets after they have been standardized to the least abundant sample. Individual-based rarefactions (based on 1000

permutations and with species richness as a diversity index) were performed using Ecosim version 7.72 (Gotelli and Entsminger 2010) for: 1) all material collected in 2007 (2007 all), 2) material collected by each collecting method at the permanent sites (2007 Malaise, 2007 pan, and 2007 sweep (p), 3) material collected in 2007 by net sweeping at both permanent and occasionnal sites (2007 sweep (all)), and 4) all material collected at Churchill before 1965 and found in collections (pre-1965). Total species richness was estimated for all data sets using two non-parametric estimators: the abundance-based coverage estimator (ACE) (Chao and Lee 1992) and the classical Chao 1 index (Chao 1984). Computation of 95% asymmetrical confidence intervals (CIs) for the Chao 1 index allowed for the comparison of richness estimates between assemblages. These were calculated in EstimateS version 8.2.0 (Colwell 2009). These estimators, which rely mostly on information from rare species, were especially relevant for the pre-1965 assemblage, as potential biases in museum specimen abundance could have affected the shape of the rarefaction curve.

Since calculations of species richness estimators and rarefaction curves both involve abundance data, all 12 species with problematic females were pooled into six species complexes and the five species with no abundance data in pre-1965 were excluded.

Differences in species composition were investigated to determine the taxonomic overlap between: 1) temporal assemblages (2007 sweep (all)/2007 all and pre-1965), and 2) collecting methods (2007 Malaise, 2007 pan, and 2007 sweep (p)). The presence or absence of non-overlapping species between pre-1965 and 2007 primary data sets may either be the result of a failure to detect or an actual change in the distribution of species.

As the number of pre-1965 species collected in the modern survey largely increased when all specimens collected in 2007 were pooled together regardless of collecting technique, I decided to investigate for repeated trends in factors possibly involved in the presence or absence of non-overlapping taxa not only for material collected by net sweeping, but for all material from both time periods. All species unique to a time period were assessed in terms of collecting dates, collecting techniques, abundance in the samples and individual distribution ranges.

While not enough data are available on the distribution range and ecological requirements of species in my study to model their past and present distribution range adequately, I can still determine where Churchill lies in relationship to their known Nearctic distribution range. Temperature is a major determinant of insect distribution (Hill *et al.* 1999; Bale 2002) and the range limits of insect species may therefore be expected to follow certain isothermal shifts (de Groot *et al*. 1995). Since latitude alone is not a good indicator of temperature across a large region such as the northern Nearctic, I first calculated the 1965 July isothermal line passing through Churchill based on the average temperature of the month (11^{o}C) and then calculated the 2007 July 11^{o}C isothermal line to establish if there has been a discernable northward displacement of the July 11^{o}C isotherm in the area of Churchill (Figure 3.2) between the two time periods. The July isotherm was selected because it represents summer heat availability by which insects may be limited in their distribution (Bale *et al*. 2002). Since the distance between the 1965 and the 2007 July $11\ ^{o}$C isothermal lines is about 300 km in the area of Churchill (Figure 3.2), it is possible that the presence or absence of some non-overlapping species between time periods might have been influenced by isothermal

changes in the region. Therefore, in an attempt to identify repeated patterns in the distribution of non-overlapping species known from at least three other Nearctic localities, I evaluated the position of all distribution points relative to the 1965 July isothermal line (instead of latitude) to determine if they were located north or south of Churchill and then categorized Churchill as either: a) a locality within the known range, b) the northern-most locality, or c) the southern-most locality.

Pre-2007 Nearctic distribution records for non-overlapping species from localities other than Churchill were compiled from label information of material housed in the CNC and supplemented with Malloch (1918, 1919, 1920, 1923), Collin (1930), Huckett (1932, 1934a, b, 1936, 1954, 1965a) Snyder (1949a, b, 1954) and Arntfield (1975). Geographic coordinates (decimal degree) for all localities were obtained from Roughley and Alperyn (2009), Google Earth (2010) and D. Sikes, University of Alaska, USA (pers. comm.), and mapped in ArcMap (Environmental Systems Resource Institute 2009). The July 11^{o}C isothermal lines were created in ArcGIS with *Inverse Distance Weighted (IDW),* a method of data interpolation which, in the context of isotherm creation, estimates the temperatures at locations where no measured values are available (Anderson 2010). Temperature data of approximately 2100 Canadian localities (Environment Canada 2010) and 19 locations in Alaska (Alaska Climate Research Center 2008) were used.

RESULTS

To facilitate the comprehension of the following section, general results on overall abundance and species richness (observed, rarefied and extrapolated) can be consulted in Table 3.1. A detailed list of species abundance arranged by collecting methods (2007 only) and collecting periods can be consulted in Appendix D.

2007 survey

A total of 9816 specimens were collected through the standardized protocol at the nine permanent sites and 478 by net sweeping at the occasional sites. Together, these 10, 294 specimens represented 155 species (143 named species and 12 morphospecies) in 24 genera, a number of species that dropped to 149, including 13 singletons and 30 doubletons, when species with undistinguishable females were pooled (Appendix D). The assemblage was dominated by *Spilogona*, a genus represented by 51 species (34%) and 2863 specimens (28%). The rarefaction curve for this assemblage (2007 all) did not reach an asymptote (Figure 3.3) indicating that more species could be present in the area. This result is consistent with the ACE and Chao 1 index, which respectively estimated that 84 and 83% of overall species richness was collected in the 2007 survey (Table 3.1). The contemporary sub-assemblage containing only material caught by net sweeping (2007 sweep (all)) yielded a total of 92 species, a number adjusted to 86 when species were pooled in complexes. Among these, 30 were collected as singletons and 10 as doubletons.

Species richness and composition by collecting methods at permanent sites

Close to 84% of all specimens collected at the permanent sites were collected by Malaise traps and this device collected almost twice as many species as the other two methods (Table 3.1, Appendix D). Species richness rarefied at 1334 individuals was significantly higher for Malaise trap (86.8 ± 3.8) than pan trap (77.0), but when species richness was rarefied at 270 individuals for the comparison of all three collecting methods (2007 Malaise, 2007 pan, 2007 sweep (p)), estimated species richness was similar with overlapping standard deviations, indicating that the difference was not significant between them (Figure 3.4, Table 3.1). This result, however, must be interpreted in light of the fact that species richness was rarefied near the bases of the curves. Results for the ACE and Chao 1 estimates of species richness ranked the Malaise trap assemblage as the most species rich and net sweeping as the least, with no overlap in CIs of the Chao 1 index between Malaise trap and the other two methods (Table 3.1).

Species composition between collecting methods did not completely overlap with Malaise traps collecting many species not captured by the other devices. Forty-three species were collected only by Malaise traps, one only by pan traps and six only by net sweeping (permanent) (Table 3.1).

Pre-1965 data set

From the literature, 105 species were listed for Churchill (Appendix D). Following the identification of uncurated material and reassessment of specimens determined by previous curators, the 2881 museum specimens yielded 110 species (Appendix D). A total of 99 species was both listed in the literature and found in

collection material, six species listed in the literature could not be linked to specimens, and collection specimens yielded 11 species not listed in the literature (Appendix D). Overall, the combination of taxa listed in the literature and those found in collections amounted to 116 species, a number that dropped to 105 when species complexes were taken into account and species with no abundance data were excluded (Table 3.1, Appendix D). Of those 105 taxa, 20 were found as singletons and 13 as doubletons. The genus *Spilogona* was also dominant in this assemblage and represented by 38 species (36%) and 869 specimens (44%). The rarefaction curve for the pre-1965 assemblage did not reach an asymptote, a result consistent with the ACE and Chao 1 index, which respectively estimated that 86 and 87% of overall species richness was collected.

Historical vs contemporary data sets comparisons

Species richness and composition

Rarefied species richness standardized at 740 specimens was lower for pre-1965 (77.1 ± 3.4) than for 2007 sweep (all) (85 ± 0.0), a trend also observed with Chao 1 estimated species richness (Figure 3.4). While there was no overlap in standard deviation of rarefied species richness between these curves, confidence intervals of Chao 1 estimates overlapped extensively (Table 3.1) and ACE-estimated species richness was slightly higher for pre-1965 than for 2007 sweep (all) (Table 3.1).

When species collected by net sweeping at both permanent and temporary sites, historical collections and/or in the literature were taken into account, the 2007 sweep (all) and pre-1965 assemblages shared 75 species, indicating that 65% of the 116 historical taxa were collected again in 2007 by this method. When all material collected in the 2007

survey regardless of collecting method was combined (2007 all), the percentage of historical species in the contemporary assemblage climbed to 87%, with 101 of the 116 historical species recovered in 2007. Percentages of historical species recovered in the contemporary assemblage were similar when species with undistinguishable females were pooled and those with no abundance data were excluded (64% for 2007 sweep (all) and 88% for 2007 all).

New records

A large number of species of Muscidae found in Churchill in 2007 and/or in the pre-1965 assemblage represent new distribution records at various geographical levels. The combination of the 116 species found in the historical assemblage with the 155 collected in 2007 resulted in a total of 170 species of Muscidae for the area of Churchill. Fifty-four are new mentions for the area of Churchill (including two new generic records: *Stomoxys* Geoffroy and *Haematobia* Le Peletier and Serville) and 42 of these are new records for the province of Manitoba (31 unique to 2007, nine found in material from both time periods, and two only found in unsorted pre-1965 museum specimens but not again in 2007) (Appendix D). *Helina humilis* (Stein) is a new record for Canada while *Spilogona griseola* (Collin) is a new record for the Nearctic region. While I am confident that none of the 12 morphospecies listed here (Appendix D) are currently known from the Nearctic region, further examination will be necessary to determine their status as either new species or new records.

Non-overlapping species

When the full assemblages (2007 all and pre-1965) were compared, 54 species were unique to 2007 all and 15 to pre-1965 (Table 3.2). Of the 54 new records, 36 (67%) were not collected by net sweeping and all of those were collected by Malaise traps (eight also collected by pan traps) (Table 3.2). In addition, more than half of the new 2007 records were rare and collected as either singletons (18) or doubletons (10) (Table 3.2). Two of the 15 species unique to the pre-1965 data set could not be retraced in collection material but collecting dates for the remaining 13 species all fell within the date range of the 2007 inventory; of those, seven were found as singletons and three as doubletons (Table 3.2).

Thirty-two of the new 2007 records had enough data to assess their distribution range. Churchill (in 2007) was categorized as a locality within the known distribution range for 17 species (see Figure 3.5 for examples) and as the northern-most locality for 15 species (see Figure 3.6 for examples) (Table 3.2). For species only found in pre-1965, Churchill (in 1965) was categorized as within the known distribution range for nine species, as the northern-most locality for three species, and as the southern-most locality for two species (see Figure 3.7 for an example) (Table 3.2).

DISCUSSION

No major differences were found in estimated species richness and composition of Muscidae between historical and contemporary assemblages collected by net sweeping in Churchill. When all material collected by different methods in 2007 was pooled, 87% of historical species were recovered in the 2007 survey. These results contrast sharply with trends reported for microgastrine wasps of Churchill by Fernández-Triana *et al.* (2011), who reported a little more than a 40% increase in estimated species richness in their contemporary (2005-2007) vs historical assemblage (1940-1950) and the recovery of approximatly 50% of historical species in their contemporary survey. It is possible that specialized hymenopteran parasitoids might be more sensitive to recent environmental changes in the Churchill area than ecologically diverse muscid flies, especially if their lepidopteran hosts are also affected. However, Fernández-Triana *et al.* (2011) did not correct for differences in collecting methods between surveys, a factor which was found here to have an important impact on species richness and composition of muscid fly assemblages. In this research, although I controlled for collecting methods between surveys, it is likely that overall sampling efforts were different between time periods and that some habitats and microhabitats sampled have changed over time, as the Churchill landscape has been moderately but continuously altered by human activity since 1965 (Newton *et al.* 2002; Edye-Rowntree *et al.* 2006). Since occasional sites were selected in 2007 either because they were listed in the literature or for special habitat features, the slightly higher rarefied species richness of the contemporary assemblage collected by net sweeping compared to the historical assemblage may be an indication that a wider range of habitats and microhabitats was sampled in 2007 than in previous surveys.

Collecting techniques had a considerable influence on the detection, in 2007, of species collected pre-1965 with the addition of Malaise and pan traps to the survey design adding 26 species not collected by net sweeping. For most organisms, sampling methods undoubtedly affect not only the abundance of species in a sample but their composition as well (Longino *et al.* 2002). In higher Diptera, including Muscidae, Malaise traps have been reported to yield more specimens and a wider array of taxa than other collecting methods (Marshall *et al.* 1994; Savage *et al.* 2011), but I report here the first assessment and comparison of this collecting method with two others for muscid flies using species richness and composition. The difference in species composition between net sweeping and the other two methods can be explained, at least in part, because some muscid species and/or gender (especially males) are invariably more often collected using net sweeping due to specific behaviour such as hovering or swarming or because they are attracted to transient resources such as decomposing organic matter from which they can easily be swept.

I therefore conclude that Malaise traps and standardized net sweeping are sufficient to conduct surveys of muscid flies in northern habitats, but I would recommend at least 120 standardized sweeps instead of the 60 conducted here to increase specimen numbers collected by this method. Pan trap servicing can be quite labour intensive, as it must be done often to prevent overflowing or complete evaporation and involves the transport of large amounts of clean (and dirty) preserving fluid, an issue that can be highly impractical in remote northern localities with limited road access. However, in situations where Malaise traps cannot be properly operated such as in areas of high wind

exposure or when research questions require large numbers of replicated samples, pan traps may be necessary to complement net sweeping.

While the nature of my data sets restricted the statistical comparison of species richness and composition of assemblages between time periods to specimens collected by net sweeping, I assessed which factors (in addition to collecting method) might have influenced the presence of non-overlapping species between time periods for the complete pre-1965 and 2007 assemblages. The Malaise trap played an important role in the collection of many pre-1965 species missed by net sweeping in 2007 and in the large number of new species records for Churchill in 2007. I acknowledge that these observations are speculative, but I consider relevant to include them here as they address the specific ecological requirements of individual species rather than treat them as random effects. These observations should therefore be taken into account in the design and interpretation of results of future studies of northern muscid diversity.

I collected 54 new records for the area in 2007, but 36 of these were not collected by net sweeping and their absence in the pre-1965 assemblage is possibly related to collecting technique rather than an actual distribution change. Notably, all six species of *Mydaea* R.-D. collected in 2007 (including four new records) were relatively abundant in samples (ranging from three to 42 individuals), but not one of the 110 *Mydaea* specimens captured in 2007 was caught using net sweeping.

Among new records collected only by Malaise traps are two haematophagous species, the stable fly, *Stomoxys calcitrans* L. and the moose fly *Haematobia alcis* (Snow). The presence of the synanthropic stable fly in the 2007 assemblage is not surprising, as it is a widely distributed species with great dispersal capacities that may

breed in manure and feed on the blood of a wide range of vertebrates (Greenberg 1971). However, overwintering temperatures at Churchill are unsuitable for this species (Jones and Kunz 1997), so *S. calcitrans* is most probably an adventive species in the low Arctic region. The moose fly, on the other hand, is rarely collected and feeds exclusively on the moose (*Alces alces* L.) as adult and requires fresh moose dung for larval development. While the specimen collected from Churchill in 2007 currently represents the northernmost confirmed locality for *H. alcis* (Figure 3.8), the moose range extends further north into Nunavut (Feldhamer *et al.* 2003) where the moose fly may also be present.

Some of the new 2007 records caught by net sweeping are species known to be associated with or attracted to specific substrates. *Hydrotaea aenescens* (Wiedemann) (Figure 3.9), the synanthropic "dump fly", was collected by net sweeping at the municipal garbage depot. While garbage was sampled in Churchill by Webb (1956), the dump fly was not recorded then; the storage of human refuse (including recycling and compost) in a specialized building since 2004 (Eliasson 2004) might have provided warmer conditions favourable to the establishment of this species which, like *S. calcitrans*, might not be able to overwinter in the wild in Churchill (Hogsette *et al.* 2002). The dump fly was also collected, along with *Hydrotaea cristata* Malloch, on rotting grain piles at the occasional sites. While grain has been shipped through Churchill since 1931 (Taylor 1993), there are no references in the literature that the substrate was specifically sampled then, possibly explaining the absence in the historical assemblage of *H. cristata*, which is known from localities further north than Churchill (Table 3.2, Figure 3.5).

More than half of the new records for 2007 were found in samples as either singletons or doubletons. The trend was even more pronounced in taxa unique to the pre-1965 assemblage as only three of the 15 species were represented by three or more specimens. Documenting changes in distribution of species rare in samples, particularly when dealing with a fixed locality, requires a strict respect of the assumptions regarding repeatability of surveys such as collecting techniques and effort as well as collector's expertise (Shaffer *et al.* 1998). As some of these assumptions were relaxed in the present study, especially regarding sampling effort, it would be premature to translate the presence/absence of non-overlapping species represented by only one or two specimens into a permanent distribution change (especially if little is known of their ecological requirements) until more specimens are collected and/or traced in museum collections.

The position of Churchill relative to the Nearctic distribution range was evaluated for all non-overlapping taxa with at least three data points. While the position of Churchill as the northernmost locality for nearly half of the new records (15/32) might suggest a repeated pattern of northern range extension in the contemporary assemblage, once all rare taxa (two or fewer specimens) and those not collected by net sweeping were removed from the list, only four species still fit that pattern: *H. aenescens* (Figure 3.9), *Hydrotaea scambus* (Zetterstedt), *Coenosia mollicula* (Fallén) and *Phaonia serva* (Meigen) (Table 3.2, Figure 3.6). The possibility of a northern range extension in these taxa should be further investigated at other low Arctic localities, especially as they all differ substantially in both adult and larval requirements.

For taxa unique to the pre-1965 data set, all three species represented by more than two specimens had a distribution range extending north and south of Churchill in

1965. One interesting feature of the list of species unique to pre-1965 is the disproportionate representation of *Spilogona* species among them (73% compared to 35% for the pre-1965 assemblage as a whole). Unlike most other muscids in my data set, *Spilogona* and other members of the tribe Limnophorini breed in various damp or aquatic substrates, including boggy pools in fens (Skidmore 1985). While my results are in no way as marked as those of Axford *et al.* (2009) for high arctic chironomids, the dominance of aquatic and semi-aquatic taxa among those not collected again in 2007 suggests that the decrease in water cover reported for the area of Churchill over the last three decades (Ballantyne 2009) might have reduced the availability of breeding habitats for the Limnophorini. As northern freshwater ecosystems are expected to be drastically altered under current trends of climatic changes in the Arctic (Arctic Climate Impact Assessment (ACIA) 2004), I therefore recommend that this group be prioritized in further Diptera surveys in Churchill (and northern localities in general), not only to increase the probabilities of collecting some of the taxa unique to the pre-1965 data set but to monitor the population dynamics of a group closely associated with wet tundra environments.

Climate obviously has an impact on northern muscid fly biology, as the emergence phenology of muscid flies at a high Arctic locality in northern Greenland is now considerably earlier in the spring than a decade ago (Høye and Forchhammer 2008). However, it has been shown that adjustments in species richness and composition of biological communities may lag significantly behind climate changes (Menéndez *et al.* 2006). I found that the muscids of Churchill appear so far to have been largely resilient to climatic and anthropogenetic habitat changes in terms of species richness and composition at the regional level. Consequently, the new baseline data presented here

will enable the monitoring of Muscidae resilience in a future where environmental changes are predicted to intensify (IPCC 2007).

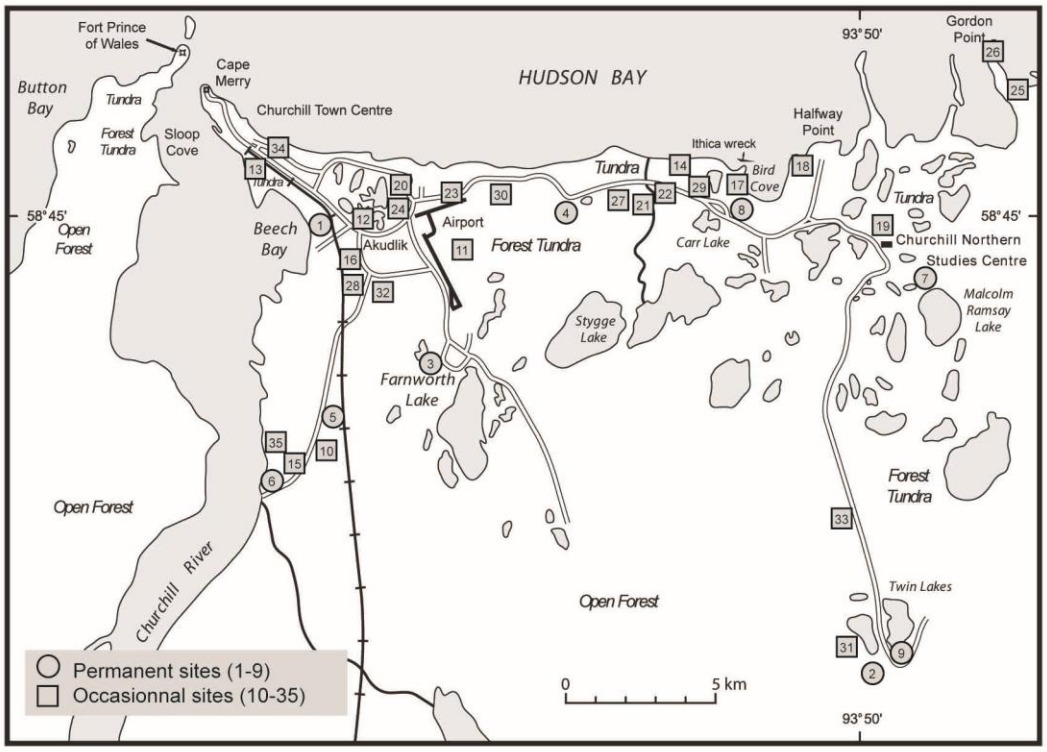


Figure 3.1. Map of Churchill, Manitoba, and location of the nine permanent sites and 26 temporary sites sampled for Muscidae in 2007. Original background map courtesy of Dr. P. Kershaw. See Appendix B for coordinates and name/description of sites identified by a number on the figure.

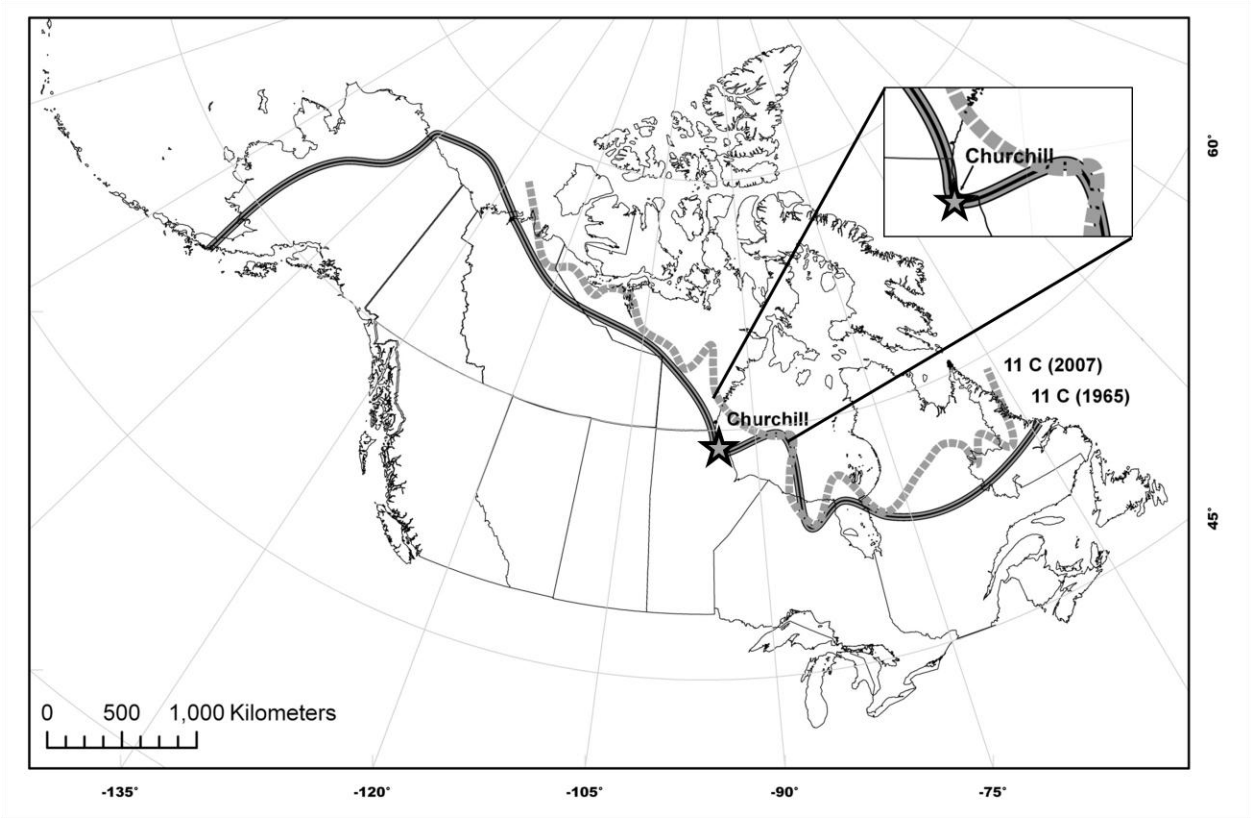


Figure 3.2. Canadian and Alaskan 11 ^{o}C July isotherms for 1965 (solid line) and 2007 (stippled line). Inset indicates that the 11 ^{o}C July isotherm has shifted by approximately 300 km north of Churchill between 1965 and 2007.

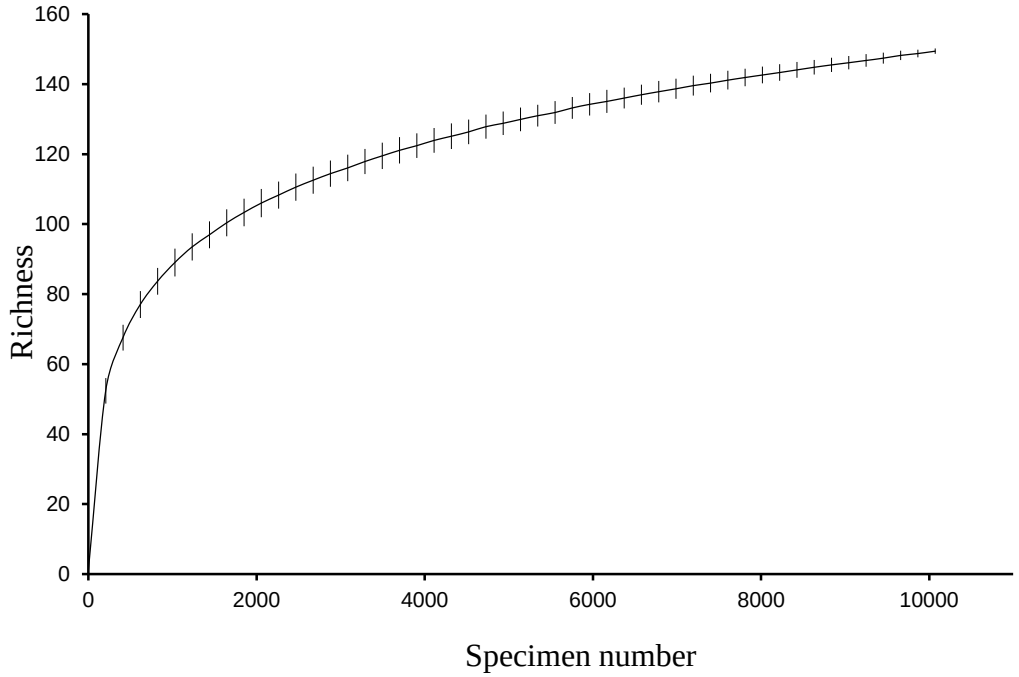


Figure 3.3. Individual-based rarefaction curve of species richness (mean ± 1 SD) of all Muscidae collected in Churchill in 2007 (2007 all). Species numbers reflect the merging of twelve species with indistinguishable females into six complexes.

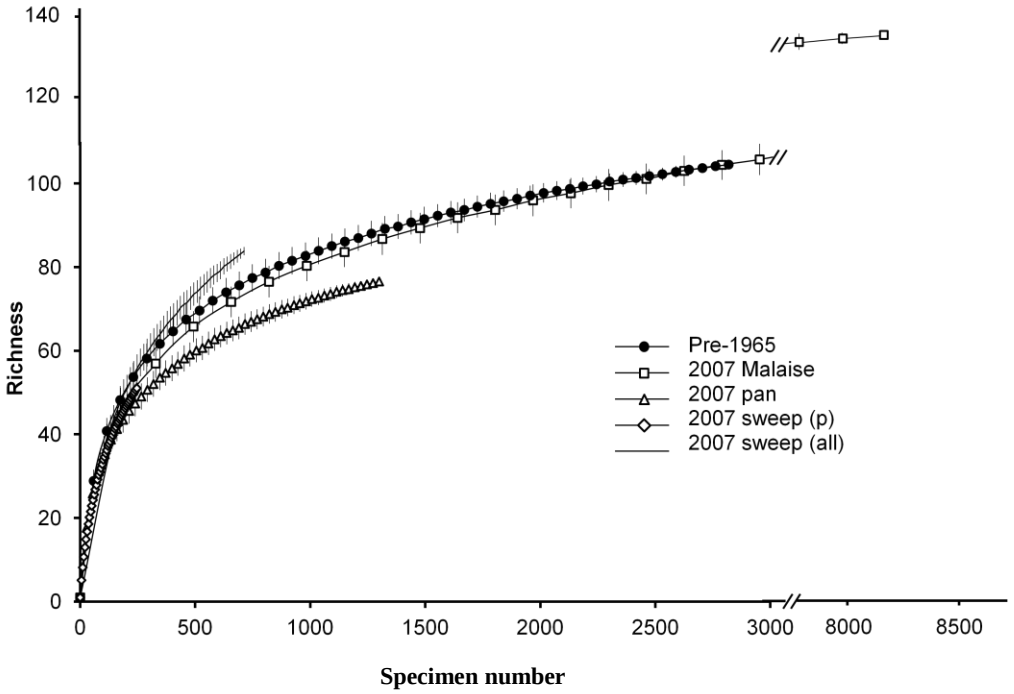


Figure 3.4. Individual-based rarefaction curves of species richness (mean ± 1 SD) of Muscidae found in pre-1965 museum material (pre-1965), collected in 2007 at the permanent sites by each sampling device (2007 Malaise, 2007 pan, 2007 sweep (p)), and collected in 2007 by net sweeping at both permanent and temporary sites (2007 sweep (all)) in Churchill (Manitoba) (see Table 3.1 for exact values). Species numbers reflect the merging of twelve species with indistinguishable females into six complexes. X-axis and 2007 Malaise rarefaction curve broken to improve visual display of rarefaction curves with fewer individuals.

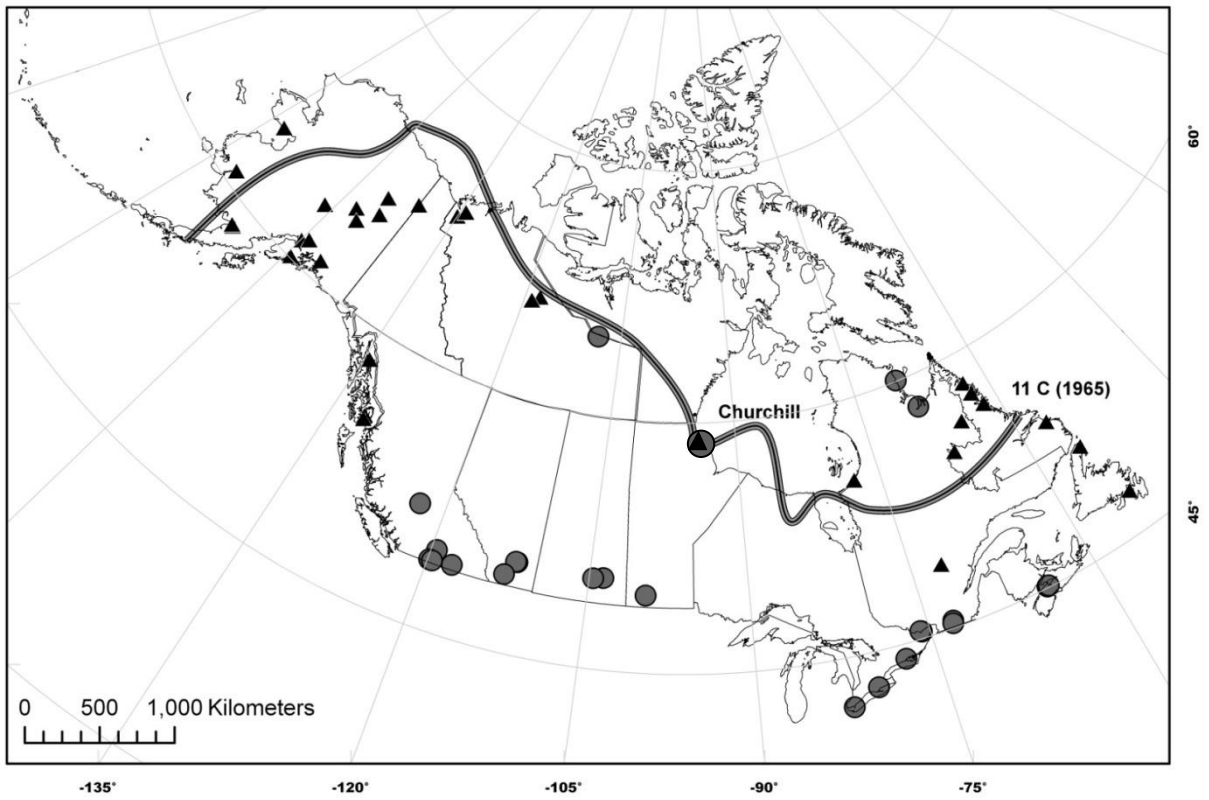


Figure 3.5. Distribution of *Hydrotaea cristata* Malloch (triangle) and *Hydrotaea floccosa* (Fallén) (circle) in Canada and Alaska. Solid line: 1965 11^{o}C July isotherm passing through Churchill (Manitoba).

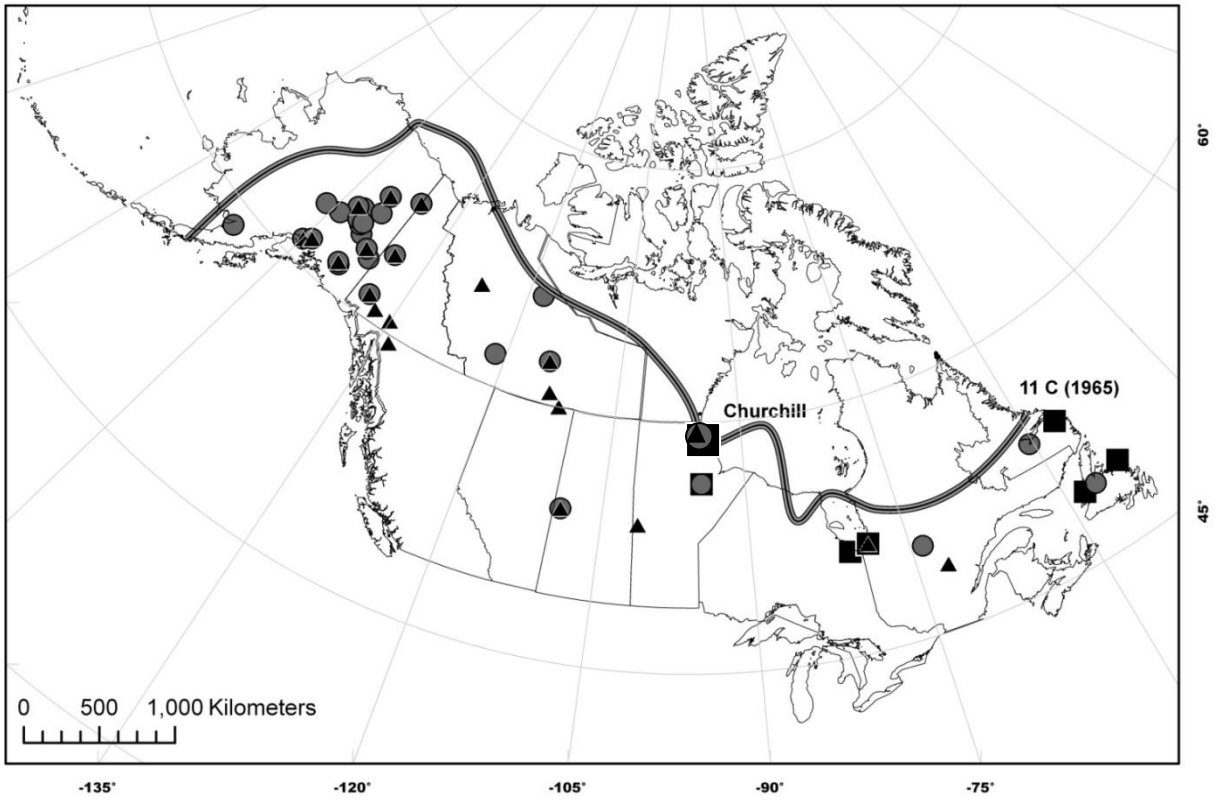


Figure 3.6. Distribution of *Hydrotaea scambus* (Zetterstedt) (triangle), *Coenosia mollicula* (Fallén) (circle) and *Phaonia serva* (Meigen) (square) in Canada and Alaska. Solid line: 1965 11°C July isotherm passing through Churchill (Manitoba).

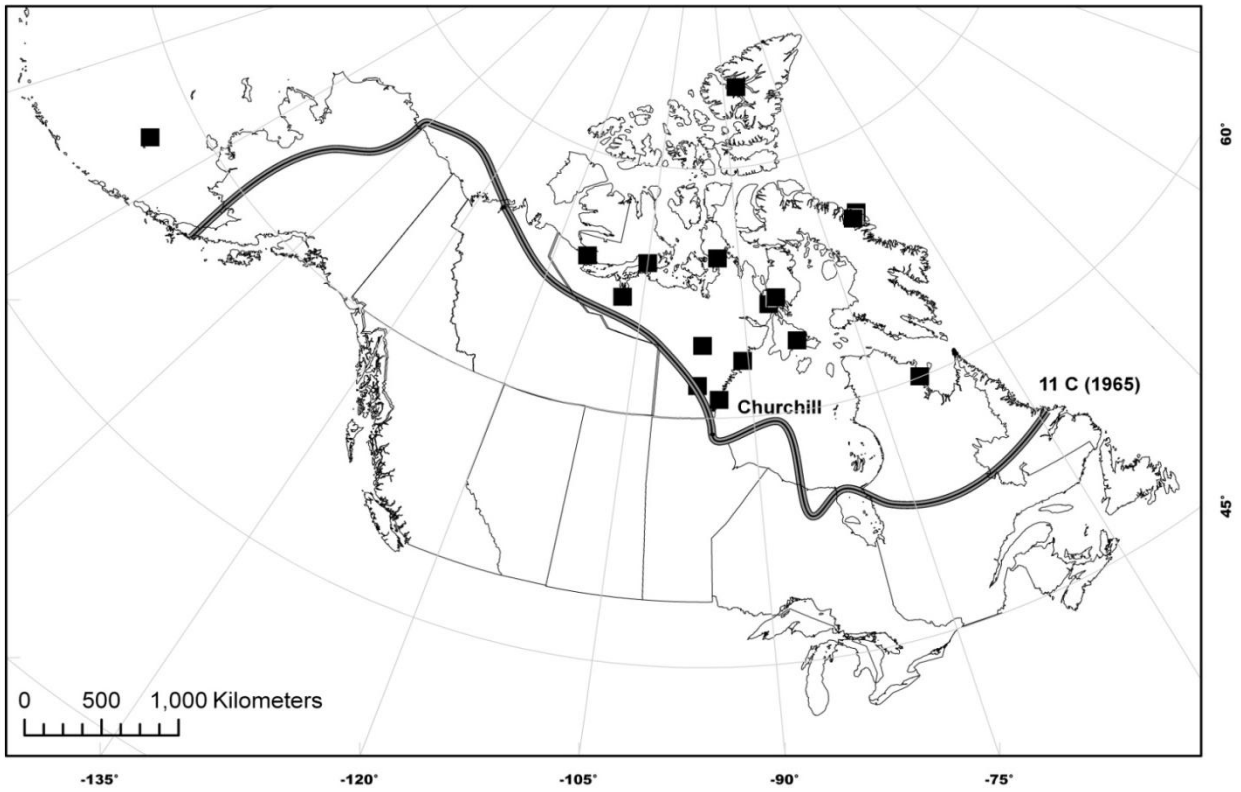


Figure 3.7. Distribution of *Drymeia segnis* (Holmgren) in Canada and Alaska. Solid line: 1965 11^{o}C July isotherm passing through Churchill (Manitoba).

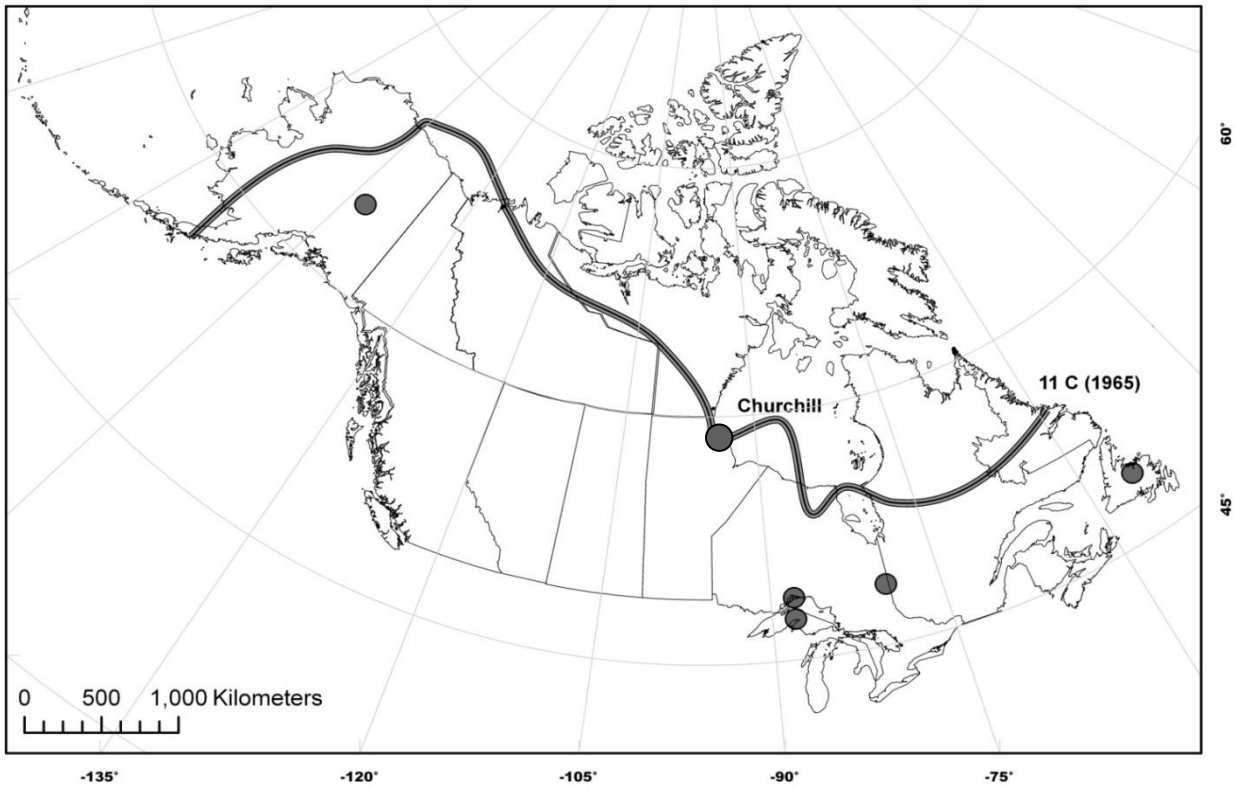


Figure 3.8. Distribution of *Haematobia alcis* (Snow) in Canada and Alaska. Solid line: 1965 11°C July isotherm passing through Churchill (Manitoba).

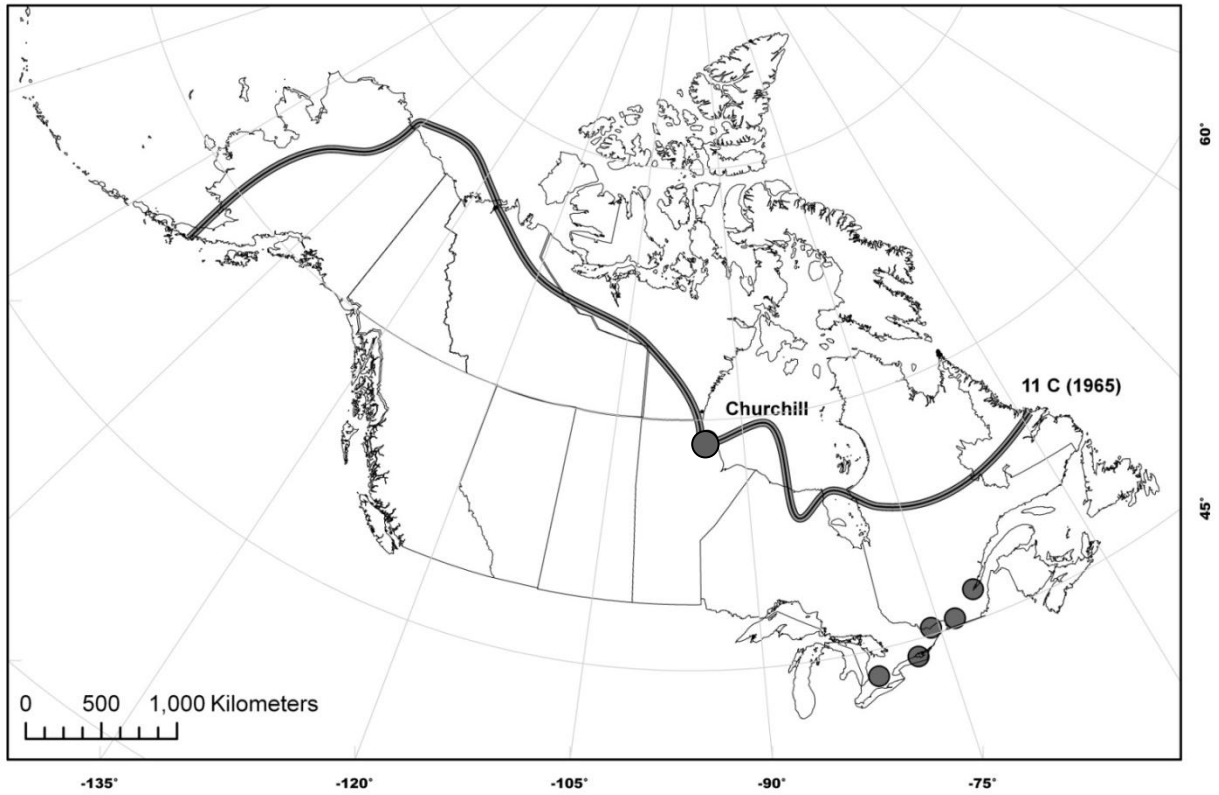


Figure 3.9. Distribution of *Hydrotaea aenescens* (Wiedemann) in Canada and Alaska. Solid line: 1965 11^{o}C July isotherm passing through Churchill (Manitoba).

Table 3.1. Total number of specimens (n), observed species richness (S_{obs}), unique species (S_{unique}), rarefied species richness ($S_{rarefied}$) (richness ± SD), ACE estimates of species richness and Chao 1 estimates of species richness with 95% confidence intervals (CI) of Muscidae found in pre-1965 museum material (pre-1965), collected in 2007 (2007 all), collected in 2007 at the permanent sites by each sampling device (2007 Malaise, 2007 pan, 2007 sweep (p)), and collected in 2007 by net sweeping at both permanent and temporary sites (2007 sweep (all)) in Churchill (Manitoba). Species numbers reflect the merging of twelve species with indistinguishable females into six complexes.

Data set	**n**	**S_{obs}**	**S_{unique}**	**$S_{rarefied}$**	**ACE**	**Chao 1 (CI)**
2007 all	10294	149	54*	---	178.2	179.0 (161.0-223.9)
2007 Malaise	8212	134	43**	52.7 ± 3.6[a]	163.5	154.3 (141.4-185.4)
2007 pan	1334	77	1**	49.5 ± 3.2[a]	89.4	87.7 (80.3-114.3)
2007 sweep (p)	270	53	6**	53.0 ± 0.0[a]	76.1	83.3 (63.5-140.3)
2007 sweep (all)	748	86	---	85.0 ± 0.0[b]	119.7	130.0 (102.7-199.6)
Pre-1965	2881	105	13*	77.1 ± 3.4[b]	121.9	120.4 (110.3-149.5)

* Species unique by temporal assemblage.

** Species unique by collecting method for 2007.

[a]Species richness rarefied at 270 individuals.

[b]Species richness rarefied at 740 individuals due to exclusion from calculations of species with no abundance data in the pre-1965 data set.

Table 3.2. List of non-overlapping species of Muscidae between all species found in pre-1965 museum material and/or listed in the literature for Churchill (Manitoba) and all species collected in 2007. Specimen abundance listed by collecting device (M: Malaise trap; P: pan trap; S: net sweeping) and position of Churchill relative to the Nearctic distribution of taxa known from at least three other Nearctic localities.

	Abundance			Churchill position		Abundance			Churchill position
	M	P	S			M	P	S	
Pre-1965 only					Pre-1965 only				
Drymeia segnis	-	-	N/A	Southern	*S. mydaeinaformis*	-	-	1	Within
Hebecnema vespertina	-	-	1	Northern	*S. norvegica*	-	-	2	Within
Lispe johnsoni	-	-	1	---	*S. nutaka*	-	-	10	Within
Lophosceles minimus	-	-	2	Southern	*S. obscura*	-	-	1	Within
Spilogona acuticornis	-	-	N/A	Northern	*S. pseudodispar*	-	-	2	Within
S. arenosa	-	-	4	Within	*S. quinquelineata*	-	-	1	Within
S. brevicornis	-	-	1	Northern	*S. tundrae*	-	-	4	Within
S. monacantha	-	-	1	Within					

Table continued on next page

Table 3.2. (concluded)

	Abundance			Churchill position		Abundance			Churchill position
	M	P	S			M	P	S	
New 2007 records					New 2007 records				
Coenosia frisoni	2	0	0	Northern	*P. protuberans*	36	20	2	Within
C. mollicula	73	11	1	Northern	*P. serva*	5	3	1	Northern
C. remissa	2	0	0	Northern	*Spilogona confluens*	2	0	0	---
Drymeia groenlandica	1	0	0	Within	*S. flavinervis*	4	0	0	---
Haematobia alcis	1	0	0	Northern	*S. forticula*	133	2	3	---
Helina fulvisquama	63	6	2	Within	*S. genualis*	1	0	1	Within
H. humilis	2	1	0	---	*S. griseola*	44	3	1	---
H. maculipennis	6	1	0	Northern	*S. incerta*	1	0	0	---
H. marguerita	1	3	0	Northern	*S. narina*	2	0	0	Northern
H. nigribasis	4	4	0	---	*S. reflecta*	10	0	0	Northern
H. spinosa	0	0	1	Within	*S. setipes*	1	0	0	---
Hydrotaea aenescens	0	0	10	Northern	*S. tornensis*	1	0	0	Within
H. cristata	0	0	7	Within	*S. trigonifera*	1	0	0	Within
H. floccosa	0	0	1	Within	*S.* sp. 1	2	0	0	---
H. pilitibia	3	0	1	Within	*S.* sp. 2	1	0	0	---
H. ringdahli	1	0	0	Within	*S.* sp. 3	1	0	0	---
H. scambus	2	0	1	Northern	*S.* sp. 4	1	0	0	---
Lophosceles impar	2	0	0	Northern	*S.* sp. 5	1	0	0	---
Limnophora sp. 1	44	39	6	---	*S.* sp. 6	1	0	0	---
Lispocephala tinctinervis	1	0	0	Northern	*S.* sp. 7	0	0	1	---
Mydaea furtiva	12	1	0	Within	*S.* sp. 8	1	0	0	---
M. obscurella	21	21	0	Within	*S.* sp. 9	1	0	1	---
M. occidentalis	12	5	0	Northern	*S.* sp. 10	3	0	0	---
M. pseudonubila	2	2	0	---	*S.* sp. 11	1	0	0	---
Phaonia apicalis	2	0	0	---	*Stomoxys calcitrans*	2	0	0	Within
P. atrocyanea	4	0	0	Within	*Thricops albibasalis*	26	6	1	Within
P. inenarrabilis	1	0	0	Northern	*T. diaphanus*	3	0	1	Within

Note: --- indicates that fewer than three data points were available for that species.

CHAPTER 4

DNA BARCODING OF THE MUSCIDAE (DIPTERA) OF CHURCHILL, MANITOBA, CANADA, REVEALS HIGH CORRESPONDENCE BETWEEN MORPHOLOGICAL AND MOLECULAR SPECIES LIMITS

ABSTRACT

The performance of DNA barcoding to discriminate between species of muscid flies was assessed based on sequences from 944 specimens from Churchill, Manitoba, Canada, and 32 from other regions. The Folmer region of the COI gene was an effective marker to identify 147 species of Muscidae, with 98.6% correspondence between barcode clusters and traditional morphology. Taxonomic reassessment of material from the four clusters with intraspecific distances greater than 2% revealed clear morphological differences in two of them and led, among other things, to the reinstatement of *Phaonia luteva* (Walker) to full species rather than as a subspecies of *Phaonia errans* (Meigen). Shared haplotypes between *Thricops septentrionalis* (Stein) and *Thricops spiniger* (Stein) indicate that either COI is not an appropriate marker to discriminate between these two morphologically distinct taxa or that they belong to one polymorphic taxon. Fifteen other barcode clusters, representing approximately 10% of species, had a minimum interspecific distance of less than 2% with their nearest neighbour, but these did not share haplotypes and clear morphological differences distinguished all of them. These results therefore indicate that minimum interspecific distance between muscid species is often lower than the 2% sequence divergence threshold generally applied to delineate insect species. DNA barcoding enabled the association of males and females in problematic taxa, the association of damaged or atypical specimens with their conspecifics, and the confirmation that some taxa in my data set are not currently recorded in the literature for the Nearctic region (new species or Nearctic records).

Keywords: insects, muscid flies, cytochrome *c* oxidase subunit 1 (COI), DNA barcodes, threshold.

INTRODUCTION

Biodiversity is often the framework of community ecology studies, and accurate species-level identification is essential to document and monitor the ecological significance of insect diversity (Krebs 2008; Sperling and Roe 2009). However, the important time investment associated with morphology-based identification, description and revision of species (Meir *et al*. 2006), as well as a severe lack of taxonomic expertise, have created a bias towards well-known and charismatic groups (Sheffield *et al*. 2009; Smith and Fisher 2009; Smith *et al*. 2009). Since taxonomic knowledge and identification tools are still weak or absent for many groups, especially among the hyper-diverse class Insecta, a number of alternatives to morphology-based identifications has been suggested. Among these is the integration of genetic data in the taxonomic workflow, and most especially DNA barcoding (Janzen *et al*. 2009; Smith and Fisher 2009; Zhou *et al*. 2010).

What is DNA barcoding

Various approaches have been developed to obtain molecular characters, especially DNA sequences, which could assist in the identification of species with insufficient or ambiguous morphological features (Sperling and Roe 2009). The name "DNA barcoding" was proposed by Hebert *et al*. (2003a) to indicate a large-scale and standardized method that uses species-level variations in a specific gene to discriminate between taxa. While different regions of the mitochondrial and nuclear genomes have been used for phylogenetic purposes, the most frequently used region to identify animal species is the 658 base-pairs "Folmer region" found at the 5' end of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) (Ratnasingham and Hebert 2007). The

Folmer region was selected as the main DNA barcoding region in animals because of its typically low variability within species combined with differentiation between species (Hebert *et al*. 2003a, b), and because it is easy to isolate in most groups as it is surrounded by regions of conserved sequences (Consortium for the Barcode of Life 2011).

DNA-based identification of a specimen depends on the comparison of its DNA barcode with those of expert-determined individuals (Hebert *et al*. 2003a). However, DNA barcoding is still a relatively new technique, and no reference DNA barcode libraries are available for most groups. The collaboration of expert taxonomists is therefore required to develop such reference libraries and test the ability of the method to discriminate between species (Hebert *et al*. 2003a). When reference sequences become available for public release (usually with publication of results for a specific work), they can be consulted through the following web sites: Barcode of Life Data System (BOLD) (www.boldsystems.org), Genbank at the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov), DNA Data Bank of Japan (DDBJ) (www.ddbj.nig.ac.jp), and the European Molecular Biology Laboratory (EMBL) (www.embl.org) (Ratnasingham and Hebert 2007). Among those listed, BOLD is unique in that it is an informatic workbench that focuses on DNA barcoding, allowing for the analysis of deposited sequences and the consultation of collection, photographic, and taxonomic information related to them (Ratnasingham and Hebert 2007).

Species identification with DNA barcoding

In DNA barcoding, various approaches are used to assess species limits. Among these are: the calculation of boostrap values to determine cluster support (Ward *et al*. 2005), the examination of the clustering patterns to uncover monophyletic and non-monophyletic taxa (Robinson *et al*. 2009), and the comparison of genetic divergences to a pre-selected threshold (Hebert *et al*. 2003b). Hebert *et al*. (2003b) determined that for insects intraspecific sequence distance rarely exceeds 2%, but a number of works published since then have pointed to different levels of maximum (and average) intraspecific divergence (Cai *et al*. 2005; Cywinska *et al*. 2006; Smith *et al*. 2006; Carew *et al*. 2007; Rivera and Currie 2009). For interspecific distance, 2% is usually the minimum level of divergence between two distinct species. To determine a threshold that accurately represents the whole range of variations present in the studied taxa, series of individuals from a range of geographic regions should be included in analyses (Moritz and Cicero 2004; Elias *et al*. 2007).

So far, DNA barcodes have been successful in the species identification of animals such as fishes (Ward *et al*. 2005), birds (Hebert *et al*. 2004b), mammals (Hajibabaei *et al*. 2007), and arthropods such as butterflies (Janzen *et al*. 2005; Hajibabaei *et al*. 2006), black flies (Diptera: Simuliidae) (Rivera and Currie 2009), tachinid flies (Diptera: Tachinidae) (Smith *et al*. 2006), parasitoid wasps (Hymenoptera) (Smith *et al*. 2008; Fernández-Triana *et al*. 2011), bees (Hymenoptera: Apidae) (Sheffield *et al*. 2009), spiders (Arachnida) (Barrett and Hebert 2005; Robinson *et al*. 2009), ants (Hymenoptera: Formicidae) (Smith and Fisher 2009), mayflies (Ephemeroptera),

stoneflies (Plecoptera), caddisflies (Trichoptera) (Zhou *et al*. 2010), and springtails (Collembola) (Hogg and Hebert 2004).

Despite the good performance of DNA barcoding reported across many taxa, it has failed to discriminate species in a number of instances. For example, DNA barcoding had limited success in the identification of taxa such as the Ithomiinae (Lepidoptera: Nymphalidae) (Elias *et al*. 2007; Dasmahapatra *et al*. 2010), *Copelatus* Erichson (Coleoptera: Dytiscidae) (Monaghan *et al*. 2006), and *Protocalliphora* Hough (Diptera: Protocalliphoridae) (Whitworth *et al*. 2007). The poor performance of DNA barcoding in those studies was attributed to recent speciation events generating small molecular distance between species and overlaps between intraspecific and interspecific distances (Monaghan *et al*. 2006). The presence of *Wolbachia* Hertig infection, which appears to cause mitochondrial DNA introgression (the insertion of other species alleles in the genome of an organism (Frankham *et al*. 2004)) thereby preventing the proper definition of molecular species boundaries, was also invoked to explain overlapping or small genetic distances (Whitworth *et al*. 2007).

In spite of uneven performances across taxa, DNA barcoding as an identification tool has proven to be valuable for biodiversity monitoring, especially when used in association with species morphology (Smith *et al*. 2006; Zhou *et al*. 2010; Fernández-Triana *et al*. 2011) and ecology (Hebert *et al*. 2004a; Smith *et al*. 2007, 2008) instead of as a complete surrogate for specimen identification.

In addition to the utility of DNA barcoding for recognizing known species, it has been used in the delimitation of biological entities through the detection of cryptic species (Hebert *et al*. 2004a; Smith *et al*. 2007; Zhou *et al*. 2010; Fernández-Triana *et al*.

2011), which are genetically distinct taxa with no discernable morphological differences, and polymorphic species (Rivera and Currie 2009), which are genetically homogeneous taxa with significant morphological variations. DNA barcodes have also been useful to associate conspecific individuals of different life stages (Rivera and Currie 2009) or gender (Li *et al*. 2010).

As of 2010, close to 800,000 high quality arthropod DNA barcodes (at least 500-bp long) were available in the BOLD database (www.boldsystems.org) but only 92,000 of these were determined to species level. Diptera was the third most sequenced and identified order of insects, with 26,860 out of approximately 65,000 available sequences determined to species level. Despite the relatively high number of sequences available on BOLD for some groups such as tachinid flies (>18,000 determined sequences), most have received little or no taxonomic attention due primarily to a lack of taxonomic expertise and ressources. Muscid flies, for example, only had 255 out of 3200 available sequences identified to species as of 2010.

Muscidae

The Diptera family Muscidae is a large and ecologically diverse taxon containing over 5100 species worldwide (Foottit and Adler 2009) and at least 700 in the Nearctic region (Huckett and Vockeroth 1987). Muscid flies can be found in a broad range of terrestrial and aquatic habitats but they are especially diverse and abundant in northern and alpine environments. In northern Canada and Alaska, they represent about a quarter of all Diptera species and close to 10% of overall insect diversity (Danks 1981). Adults can be saprophagous, predaceous, haematophagous, or anthophilous, while immatures are

mostly saprophagous and/or predaceous (Skidmore 1985). In most habitats, especially in northern environments, muscids provide ecological services such as pollination, decomposition, predation, and serve as a food source for other vertebrate and invertebrate animals (Larson *et al*. 2001; Savage 2002; Vockeroth 2002; Courtney *et al*. 2009). But in spite of their beneficial ecological contributions, muscid flies are mostly renowned as medical, veterinary and agricultural pests, such as the house fly, *Musca domestica* Linnaeus, the stable fly, *Stomoxys calcitrans* (Linnaeus), and various shoot flies of the genus *Atherigona* Rondani.

For Muscidae, as for many other Diptera, adult identification is based on chaetotaxy (the number and organization of various bristles), wing venation, and genitalic structures (Huckett 1965a; Huckett and Vockeroth 1987). Their morphology-based identification is often difficult, especially for non-experts, and may require genitalic dissections, which require a substantial amount of time. The identification of adult Muscidae is further complicated by sexual dimorphism (different criteria are therefore required to identify specimens for each gender) and by the lack of morphological features to differentiate females of some species. The problematic association of conspecific specimens belonging to different genders may in turn be further exacerbated due to the fact that some species are only described based on males. The lack of well-illustrated, easy-to-follow identification keys and unambiguous original or revised descriptions (including genitalic illustrations for both sexes) in some genera also complicate their identification, potentially leading to identification dead-ends or errors.

In spite of the great potential of DNA barcoding for biodiversity monitoring (Hajibabaei *et al*. 2007), the few published studies involving muscid flies and COI used

sequence data to perform phylogenetic analyses (Savage *et al*. 2004; Schuehli *et al*. 2004, 2007; Kutty *et al*. 2008), compare haplotype diversity between populations (Cummings and Krafsur 2005; Tulio de Oliveira *et al*. 2005; Marquez *et al*. 2007), and identify necrophagous species in forensic entomology (Cai *et al*. 2005). Unfortunately, these studies generally focused on COI fragments other than the Folmer region, and thus the comparison of these published sequences with those available on BOLD is only possible for genetic areas where they overlap. Moreover, all of these studies included only a few species, each often represented by one individual, therefore preventing the rigourous assessment of species limits for closely related taxa and the calculation of intraspecific distances.

A muscid survey conducted in 2007 in Churchill, Manitoba (see Chapter 3) yielded a large number of fresh specimens. From this material, over 10,000 specimens were initially identified to species level based on morphology alone. Several specimens collected in 2007 could not be identified to species because their morphology did not correspond to available descriptions or because they were too damaged. Moreover, female specimens of twelve species could not be distinguished from close relatives, and these had to be combined into six species complexes in all analyses of species richness and composition requiring abundance data. The identification of the Churchill material was further complicated because species from northern populations often present anatomical variations related to cold-climate adaptations (*e.g*. darker colouration) not seen in populations from temperate regions (Danks 1981).

Objectives

Because DNA barcoding has failed to delimit/identify species in some taxa, it is important to assess its performance before it can be used as a reliable molecular marker to identify Muscidae. Consequently, by conducting the first large-scale barcoding study on this family, the primary objective of this work was to assess the performance of DNA barcoding in the discrimination of northern Nearctic muscid fly species, primarily from Churchill, by determining the levels of correspondence between morphology-based species limits currently accepted in the literature and DNA barcoding clusters. The second objective was then to use the extensive reference library of DNA barcodes generated in this work and its accompanying information pertaining to intraspecific and interspecific distances to address taxonomic problems such as cryptic or polymorphic taxa, anatomical variations related to cold-climate adaptations, and male-female associations. In addition, by sequencing the muscid fauna of Churchill, this study will contribute to the understanding of the local fauna of a very rich arctic region.

MATERIAL & METHODS

Specimen selection

A total of 1092 muscid specimens representing 24 genera were selected for sequencing of the Folmer region of COI with a minimum of two males and two females of each species included whenever possible; more specimens were included for problematic taxa. Most specimens were selected among material identified in Chapter 3 and therefore collected in 2007 in Churchill, Manitoba, Canada (see Chapter 3 for collection protocol). In order to increase the number of individuals belonging to rare or problematic species and to investigate whether the addition of material from geographically distinct populations would increase levels of intraspecific variation, the 1036 Churchill specimens were supplemented with 56 specimens collected between 2005 and 2008 from various Nearctic and Palearctic localities (Appendix E). Voucher specimens were deposited in the Bishop`s University Insect Collection, Sherbrooke, Québec, Canada (BUIC), the J.B. Wallis/R.E Roughley Museum of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada (JBWM), and the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada (CNC).

DNA processing and alignment

Leg-tissue samples consisting of one (occasionally two) legs were removed from specimens and deposited in 96-well glass fibre plates prefilled with 30 µl of 95% ethanol using a channel pipettor (International Barcode of Life Project 2008). All instruments used to remove leg tissues were cleaned in 70% ethanol and sterilized by flame between

each specimen. DNA was extracted from tissue samples with an automated process for invertebrates at the Canadian Centre for DNA Barcoding (CCDB), University of Guelph, Guelph, Ontario, Canada, following standard protocols (Ivanova *et al*. 2006, 2007). The Folmer region of COI was amplified using LepF1/LepR1 primers. When these primers failed to amplify full-length sequences, the following alternatives were used: LCO1490_t1/HCO2198_t1, LepF1/C_ANTMR1D, MLepF1/HCO2198_t1, MLepF1/LepR1, LepF1/MLepR1 (see Table 4.1 for details). For a 12.5 µl reaction, the Polymerase Chain Reaction (PCR) mixture included: 6.25 µl 10% trehalose, 2 µl ddH_20, 1.25 µl 10X buffer, 0.625 µl 50 mM $MgCl_2$, 0.125 µl 10 mM primer A, 0.125 µl 10 mM primer B, 0.0625 µl 10 mM dNTPs, 0.06 µl Polymerase (5 U/ µl) (Platinum® *Taq* dNA Polymerase form Invitrogen™), and 2 µl per well of DNA extract (Ivanova and Grainger 2007a). The thermocycle program (Mastercycler® ep gradient) was conducted under the following conditions: initial denaturation at 94 ºC (1 minute); 5 cycles of 94 ºC (30 seconds), annealing at 45-50 ºC (40 seconds), and extension at 72 ºC (1 minute); 30-35 cycles of 94 ºC (30 seconds), 51-54 ºC (40 seconds), and 72 ºC (1 minute); and extension at 72 ºC (10 minutes) followed by an indefinite hold at 4 ºC (Ivanova and Grainger 2007a).

PCR products were checked with agarose gel electrophoresis (2% agarose E-gel® 96 gel) before being sent to cycle sequencing (Ivanova and Grainger 2007a). Sequencing reaction mix containing 0.25 µl Big Dye v3.1, 1.875 µl 5 X Sequencing Buffer, 5 µl 10% trehalose, 1 µl 10 µM Primer and 0.875 µl H_20 (Inavova and Grainger 2007b) to which 2 µl of unpurified and diluted (four times) PCR product were added. Cycle sequencing was run in Eppendorf thermocyclers with the following program: 96 ºC (1 minute); 15 cycles

of 96 ºC (10 seconds), 55 ºC (5 seconds), and 60 ºC (1 minute, 15 seconds); 5 cycles of 96 ºC (10 seconds), 55 ºC (5 seconds), and 60 º C (1 minute, 45 seconds); an addition extension at 60 ºC (15 seconds); 15 cycles of 96 ºC (10 seconds), 55 ºC (5 seconds), and 60 º (2 minutes); and final extension at 60 ºC (1 minute) (Ivanova and Grainger 2007b). The EdgeBio® AutoDTR™ 96™ cycle sequencing clean-up was done before final products were analyzed with a 3730xl DNA Analyzer (Applied Biosystems) (Ivanova and Grainger 2007b).

As suggested in Hajibabaei *et al*. (2005), a preliminary sample of 104 specimens was first sent to the CCDB to test the performance of the standard amplification protocol before the complete set of samples could be processed. To assess the influence of killing agents (70 or 95% ethanol, Dichlorvos insecticide cubes (Vapona®), solution of propylene glycol + soapy water, or sodium cyanide/ethyl acetate) and drying techniques (air dry or hexamethyldisilazane (HMDS) (see Brown 1993 for protocol)) on DNA quality, the preliminary sample contained specimens killed and dried with each agent/technique (see Table 4.2 for killing agents and drying techniques of these 104 specimens).

Only high quality sequences of at least 600 bp and containing less than 1% missing nucleotides (Ns) were retained for data analysis to reduce intraspecific variations due to sequence length (Ratnasingham and Hebert 2007). Sequences, specimen photographies, taxonomic data, and collection data of specimens barcoded and included in this work are available in BOLD (www.boldsystems.org) and sequences also on Genbank (www.ncbi.nlm.nih.gov/genbank) (see Appendix E for BOLD Sample ID numbers and GenBank accession numbers). Since 134 additional high quality COI

sequences from muscid specimens collected at Churchill between 2005 and 2008 and already deposited in the voucher specimen repository of the Biodiversity Institute of Ontario, University of Guelph (BIOUG Collection), were available, these were added to my data set. All sequences from the final data set were translated using the invertebrate mitochondrial code, and manually aligned in Mesquite version 2.74 (Maddison and Maddison 2010). The alignment was subsequently uploaded to BOLD (Ratnasingham and Hebert 2007) and MEGA version 5 (Tamura *et al*. 2011) for data analysis.

Data analysis

Sequence composition was analyzed in MEGA using the *Nucleotide composition* and the *Nucleotide pair frequencies* applications. Mean frequencies (%) of each nucleotide and pairs of nucleotide (A + T and C + G) were calculated to evaluate if nucleotide frequencies were comparable to those typical of insects in general for this gene region.

A Neighbor-joining (NJ) tree was built in MEGA for the original data set using the default parameters of BOLD: Kimura 2-Parameter distance model (Kimura 1980) with pairwise deletion of gaps/missing data and inclusion of all substitutions (transitions and transversions). These parameters are recommended by Nei and Kumar (2000) when missing data or gaps are not distributed evenly among aligned sequences as in the case of this data set. Cluster monophyly was assessed to determine the performance of COI in the recovery of morphological species limits and individual branch support was assessed by bootstrapping with 1000 replicates (Pattengale *et al*. 2009) (support considered high for values of 95% and higher). Monophyly at the generic level was also assessed to

determine if generic identification of a specimen with no reference barcode could be obtained based on the identification of its neighbours. Genetic distances, based on the same parameters as those used for building the NJ tree, were computed in BOLD and confirmed in MEGA. Intraspecific distances were calculated on a data subset composed of all species with two or more individuals, while interspecific distances (distance to nearest neighbour) were calculated with all taxa included.

To assess if the addition of specimens from localities other than Churchill had an influence on intraspecific distances, maximum intraspecific distances calculated with and without specimens from other regions were compared using randomized permutations in PERM (Duchesne *et al*. 2006) (permutations = 1000, iterations = 10) for all species with material from at least two localities.

As the efficacy of DNA barcoding to discriminate between species lies in the capacity of the chosen marker to display levels of intraspecific variability that are lower than the minimum distance to its closest relative, maximum intraspecific distances were plotted against minimum interspecific distances for species with two or more individuals. Specimens of all taxa with maximum intraspecific distance > minimum interspecific distances were reassessed morphologically to investigate for: 1) potential identification mistakes, 2) the presence of morphologically distinct species not currently recognized in the literature, and 3) polymorphic species (morphologically distinct individuals sharing identical sequences). Following the recommendations of Hajibabaei *et al*. (2007), I also reassessed specimens of all taxa with more than 2% intraspecific distance to investigate if they were morphologically homogeneous, and all those with less than 2% interspecific distance to determine if they were morphologically distinct. Cluster structure and

bootstrap values for taxa with intraspecific distance above 2% was also examined to identify the presence of genetically different but morphologically homogeneous lineages that may represent cryptic taxa (Zhou *et al*. 2010).

Following morphological reassessment of specimens belonging to the categories described above, decisions were made as to their taxonomic status and specimen determinations were adjusted accordingly. All genetic distances were recalculated and the number of haplotypes per species was determined using the DNA barcoding tools available at www.ibarcode.org (Singer and Hajibabaei 2009). A new NJ tree reflecting the taxonomic reassessment was built in MEGA with the graphic output showing taxa (instead of individuals), the number of haplotypes per taxon, and the number of sequences for each haplotype. To assess whether the number of sequences per taxon had an influence on maximum intraspecific distances and the number of haplotypes per taxon, linear regressions were performed in Excel (Microsoft Excel 2007).

RESULTS

As COI amplified and sequenced successfully in 100 of the first 104 specimens (96.4%) sent to the CCDB, the remaining material could be processed without changes to the standard protocol (Hajibabaei *et al*. 2005) (Table 4.2). Sequences were obtained from 100% of specimens dried with HMDS, regardless of the killing agent used, and from 92.8% of air-dried specimens (excluding specimens collected in cyanide/ethyl acetate) (Table 4.2). Success rate by killing agent for air-dried specimens ranged from 89% (propylene glycol + soapy water) to 100% (95% ethanol) (Table 4.2). Sequencing results have been received for 997 of the 1092 specimens originally submitted to the CCDB (Appendix E). Data for the last plate, filled with 95 specimens, were not yet available at time of thesis submission. Sequencing procedure was successful for 883 of the 997 specimens sequenced (Appendix E). Among the 114 specimens that failed to amplify properly were 61 specimens that were air-dried and 53 chemically dried with HMDS. Of these 114 specimens that were air-dried or chemically dried, some were small individuals (47) for which the amount of tissue sampled (1-2 leg(s) from individual with body size less than 4 mm long) might have been inadequate or specimens from museum collections (30) collected before 2001. None of the 883 successful amplifications had more than 1% missing nucleotides, but 41 were less than 600 bp long and were therefore excluded. The combination of the remaining 842 sequences, 810 from Churchill and 32 from other localities, with the 134 sequences already available on BOLD resulted in a final data set of 976 sequences representing 147 species (based on morphological concepts) from 23 genera (Appendix F).

Inspection of the final alignment revealed no stop codons, insertions, or deletions. Mean nucleotide content of COI sequences was: A (29.9%), T (39.3%), C (15.3%), and G (15.4 %). As reported for some Muscoidea (Schuehli *et al*. 2007) and other dipteran mitochondrial sequences (Hebert *et al*. 2003a; Rivera and Currie 2009), A + T (69.2%) was in higher proportion than C + G (30.8%).

Ten of the 12 species combined into species complexes in Chapter 3 clustered as distinct taxa on the NJ tree (Appendix F), therefore allowing for: 1) the distinction of the very similar *Schoenomyza dorsalis* Loew and *Schoenomyza litorella* (Fallén), and 2) the association of females that could not be associated to their conspecific males in *Coenosia tarsata* Huckett, *Coenosia verralli* Collin, *Limnophora nigripes* (R.-D.), *Limnophora rotundata* Collin, *Phaonia consobrina* (Zetterstedt), *Phaonia rugia* (Walker), *Spilogona atrisquamula* Hennig, and *Spilogona pusilla* Huckett. *Thricops septentrionalis* (Stein) and *Thricops spiniger* (Stein) formed a single cluster and even shared some identical haplotypes.

Once the identity of all individuals belonging to the species listed above was confirmed, congruence between morphology and molecular species limits on the NJ tree (Appendix F) was found in 145 of the 147 morphologically defined taxa (98.6%), the only exceptions being *T. septentrionalis* and *T. spiniger*. All individuals belonging to the 120 taxa represented by more than one specimen grouped with their conspecifics and the NJ tree (Appendix F) confirmed that 14 distinct morphospecies that could not be associated with valid names were genetically distinct from all named taxa in the data set.

Average bootstrap support of species clusters was high (99.3%), with only *Spilogona atrisquamula* (55%) and *Spilogona suspecta* (Zetterstedt) (93%) exhibiting

values below 95%. Recovery of generic limits was poor, with only six of the 15 genera represented by more than one species appearing as monophyletic (*Limnophora* R.-D., *Lispe* Latreille, *Schoenomyza* Haliday, *Lispocephala* Pokorny, *Drymeia* Meigen, *Muscina* R.-D.). The remaining nine, *Thricops* Rondani, *Hydrotaea* R.-D., *Lophosceles* Ringdahl, *Coenosia* Meigen, *Spilogona* Schnabl, *Pseudocoenosia* Stein, *Phaonia* R.-D., *Helina* R.-D. and *Mydaea* R.-D. were either paraphyletic or polyphyletic, with some represented by up to three separate apparent clades (Appendix F).

Pairwise intraspecific distances calculated for the 120 taxa with two or more individuals in the original data set ranged from 0 to 4.25% (mean of 0.2%) (Appendix G). The inclusion of 24 sequences from localities other than Churchill did not significantly alter the maximum intraspecific distance (one-tail permutation test: $P = 0.5$) of the 12 taxa to which they belonged. Four taxa had a maximum intraspecific divergence over 2%: *Helina evecta* (Harris), *Phaonia errans* (Meigen), *Spilogona contractifrons* (Zetterstedt), and *S. atrisquamula* (Figure 4.1a, Appendix G).

Interspecific distances to nearest neighbour in the original data set ranged from 0 to 11.35% with a mean of 4.90% when all 147 species were included. Fifteen taxa had less than 2% interspecific distance with their nearest neighbour (Appendix G).

Maximum intraspecific distance was higher than minimum interspecific distance in four taxa: *S. atrisquamula*, *S. contractifrons*, *T. septentrionalis*, and *T. spiniger* (Figure 4.1a, Appendix G).

Taxonomic reassessment

All specimens belonging to the 15 taxa with less than 2% interspecific distance to their nearest neighbour (Appendix G) were reassessed, and clear morphological differences were found to distinguish all of them (at least in the males) except for *T. septentrionalis* and *T. spiniger*, who were also the only ones sharing identical haplotypes (Figure 4.1a, Appendix F). Either COI does not discriminate between these two species or they are members of one polymorphic species (there are marked external morphological differences between males of both taxa, but females cannot be separated (Savage 2003)). Therefore all specimens belonging to these taxa were pooled together under the name *T. septentrionalis*/*spiniger* in order to recalculate distance measures.

In addition to *T. septentrionalis* and *T. spiniger*, four other taxa had maximum intraspecific distance > minimum interspecific distance and/or intraspecific distance > 2% (Figure 4.1a). These were also reassessed to look for morphological variation within clusters. Subtle but consistent morphological differences in external characters and male genitalia were found between specimens belonging to the two internal clusters of *S. contractifrons* (Appendix F), one corresponding to the description of the nominal species, therefore renamed *S. contractifrons*, and the second renamed *Spilogona* sp. 12 as it did not correspond to any known Nearctic or Palearctic species (Figure 4.2). A similar pattern was found for the two internal clusters of *P. errans* (Appendix F), which were renamed *P. errans* and *Phaonia luteva* (Walker) (Figure 4.2), as material from each barcode cluster corresponded to a distinct Nearctic subspecies or variety of *P. errans* recognized by various authors (Malloch 1923; Huckett 1965b; but see Huckett 1965a for synonymy details). Conversely, no morphological differences could be found between the

three internal clusters of *H. evecta* and the two of *S. atrisquamula* (Appendix F). As both of these taxa formed monophyletic barcode clusters on the NJ tree (Appendix F) and displayed no more than 2.5% maximum intraspecific distance (Figure 4.1b), I elected to maintain the original determination of their specimens until more molecular and/or ecological data are available to support the recognition of morphologically cryptic species for their internal lineages (Figure 4.2).

Taxonomic changes implemented following the reassessment of taxa with a high intraspecific distance and/or low interspecific distances brought number of species to 148 (121 of which were represented by two or more individuals). Pairwise intraspecific distances in the post-reassessment data set decreased slightly, now ranging from 0 to 2.5% (average 0.18%) (Figure 4.3b), whereas distance to nearest neighbour remained similar on average (4.88%) but now ranged from 1.23 to 11.35% (Figure 4.3a). Seventeen species shared less than 2% interspecific distance with their nearest neighbour (Figure 4.1b, Appendix G). Maximum intraspecific distance was slightly higher than minimum interspecific distance in only two taxa, *T. septentrionalis*/*T. spiniger* and *S. atrisquamula*, and this last species, along with *H. evecta*, were the only two with an intraspecific distance greater than 2% (Figure 4.1b, Appendix G).

Of the 121 taxa represented by two or more individuals, 37 (30.58%) were represented by one haplotype, 42 (34.71%) by two, and the remaining 42 (34.71%) were represented by three or more haplotypes. The number of sequences per taxon was slightly correlated with an increase in maximum intraspecific distances ($R^2 = 0.09$, $P < 0.01$) and more strongly correlated with an increase in the number of haplotypes per taxon ($R^2 = 0.32$, $P < 0.01$).

Of the 148 taxa mentioned in this Chapter, 143 were also listed in Chapter 3; 12 species were only listed in Chapter 3 as they failed to produce a sequence, and five additional species, *Coenosia* sp. 1,*Hydrotaea* sp. 1, *Mydaea flukei* Snyder, *Phaonia luteva* (Walker) and *Spilogona* sp. 12 were mentioned for the first time in this Chapter.

DISCUSSION

DNA amplification and sequencing process

Success of the amplification and sequencing process is considered high when DNA barcodes (sequences with a minimum length of 500 bp and less than 1% ambiguous or missing nucleotides (Ratnasingham and Hebert 2007)) are obtained for more than 95% of the samples (Hajibabaei *et al*. 2005). The high sequencing success observed in the test assemblage for chemically dried specimens regardless of killing agents and air-dried material preserved in 95% ethanol (Table 4.2) are consistent with those of Austin and Dillon (1997). But while the overall amplification was successful at 96.4% for the test assemblage, it dropped to 89% for the complete data set. Unlike trends observed in the test assemblage (Table 4.2), failures to amplify in the complete data set do not appear to be related to drying conditions, as among the 114 sequences that failed to amplify, 53.5% were air-dried and 46.5% chemically dried. However, the influence of killing agent was difficult to assess for specimens outside of the test assemblage, as this information was not recorded for all individuals. Small specimen size and more than eight years elapsed since collection date appear to be the two main reasons for amplification failures in the complete data set, and these factors were also found to have a negative influence on amplification success rates in other insects (Dillon *et al*. 1996; Nagy *et al*. 2010; Van Houdt *et al*. 2010). In future barcoding studies of muscid flies, specimens smaller than 4 mm might require the removal of more than one leg to obtain the minimum amount of tissue required for amplification or, as a less destructive but more labour intensive alternative, a second amplification of the original PCR product. Hajibabaei *et al*. (2005) recommended the primer should be redesigned when amplification success is low. Since

about a quarter of the specimens that failed to amplify were museum specimens older than eight years, the development of degenerated primers (a combination of primers) allowing for the production of overlapping sequences (Van Houdt *et al*. 2010; Virgilio *et al*. 2010) would possibly allow the recovery of full DNA barcodes for these specimens. It has also been suggested that mini-barcodes, smaller fragments of approximately 350 bp, could also be successfully used to identify older specimens (Hajibabaei *et al*. 2005; Meusnier *et al*. 2008; Virgilio *et al*. 2010).

DNA barcoding as an efficient tool for identification of muscid flies

With congruence over 98% between morphological and molecular species limits, the Folmer region of the COI gene appears to be a highly effective marker to identify species of northern Muscidae. Congruence over 90% have been reported in the Lepidoptera (Hebert *et al*. 2003a; Janzen *et al*. 2005; Ward *et al*. 2005; Hajibabaei *et al*. 2006), the Ephemeroptera, Plecoptera and Trichoptera (Zhou *et al*. 2010), and the Hymenoptera (Fernández-Triana *et al*. 2011). In the Diptera, however, congruence ranged from 40 and 100% depending on the family (Cywinska *et al*. 2006; Smith *et al*. 2006, 2007; Whitworth *et al*. 2007; Rivera and Currie 2009). Interestingly, among the Diptera, high congruence levels comparable to those reported here have only been reported in mosquitoes (Diptera: Culicidae) (Cywinska *et al*. 2006), black flies (Diptera: Simuliidae) (Rivera and Currie 2009), and tachinid flies (Smith *et al*. 2006), whereas much lower values were found in calyptrate taxa such as the Calliphoridae (*Protocalliphora*) (Whitworth *et al*. 2007). Mitochondrial DNA introgression caused by *Wolbachia* infection has been suggested as a potential explanation for the lack of

correspondence between COI and species limits defined by morphology and/or other molecular markers in some taxa (Whitworth *et al*. 2007; Nice *et al*. 2009). In the present work, the strong correspondence between morphological species and genetic clusters indicates that introgression is not common in my data set.

The high levels of congruence between morphological and molecular species limits reported here allowed for the association of conspecific specimens from different sexes and facilitated the determination of damaged or atypical specimens. Furthermore, it allowed me to confirm separate species limits for many taxa not currently recorded in the literature for the Nearctic Region (new species or new mentions for the Nearctic). In Chapter 3, 12 morphospecies could not be associated to any known Nearctic species using morphology. The examination of the NJ tree (Figure 4.2) and genetic distances confirmed that 11 of these morphospecies (material of *Spilogona* sp. 1 failed to amplify), along with three more mentioned in this Chapter for the first time (*Coenosia* sp. 1, *Hydrotaea*, sp. 1 and *Spilogona* sp. 12), were distinct from all other species included in my data set. While I am confident that these morphospecies are not currently known from the Nearctic region, further research is necessary to determine their status as either new species or new records (except for *Spilogona* sp. 12, see above).

Species of *Graphomya* Robineau-Desvoidy are notoriously difficult to identify to species based on morphology, and characters used by Arntfield (1975) in his key to the Nearctic fauna of the group appear to be highly variable. Unfortunately, sequences from the 22 specimens of *Graphomya* submitted to the CCDB (Appendix E) were not available in time for thesis submission. As I anticipate levels of correspondence between molecular and species limits as currently defined by Arntfield (1975) to be much lower than those

reported for other genera in this work, I believe that this group should be prioritized in future barcoding studies of the Muscidae.

In contrast to the results obtained at the species level for my data, generic limits were poorly supported by COI in NJ trees, with more than half of the genera represented by two or more species being para- or polyphyletic. It appears then that muscid specimens cannot be reliably identified to genus using COI based solely on association with closely related taxa, at least when based on the NJ method of tree building. In the literature, the percentage of insect genera forming monophyletic clusters based exclusively on COI ranges from 50 to 100% with values similar to mine reported for ithomiine butterflies (50-61% depending on the clustering method used) (Elias *et al*. 2007) and black flies (62.5%) (Rivera and Currie 2009), whereas much higher values were reported in bees (100%) (Sheffield *et al*. 2009).

Intraspecific distance

Several factors, such as the number of sequences or the inclusion of sequences from a range of geographic localities, can influence the extent of genetic divergences measured within a taxon (Lukhtanov *et al*. 2009). Bearing in mind that only 12 species could be included in the analysis, the inclusion of sequences from individuals of these species from localities other than Churchill (arctic/alpine regions of Québec and Sweden) did not increase maximum intraspecific distances. These results are comparable to those of Hebert *et al*. (2009), who reported low intraspecific variation among 11,289 sequences of lepidopteran species (1327 species in 62 families) collected from different localities in eastern North America. While it appears then that global sequence libraries of Muscidae

and Lepidoptera may serve as references for local species identification, this may not be the case for all taxa, as Zhou *et al*. (2011), reported an increase in maximum intraspecific divergence and a decrease in distance to nearest neighbour with an expansion of the geographic range of sequenced specimens in caddisflies.

In Diptera, ranges of 3.00 to 5.40% and 0.17 to 1.20% have been reported for maximum and average intraspecific distances of COI, respectively (Cai *et al*. 2005; Cywinska *et al*. 2006; Smith *et al*. 2006; Carew *et al*. 2007; Rivera and Currie 2009). The values reported here for the post-reassessment data set are lower or at the lower end of these ranges (2.5% maximum and 0.18% average intraspecific distances), with only two out of 121 species represented by more than one individual exhibiting levels of intraspecific distance above 2%. This may reflect a low incidence of cryptic species in my data set.

Reassessment of material with intraspecific distances exceeding 2% in the original data set led to the discovery of *Spilogona* sp. 12, a taxon which can be clearly separated from the similar *S. contractifrons* based on male genitalic structures, and confirmed to be a new species by A. Pont and J. Savage (pers. comm.). The same process led to the reinstatement of *Phaonia luteva* as a species distinct from *P. errans*. Malloch (1923) recognized three distinct Nearctic varieties of *Phaonia errans*: a yellow-legged variety, *Phaonia errans errans* (Meigen); a dark-legged variety, *Phaonia errans varipes* (Coquillet); and a variety with rufous-yellow legs and distinctive chaetotaxy, *Phaonia errans completa* Malloch. Huckett (1934c) synonymized *varipes* Coquillet with *Anthomyia luteva* Walker and treated the dark-legged form as *Phaonia errans* var. *luteva* in later publications (Huckett 1965a, b). Since specimens of *Phaonia errans sensu lato*

clustered here into distinct yellow and dark-legged branches separated by more than 2% interspecific distance (higher than for many other taxa in this work), I concluded that *P. luteva* should be recognized as a full species distinct from *P. errans*. Specimens of *Phaonia errans* var. *completa* were not available for DNA extraction in the context of this work but the distinctive leg colour and chaetotaxy of this taxon suggest that it might also be a separate species rather than a regional variety of *P. errans*.

One of the strengths of DNA barcoding is the possibility of revealing cryptic species (Hebert and Gregory 2005). A large genetic distance among COI sequences for a taxon may therefore indicate the presence of one or more morphologically "hidden" species. In Diptera, cryptic species appear to be especially common in parasitoid flies of the family Tachinidae (Smith *et al*. 2006, 2007), but no information was available for muscid flies prior to this study. In my post-reassessment data set, only *H. evecta* and *S. atrisquamula* demonstrated maximum levels of intraspecific distances greater than 2% (but still no higher than 2.5%) coupled with homogeneous morphological characters. As there is nothing among the scant information currently available on the ecology of these two species suggesting the presence of distinct internal lineages (Skidmore 1985), I recommend the analysis of further molecular data such as the Internal Transcribed Spacers (ITS) region of the ribosomal DNA. ITS appears to be variable enough to successfully detect the presence of cryptic species in closely related Diptera taxa and separate species when morphology is unreliable, as demonstrated in *Belvosia* (Diptera: Tachinidae) (Smith *et al*. 2006) and *Chrysomya* (Diptera: Calliphoridae) (Nelson *et al*. 2008).

Interspecific distance

As with intraspecific distance values reported here, minimum (1.23%) and average (4.88%) interspecific distances for the post-reassessment data set were lower than most found in the literature for insects, including mosquitoes (Cywinska *et al*. 2006), black flies (Rivera and Currie 2009), bees (Sheffield *et al*. 2009), mayflies, stoneflies and caddisflies (Zhou *et al*. 2010) and springtails (Hogg and Hebert 2004), but comparable to those reported for tachinid flies (Smith *et al*. 2006). Hebert *et al*. (2003b), in their work on sequence divergences among species pairs of Arthropoda and other invertebrates, reported that more than 98% of the taxa investigated (including 177 species of Diptera, but no Muscidae) showed greater than 2% pairwise distance between them. In contrast, only 88% of taxa in the present work were separated from their nearest neighbour by a distance greater than 2%. This difference may possibly be explained by the inclusion of many species belonging to one family (including many closely related taxa), as opposed to the broad taxonomic range covered in Hebert *et al*. (2003a). Species limits of the 12% of species with distance to nearest neighbour < 2% in my data set were supported by morphological characters, but these were occasionally subtle or only detectable in the males, possibly suggesting a recent divergence time (Meier *et al*. 2006.).

A very low level of genetic divergence between species, often well below the delineated threshold, may reflect polymorphism (Hebert *et al*. 2004a). Of all the taxa included in this work, only two were below 1.23% and even shared identical haplotypes. Males of *T. septentrionalis* and *T. spiniger* have traditionally been separated based on the number of posteroventral spines on the fore tibia and the length/width ratio of tarsomere four, but no characters have been found to distinguish females (Savage 2003). Both taxa

are found in alpine and northern habitats, and, with the exception of one (but confirmed) mention of *T. spiniger* from the Kamtchatka Peninsula (Savage 2003), they share a mostly overlapping Nearctic distribution (Huckett 1965a; Savage 2003). In a phylogenetic analysis of *Thricops* based on COI, COII, and the nuclear gene *white*, Savage *et al*. (2004) treated the two species as distinct but very closely related. Savage *et al*. (2004), however, included only one specimen of each taxon in the analysis, therefore preventing an assessment of intraspecific vs interspecific distances. Based mostly on geographical distribution data for these two taxa, I suspect that *T. septentrionalis* and *T. spiniger* may belong to one polymorphic species. In order to test this hypothesis, and before permanent changes are made to their taxonomic status, the genetic distance between *T. septentrionalis* and *T. spiniger* should be further assessed with other markers capable of distinguishing between closely related species as done by Whitworth *et al*. (2007), who found that COI and COII underestimated species numbers in the genus *Protocalliphora* but that the analysis of amplified fragment length polymorphism (AFLP) generated clusters corresponding to morphological *Protocalliphora* species limits.

Conclusion

In summary, DNA barcoding retrieved most of the morphologically defined species included in this study, demonstrating its capacity to discriminate between northern muscid fly species. The Folmer region of COI has been proposed as a standard marker to delimit insect species (Hebert *et al*. 2003a, b), but as the performance of this marker has been uneven in Diptera, the high levels of correspondence between morphology and barcodes in the present work came as a surprise. In addition to

supplementing (and often accelerating) the identification of a large number of well known and (relatively) unambiguous muscid species, DNA barcoding allowed me to recognize and address several issues and new discoveries in Nearctic Muscidae taxonomy. It appears therefore that the Muscidae can greatly benefit from further integration of molecular data in taxonomy and biodiversity research.

The ultimate goal of DNA barcoding is eventually to develop a global system of bioidentification for all species (Hebert *et al*. 2003b). The creation of such a database requires the commitment of expert taxonomists to provide correctly identified reference specimens to be sequenced and to address the occasionally puzzling outcomes resulting from data analysis. Therefore, through the sequencing and identification of close to 150 species and more than a thousand muscid specimens, I have created the largest DNA barcoding reference library for muscid flies in the world and I hope my data may now serve not only as a framework to accelerate species identification of muscid flies at a global scale, but as a baseline to further test the limits and applications of DNA barcoding in this group.

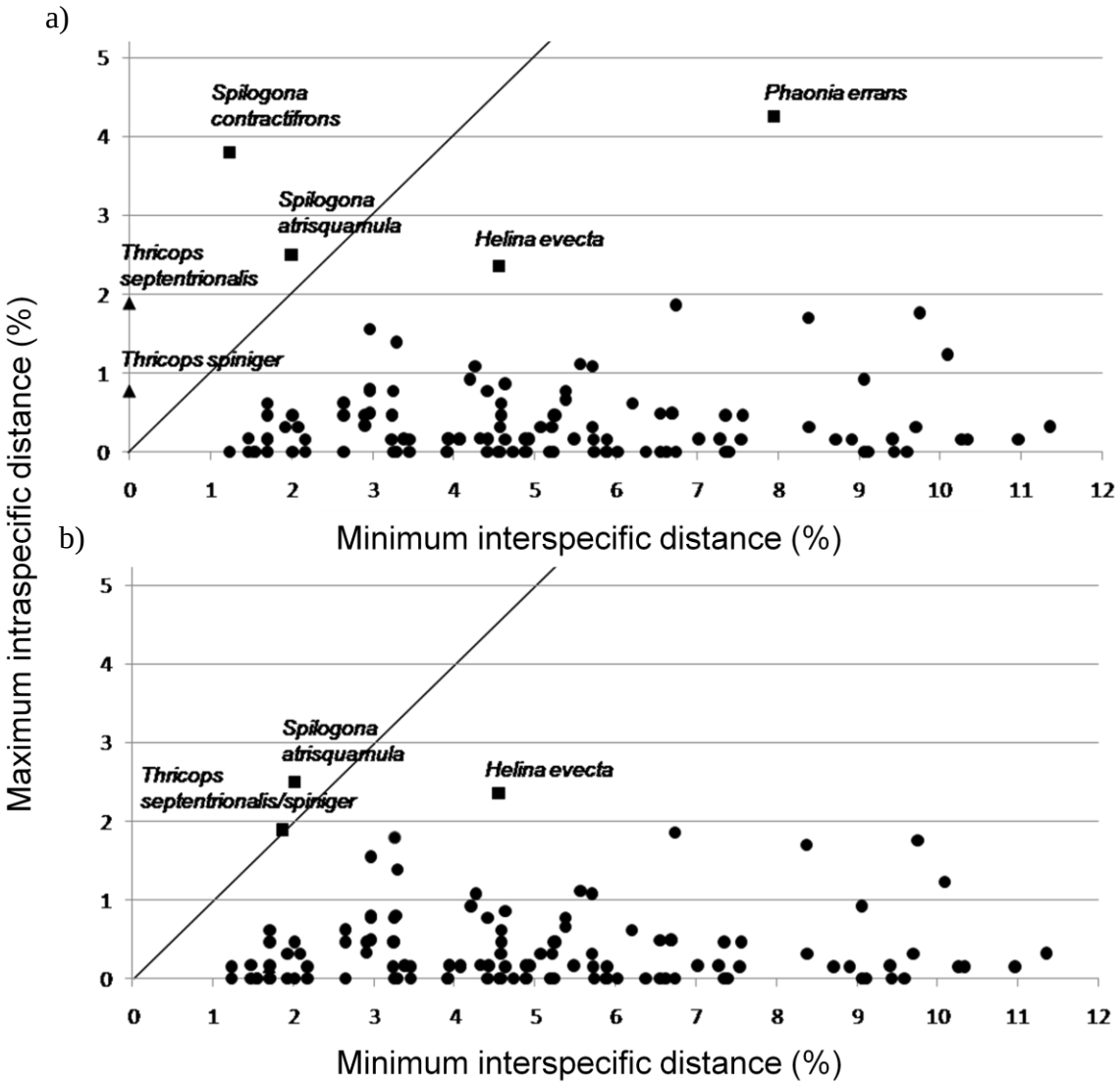


Figure 4.1. Maximum intraspecific and minimum interspecific distances of COI sequences of muscid taxa represented by two or more individuals from Churchill (Manitoba), Gaspésie (Québec), and Sweden in: a) the original data set, and b) the post-reassessment data set. Circles represent taxa with maximum intraspecific distance < minimum interspecific distance. Triangles represent taxa with 0% interspecific distance. Squares represent taxa with intraspecific distance > 2% and/or maximum intraspecific distance > minimum interspecific distance (Appendices F and G).

Figure 4.2. Kimura 2-parameter Neighbor-joining tree of 976 COI sequences of Muscidae from Churchill (Manitoba), Gaspésie (Québec), and Sweden. Each taxon is represented by a triangle where height is proportional to the number of sequences and width proportional to intraspecific distance (%). Numbers in parenthesis represent the number of haplotypes over the number of sequences for each taxon.

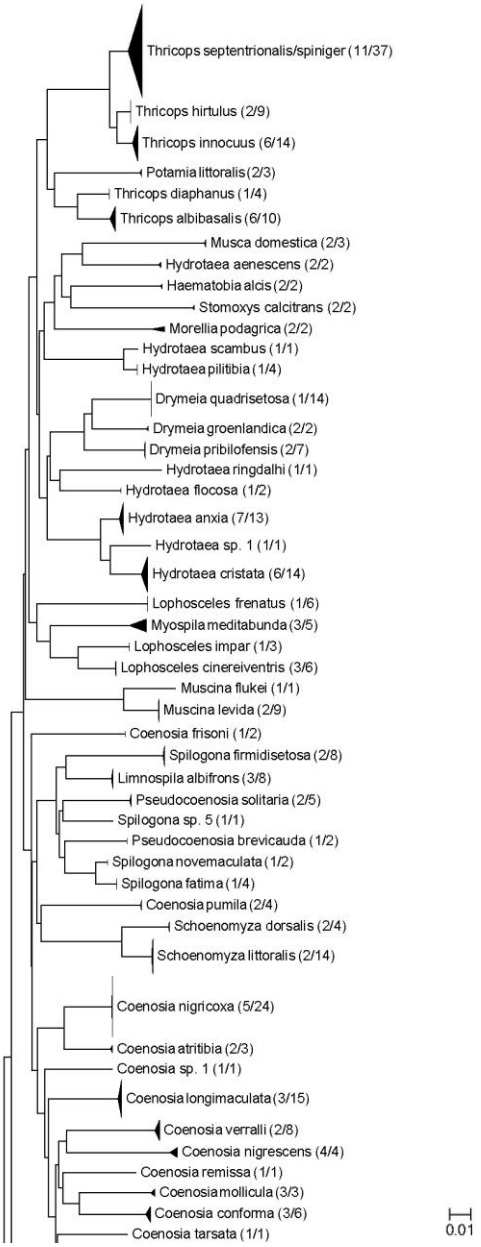
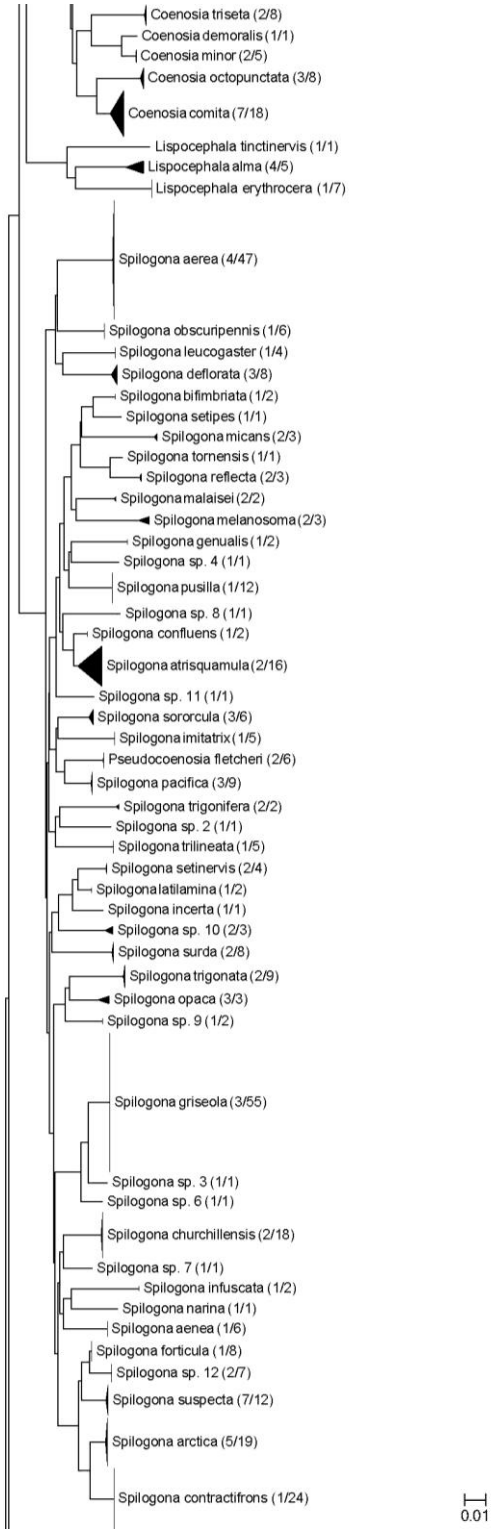
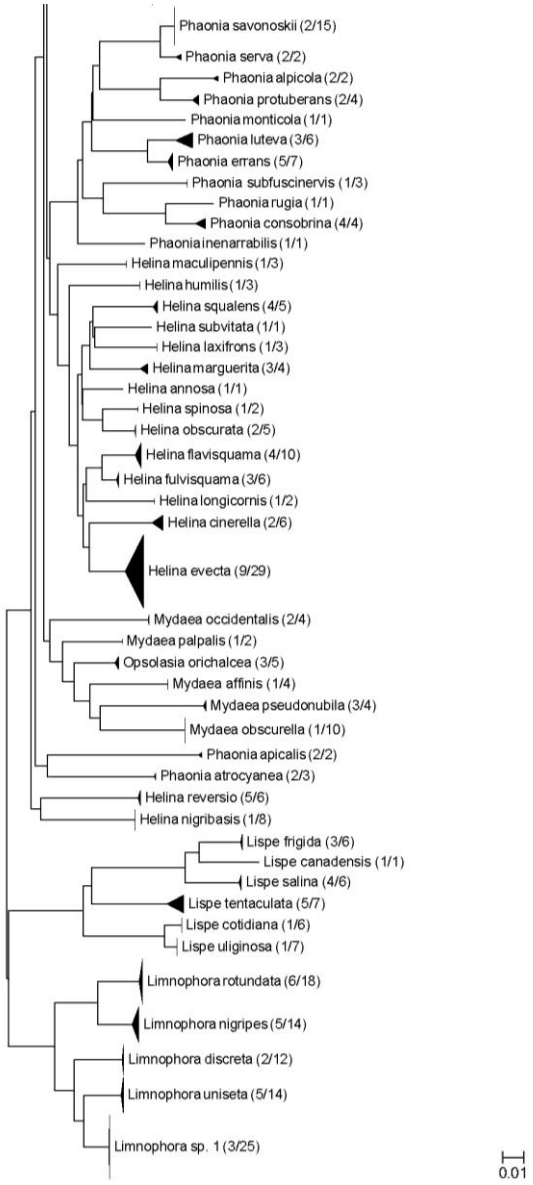


Figure 4.2. (continued)[1]



[1] *Spilogona latilamina* (Collin) is a junior synonym of *Spilogona zaitzevi* (Schnabl) (Michelsen 2006).

Figure 4.2. (concluded)



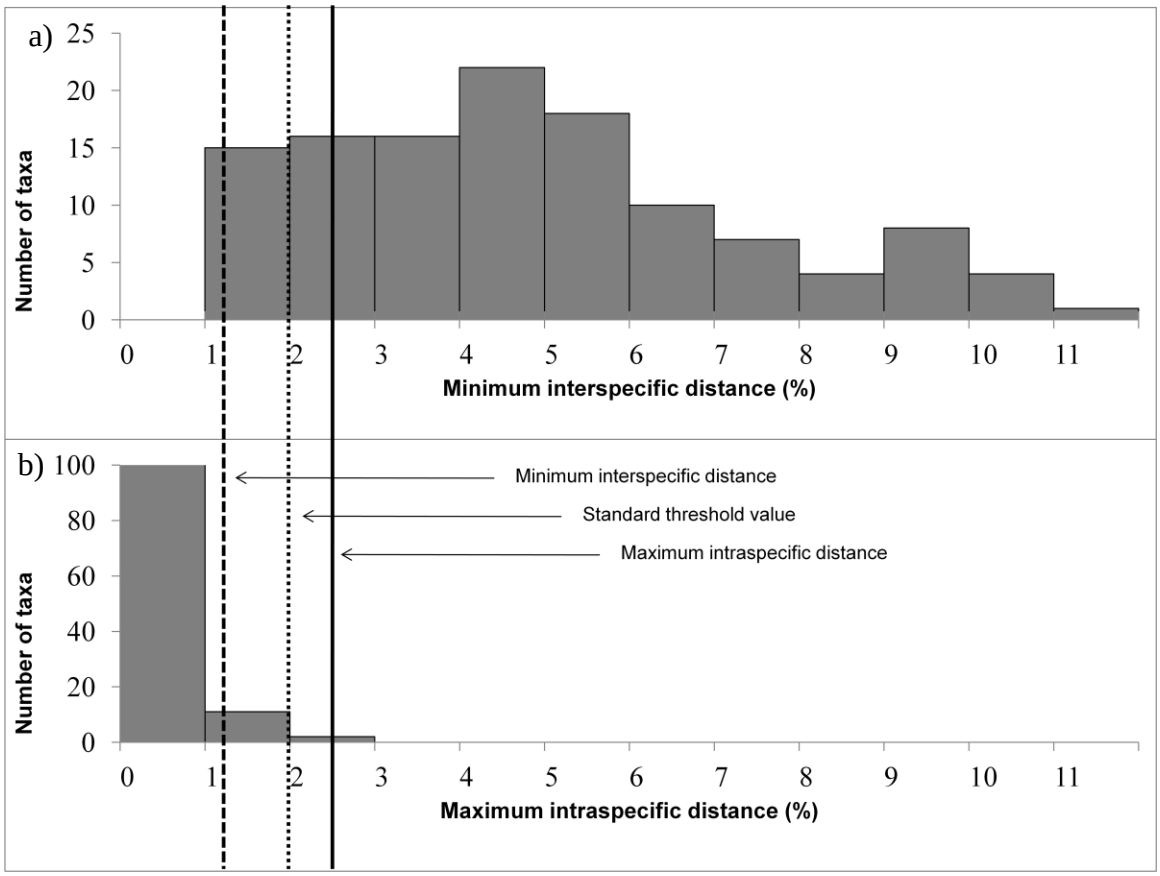


Figure 4.3. Frequencies of: a) minimum interspecific distance, and b) maximum intraspecific distance for COI sequences of muscid species represented by two or more individuals from Churchill (Manitoba), Gaspésie (Québec), and Sweden after taxonomic reassessment. Stippled line marks minimum interspecific distance, dotted line marks 2% standard threshold value commonly used to separate insect species, and solid line marks maximum intraspecific distance.

Table 4.1. Pairs of primers used at the Canadian Centre for DNA Barcoding to amplify COI sequences of muscid flies from Churchill (Manitoba), Gaspésie (Québec), and Sweden. The primers used for PCR and sequencing are available for all specimens through BOLD (www.boldsystems.org).

Primer	Sequence (5' to 3')		Reference
	Forward	Reverse	
LepF1/LepR1	ATTCAACCAATCATAAAGATATTGG	TAAACTTCTGGATGTCCAAAAAATCA	Hebert *et al*. 2004a/ Hebert *et al*. 2004a
LCO1490_t1/HCO2198_t1	TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG	CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*. 1994/ Folmer *et al*. 1994
LepF1/C_ANTMR1D	ATTCAACCAATCATAAAGATATTGG	N/A	Hebert *et al*. 2004a/N/A
MLepF1/HCO2198_t1	GCTTTCCCACGAATAAATAATA	CAGGAAACAGCTATGACTAAACTTCAGGTGACCAAAAAATCA	Hajibabaei *et al*. 2006/Folmer *et al*. 1994
MLepF1/LepR1	GCTTTCCCACGAATAAATAATA	TAAACTTCTGGATGTCCAAAAAATCA	Hajibabaei *et al*. 2006/Hajiba-baei *et al*. 2006
LepF1/MLepR1	ATTCAACCAATCATAAAGATATTGG	GCTTTCCCACGAATAAATAATA	Hebert *et al*. 2004a /Hajibabaei *et al*. 2006

Table 4.2. Amplification success rate by drying methods and killing agents for a preliminary sample (n = 104) of Muscidae specimens from Churchill (Manitoba) sent to the Canadian Centre for DNA Barcoding. Number of sequences amplified over number of specimens submitted and percentages of amplification success (%) are presented for each combination of drying method and killing agent.

	Drying method	
Killing agent	**Air (n = 51)**	**HMDS[a] (n = 53)**
Cyanide/ethyl acetate	9/10 (90%)	N/A
Propylene glycol+ soapy water	8/9 (89%)	15/15 (100%)
70% ethanol	9/10 (90%)	16/16 (100%)
95% ethanol	10/10 (100%)	10/10 (100%)
Dichlorvos[b]	11/12 (92%)	12/12 (100%)

[a]Hexamethyldisilazane

[b]Vapona®

CHAPTER 5

GENERAL DISCUSSION

The primary aims of this study were to document the contemporary diversity of muscid flies at a low arctic site and to contrast patterns of species richness and composition with those from a historical assemblage. Muscidae were selected for this study as they are one of the most diverse, abundant and ubiquitous insect taxa in the Arctic and the northern Nearctic fauna has been well documented in the past (Webb 1956; Huckett 1965a, b; Arntfield 1975; Savage 2003). An investigation of potential turnover in muscid species assemblages in Churchill since 1965 required the compilation of two databases, one historical and one contemporary. As it was crucial that species be correctly identified, morphology-based identification of specimens was supplemented with genetic data.

In this general discussion, I take a critical look at the 2007 inventory to put forward recommendations for future faunal surveys of muscid flies in Churchill. I also discuss strengths and limitations of the study and present potential avenues for future studies.

RECOMMENDATIONS FOR FUTURE INVENTORIES

The inventory of muscid flies conducted at Churchill in 2007 generated an assemblage of 160 species (155 listed in Chapter 3 with five more listed in Chapter 4), nearly 70% of all muscid species reported for the entire northern Nearctic region (Huckett 1965a; Danks 1981). It appears then that my experimental design was adequate to sample most muscid flies in the area of Churchill. However, the sampling protocol was labour intensive and the resulting data set had a number of limitations.

Therefore, here are my recommendations to improve future collecting protocols and increase the scope of collected data:

1) **Synchronize sampling initiation with snowmelt** - in the actual context of global warming, end and start dates of seasons can be shifted. Therefore, in order to cover the whole active period of flies, the collector should be ready to start the inventory as soon as the snow melts. I thus recommend arriving a week earlier than the dates at which the snow melted on previous years, as was done for the 2007 inventory, to prepare the experimental setup on time and begin collecting as soon as activity starts. The collector should be aware that some sites will take longer to become clear of snow.
2) **Sample for only 3-4 days of each week** - by collecting twice a week for 10 weeks, too many specimens were collected for the time available for processing. Thus, a more restricted number of specimens could be collected to reduce the handling time.
3) **Sample only with Malaise trap and sweep net at permanent sites, but increase sampling effort by net sweeping** - The Malaise trap was highly effective in the present work, and while it requires more time to set-up than either pan traps or net sweeping, servicing does not take longer than for the pan traps. In addition, specimens collected by Malaise traps are directly preserved in ethanol, rather than immersed in water for a number of days, thereby improving the quality of specimens for morphological identification by minimizing bristle loss and for molecular sequencing by preventing DNA degradation.

   At the permanent sites, net sweeping collected only few specimens, therefore making it hard to compare rarefied species richness between assemblages from different time periods. In order to increase the number of specimens collected by

net sweeping, I recommend increasing the number of sweeps around each Malaise trap to 120. While not as productive as Malaise traps in term of species richness, net sweeping still collected several unique species despite being carried out only four metres from the Malaise trap at the permanent sites. This may indicate that net sweeping collects species that tend to sit on the surrounding vegetation rather than fly actively.

While Malaise traps and net sweeping yielded complementary rather than fully overlapping data in terms of species collected, pan traps performed much more poorly, both in terms of species richness and species composition (it collected a single unique species). Not only did the pan traps perform poorly in terms of results but they were costly to operate as close to 100 litres of food-quality propylene glycol used as preservative had to be purchased and transported to Churchill. In addition, the disposal of pan trap fluid was problematic in Churchill since the Churchill Northern Research Centre prohibits discarding it in their septic system. Therefore, residual fluids needed to be handled as a hazardous waste and deposited in town to be picked up by a mandated operator. Ethanol, however, can be flushed in the septic system as it eventually evaporates.

4) **Standardize net sweeping at occasional sites** - Occasional sites were mostly habitats with uncommon elements that could not be regularly sampled because they were not easily accessible or received too many visitors. I recommend maintaining the sampling with net sweeping at occasional sites as I collected eight species not collected by any other sampling devices in 2007 and almost twice the number of specimens collected by net sweeping at permanent sites. However, I would suggest standardizing the collection at these occasional sites by: 1) selecting a maximum number of sites to be sampled during the inventory,

2) selecting sites sampled during previous year(s), 2) visiting each occasional site the same number of times during the summer, 3) spreading the visits over the complete period of the inventory, and 4) visiting all occasional sites on the same day to decrease the influence of weather on specimen abundance and species richness.

LIMITATIONS IN THE COMPARISON OF HISTORICAL VS CONTEMPORARY ASSEMBLAGES

Because the sampling protocol was carried out over a single year, it did not allow me to determine the effects of interannual variations on species assemblages. Thus, it was impossible to determine if historical species not collected again in the contemporary assemblage were present but missed during the collecting effort as opposed to being truly extirpated from the area.

Optimally, the comparison of species assemblages requires similar sampling effort, sample size, collecting methods, and collector expertise. To avoid violating these assumptions, the comparison of assemblages between time periods was based exclusively on material collected by the same method (net sweeping) and excluded taxa listed in the literature that could not be traced in collections.

The necessity to merge certain species into complexes due to identification problems also decreased the number of species included in the analysis. While DNA barcoding confirmed the separate identity of most taxa in these complexes, the method is too expensive and labour intensive to be applied to all material collected, and species complexes therefore had to be maintained in order to allow the inclusion of abundance data necessary to calculate rarefaction curves.

While one of the goals of this project was to emulate sampling efforts invested in the pre-1965 assemblage, it was necessary to increase the scope of historical

sampling efforts and techniques in order to create a comprehensive and reproducible sampling protocol. Interestingly, the use of Malaise trap appeared as the most likely explanation for the presence/absence of many non-overlapping species between the two temporal assemblages when all data from each time period were taken into account.

A comparison of the relative abundance of species during each time period would have allowed me to push the comparison beyond species composition and therefore detect more subtle changes in muscid assemblages between the two time periods. However, uncertainties related to collectors' bias as well as sampling efforts were too important to allow for this type of analysis. Hopefully, the contemporary baseline data assembled through this study and the repeatable sampling protocol will now permit for the comparison of relative abundance between assemblages collected in different years. Furthermore, it will enable the study of other research questions such as interannual variations in the emergence phenology of various species.

OVERLAP IN SPECIES RICHNESS BETWEEN TIME PERIODS: THE RESILIENCE OF MUSCIDAE

Contrary to my original hypothesis, the muscid fly fauna of Churchill does not appear to have been notably affected by climate and anthropogenic changes that had occurred in the area over the previous 42 years. No significant differences were observed between the estimated species richness of the historical (pre-1965) and contemporary (2007) assemblages collected by net sweeping, and most of the differences in overall species composition were attributable to rarity in samples and/or collecting methods.

Because of their ecological diversity, the Muscidae may be less affected by habitat changes than other more specialized taxa, such as parasitoids. The richness of

the 2007 assemblage, both in terms of species numbers and ecological diversity, may reflect the heterogeneity of the landscape of Churchill where many niches may still be available, even under current rates of environmental changes. In terms of biogeographical affinities, the muscid fauna of Churchill is composed of widespread species as well as primarily eastern or western elements. It is possible, therefore, that the central position of Churchill in the northern Nearctic facilitates exchanges with the surrounding areas, thereby contributing to the stability in species richness and composition of muscid flies of Churchill reported here.

A prediction made earlier in this work was that in the context of recent climate changes, the biodiversity of Muscidae is likely to change in the area of Churchill. Knowing that climate is one of the most influential abiotic factors for flies and that the temperature has increased in Churchill by almost 2 °C in the last 40 years, it is surprising to see that no marked changes were observed in the species richness and composition since 1965. It may be that Churchill has not yet reached a threshold point for muscids that would result in marked changes in species richness and/or a turnover in community composition. Moreover, despite temperature having significantly increased in Churchill, winter conditions may still be too cold for many species to overwinter successfully and to be able to establish permanently at Churchill. Thus, even if several of the new Churchill records reported here may have recently been introduced to Churchill by the transportation of organic goods such as grains, it is likely that they will not be able to overwinter there unless they can find sheltered microhabitats, which is perhaps the case for some of the species newly recorded from Churchill in 2007 (*e.g*. *Hydrotaea aenescens* (Wiedemann)). To test the hypothesis that the climate of Churchill is still too cold for many species to establish, it would be necessary to perform laboratory experiments to obtain more information on the

minimal conditions required by the various species of Muscidae to reproduce and develop. Moreover, in order to determine if new records are adventive or well established species, additional monitoring will be necessary in Churchill.

NON-OVERLAPPING SPECIES

Although estimated species richness was similar for the historical and contemporary assemblages, some taxa were unique to each assemblage. Many species were newly collected in Churchill in 2007 and although the presence of a large number of species unique to the 2007 assemblage appears to be attributable mostly to the use of Malaise traps, this does not exclude the possibility that some were introduced to the Churchill area between 1965 and 2007. Several studies that have reported changes in the diversity of insects have also noted extensions/shifts of species distribution range towards the north (Parmesan 1996, 2006). Although only four species newly recorded for the area in 2007 fit the criteria established here to indicate a potential northern extension/shift in their distribution range, it is possible that some of the new records that were either rare in samples and/or collected exclusively by Malaise traps also recently migrated northwards. Repeated sampling along a north/south transect passing through Churchill would allow for the monitoring of species range limits over time to investigate if the reported trends in recent northward isothermal shift in the Nearctic region are concurrent with a northward displacement in the distribution of muscid flies.

Among the few differences in species composition between time periods, I noted a disproportionate representation of *Spilogona* species among those taxa unique to the pre-1965 assemblage. The absence of many *Spilogona* species previously found in Churchill could be related to their semi-aquatic larval habits, as a decrease in

freshwater cover reported for the area over the last 30 years could have disturbed their life cycle. Sites in the present work were not selected for the presence of freshwater; additional sampling near aquatic and semi-aquatic habitats would therefore be required to further investigate if these *Spilogona* species have truly been extirpated from the Churchill area or if the trend reported here for that group is a sampling artefact.

DNA BARCODING

In a biodiversity study it is extremely important that species are properly identified (Gotelli 2004) in order to have an accurate vision of the monitored community and be able to report true changes if any occur. Unfortunately, erroneous identifications of specimens can happen in biodiversity research (Gotelli 2004). Because the use of morphology to identify species may require extensive taxonomic expertise depending on the group, it limits the utilization of most insects, including flies, in ecological studies. This is especially unfortunate as flies play a key role in the functioning of ecosystems, especially in northern environments where they act as decomposers, pollinators, prey and predators.

As discussed in the literature review, the combination of genetic and morphological data seems promising for the identification of a wide range of animal species. This is what DNA barcoding proposes. Once a reference genetic database is created, researchers only have to compare their new sequences with those in the database, adding more reference sequences as additional known taxa are sequenced and new species are discovered. The main objective of the study reported in Chapter 4 was precisely to create this reference database for the Muscidae and to assess if COI, the genetic marker used in DNA barcoding of animals, was able to discriminate

properly between species. In addition, I wanted to assess the degree of correspondence between morphological and molecular species limits.

DNA barcoding proved to be an extremely useful tool for the identification of Muscidae of Churchill and COI appears to be a proper marker for muscid flies because I observed very high level of correspondence between species limits based on morphology and molecular data. Such correspondence, higher than in most insect groups, could be explained by the rarity in my data set of introgressions that prevent the establishment of proper species limits when present in a species genome. It might also be explained by the quality of the taxonomic work done by dipterists such as H. C. Huckett, W. Hennig and A.C. Pont.

COI proved to be an effective marker to separate muscid species, thereby having the potential to greatly facilitate the process of specimen identification in a taxonomically difficult group. Consequently, I hope that the public genetic database produced in the present work and available on Bold (www.boldsystems.org) will stimulate further research on the diversity and ecology of muscid flies, most especially in northern regions. I don't believe it is realistic for the moment to think that biodiversity studies could possibly rely exclusively on DNA barcoding for species identifications. Like most molecular techniques, DNA barcoding demands time investment and money. Obtaining sequences for 10,000 specimens requires time and good financial support often not available for research in ecology and only a subset of the specimens collected can usually be barcoded for identification purposes. In 2011, only 323 of the 5100 species of Muscidae have been barcoded, half of these in the present work. Therefore morphological identification of Muscidae will still be required until more species are barcoded.

CONSERVATION

Environmental changes have caused an important loss of diversity in many ecosystems on earth. Several areas, such as the Arctic, have been poorly studied and we have little information on the level of resilience of their ecosystems to rapid environmental changes. Consequently, it is only through long-term monitoring programs that we will be able to fully assess the responses of insect populations and communities to environmental changes. As comprehensive baseline data are not available for most northern organisms, the current study was designed in such a way that the results could be compared to those of previous inventories as well as become the new standard for the monitoring of northern muscid flies.

Biodiversity is our natural heritage and must be preserved to ensure the availability of this heritage for future generations. Exploitation, fragmentation of habitat, pollution and climate changes are threats to arctic ecosystems. While I demonstrated in this work that the muscid fly fauna of Churchill so far appears to have been mostly resilient to recent environmental changes, it is likely that the synergic and cumulative effects of environmental changes will ultimately reach a point where changes in diversity patterns will be unavoidable. Since important changes in the diversity and community structure of faunal and floral assemblages reported across many northern groups have already been linked to environmental changes, muscid flies provide us with a unique opportunity to observe and understand how and when these changes will eventually unfold.

LITERATURE CITED

Aanes, R., Sæther, B.-E., Smith, F.M., Cooper, E.J., Wookey, P.A., and Oristland, N.A. 2002. The arctic oscillation predicts effects of climate change in two trophic levels in a high-arctic ecosystem. Ecology Letters, **5:** 445-453.

Alaska Climate Research Center 2008. Climatological data - monthly time series [online]. Available from http://climate.gi.alaska.edu/Climate/Location/TimeSeries/index.html [accessed 5 July 2010].

Anderson, S. 2010. An evaluation of spatial interpolation methods on air temperature in Phoenix, AZ [online]. Available from http://www.cobblestoneconcepts.com/ucgis2summer/anderson/anderson.htm [accessed 3 November 2010].

Arctic Climate Impact Assessment (ACIA) 2004. Impact of a warming Arctic: Arctic climate impact assessment. Cambridge University Press, Cambridge.

Arctic Monitoring and Assessment Programme (AMAP) 1998. AMAP assessment report: arctic pollution issues [online]. Available from http://www.amap.no/ [accessed 15 September 2010].

Arntfield, P.W. 1975. A revision of *Graphomya* Robineau-Desvoidy (Diptera: Muscidae) from North America. The Canadian Entomologist, **107:** 257-302.

Ausden, M., and Drake, M. 2006. Invertebrates. *In* Ecological census techniques: a handbook. *Edited by* W. Sutherland. Cambridge University Press, Cambridge, UK. pp. 214-247.

Austin, A.D., and Dillon, N. 1997. Extraction and PCR of DNA from parasitoid wasps that have been chemically dried. Australian Journal of Entomology, **36:** 241-244.

Axford, Y., Briner, J.P., Cooke, C.A., Francis, D.R., Michelutti, H., Miller, J.H., Smol, J.P., Thomas, E.K., Wilson, C.R., and Wolfe, A.P. 2009. Recent changes in a remote Arctic lake are unique within the past 200,000 years. Proceedings of the National Academy of Sciences of the United States of America, **44:** 18443-18446.

Bale, J.S. 2002. Insects and low temperatures: from molecular biology to distributions and abundance. Philosophical Transactions of the Royal Society B: Biological Sciences, **357:** 349-862.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Harrington, R., Hartley, S., Jones, T.H., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A.D., and Whittaker, J.B. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology, **8:** 1-16.

Ballantyne, K. 2009. Whimbrel (*Numenius phaeopus*) nesting habitat associations, altered distribution, and habitat change in Churchill, Manitoba, Canada. M.Sc. Thesis. Trent University, Peterborough, Canada.

Ballard, J.W. 2000. When one is not enough: introgression of mitochondrial DNA in *Drosophila*. Molecular Biology and Evolution, **17:** 1126-1130.

Barrett, R.D.H., and Hebert, P.D.N. 2005. Identifying spiders through DNA barcodes. Canadian Journal of Zoology, **83:** 481-491.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meir, R., Winker, K., Ingram, K.K., and Das, I. 2006. Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution, **22:** 148-155.

Bishop, F.C. 1913. The stable fly (*Stomoxys calcitrans* L.), an important livestock pest. Journal of Economic Entomology, **6:** 112-126.

Breeland, S.G., and Pickard, E. 1965. The Malaise trap - an efficient and unbiased mosquito collecting device. Mosquito News, **21:** 19-21.

Brook, R. 2001. Structure and dynamics of the vegetation in Wapusk National Park and the Cape Churchill Wildlife Management Area of Manitoba**:** community and landscapes. M.Sc. Thesis. University of Manitoba, Winnipeg, Canada.

Brown, B.V. 1993. A further chemical alternative to critical-point-drying for preparing small (or large) flies. Fly Times, **11:** 10.

Brown, W.J. 1937. The Coleoptera of Canada's eastern Arctic. The Canadian Entomologist, **69:** 106-111.

Buddle, C. 2009. Bylot Island and the Northern Biodiversity Program: ongoing studies about arctic entomology and arachnology. Newsletter of the Biological Survey of Canada (Terrestrial Arthropods), **28:** 63-65.

Burger, J.F., and Anderson, J.R. 1974. Taxonomy and life history of moose fly, *Haematobosca alcis*, and its association with moose, *Alces alces* in Yellowstone National Park. Annals of the Entomological Society of America, **67:** 204-214.

Cai, J.-F., Liu, M., Ying, B.-W., Deng, R.-L., Dong, J.-G., Zhang, L., Tao, T., Pan, H.-F., Yan, H.-T., and Liao, Z.-G. 2005. The availability of mitochondrial DNA cytochrome oxidase I gene for the distinction of forensically important flies in China. Acta Entomologica Sinica, **48:** 380-385.

Campbell, J.B., and Thomas, G.D. 1992. The history, biology, economics, and control of the horn fly, *Haematobia irritans*. Agri-Practice, **13:** 31-36.

Campbell, J.W., and Hanula, J.L. 2007. Efficiency of Malaise traps and colored pan traps for collecting flower visiting insects from three forested ecosystems. Journal of Insect Conservation, **11:** 399-408.

Cannings, S. 2009. Invertebrate samples from Arctic Wildlife Observatories Linking Vulnerable Ecosystems (Arctic WOLVES). Newsletter of the Biological Survey of Canada (Terrestrial Arthropods), **28:** 25.

Carew, M.E., Pettigrove, V., Cox, R.L., and Hoffmann, A.A. 2007. DNA identification of urban Tanytarsini chironomids (Diptera: Chironomidae). Journal of the North American Benthological Society, **26:** 587-600.

Carvalho, C.J.B. 1989. Classificação de Muscidae (Diptera): una proposta através da análise cladistica. Revista Brasileira de Zoologia, **6:** 627-648.

Chao, A. 1984. Non-parametric estimation of the number of classes in a population. Scandinavian Journal of Statistics, **11:** 265-270.

Chao, A., and Lee, S.-M. 1992. Estimating the number of classes via sample coverage. Journal of the American Statistical Association, **87:** 210-217.

Chazdon, R.L., Colwell, R.K., Denslow, J.S., and Guariguata, M.R. 1998. Statistical estimation of species richness of woody regeneration in primary and secondary rain forests of northeastern Costa Rica. *In* Forest biodiversity in North, Central, and South America and the Caribbean: research and monitoring. *Edited by* F. Dallmeier and J.A. Comiskey. Parthenon Press, Paris, France. pp. 285-309.

Coddington, J.A., Griswold, C.E., Davila, D.S., Peneranda, E., and Larcher, S.F. 1991. Designing and testing sampling protocols to estimate biodiversity in tropical ecosystems. The Unity of Evolutionary Biology: Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology, **2:** 44-60.

Collin, J.E. 1930. A revision of the Greenland species of the anthomyiid genus *Limnophora sens. lat.* (Diptera), with figures of the male genitalia of these and many other Palearctic species. Transactions of the Entomological Society of London, **78:** 255-281.

Colwell, R.K. 2009. EstimateS: statistical estimation of species richness and shared species from samples [online]. Version 8.2. Available from http://purl.oclc.org/estimates [accessed April 2010].

Conservation of Arctic Flora and Fauna (CAFF). 2001. Arctic flora and fauna: status and conservation. Edita, Helsinki, Sweden.

Consortium for the Barcode of Life 2011. What is CBOL [online]? Available from http://barcoding.si.edu/Originalindex.html [accessed 11 March 2011].

Couri, M.S, and Pont, A. 2000. Cladistic analysis of Coenosiini (Diptera: Muscidae: Coenosiinae). Systematic Entomology, **25:** 373-392.

Couri, M.S., and Salas, C. 2010. First record of *Coenosia attenuata* Stein (Diptera, Muscidae) from Chile, with biological notes. Revista Brasileira de Entomologica, **54:** 144-145.

Courtney, G.W., Pape, T., Skevington, J.H., and Sinclair, B.J. 2009. Biodiversity of Diptera. *In* Insect biodiversity: science and society. *Edited by* R.G. Foottit and P.H. Adler. Blackwell Publishing, Oxford, UK. pp. 185-222.

Cummings, M.A., and Krafsur, E.S. 2005. Spatial diversity in mitochondrial cytochrome c oxidase in house flies. Medical and Veterinary Entomology, **19:** 53-59.

Currie, D.C., and Adler, P.H. 2000. Update on a survey of the black flies (Diptera: Simuliidae) from the Northwest Territories and Nunavut Project. Arctic Insect News, **11:** 6-9.

Currie, D.C., Giberson, D., and Brown, B.V. 2000. Insects of Keewatin and Mackenzie. Newsletter of the Biological Survey of Canada (Terrestrial Arthropods), **19:** 48-51.

Cywinska, A., Hunter, F.F., and Hebert, P.D.N. 2006 . Identifying Canadian mosquitoes through DNA barcodes. Medical and Veterinary Entomology, **20:** 413-424.

Danks, H.V. 1981. Arctic arthropods, a review of systematics and ecology with particular reference to the North American fauna. Entomological Society of Canada, Ottawa, Canada.

Danks, H.V. 1992. Arctic insects as indicators of environmental change. Arctic, **45:** 159-166.

Danks, H.V. 1993. Patterns of diversity in the Canadian insect fauna. Memoirs of the Entomological Society of Canada, **165:** 51-74.

Danks, H.V., and Byers, J.R. 1972. Insects and arachnids of Bathurst Island, Canadian Arctic Archipelago. The Canadian Entomologist, **104:** 81-88.

Darrigo, R.D., and Jacoby, G.C. 1993. Secular trends in high northern latitude temperature reconstructions based on tree-rings. Climatic Change, **25:** 163-177.

Dasmahapatra, K.K., Elias, M., Hill, R.I., Hoffman, J., and Mallet, J. 2010. Mitochondrial DNA barcoding detects some species that are real, and some that are not. Molecular Ecology Resources, **10:** 264-273.

de Groot, R.S., P. Ketner, and Ovaa, A.H. 1995. Selection and use of bio-indicators to assess the possible effects of climate change in Europe. Journal of Biogeography, **22:** 2707-2715.

Dillon, N., Austin, A.D., and Bartowsky, E. 1996. Comparison of preservation techniques for DNA extraction from hymenopterous insects. Insect Molecular Biology, **5:** 21-24.

Disney, R.H.L., Erzinclioglu, Y.Z., de C. Henshaw, D.J., Howse, D., Unwin, D.M., Withers, P., and Woods, A. 1982. Collecting methods and the adequacy of attempted fauna surveys, with reference to the Diptera. Field Studies, **5:** 607-621.

Dredge, L.A. 1992. Field guide to the Churchill region, Manitoba. Geological Survey of Canada, miscellaneous report **53:** 1-52.

Drummond, F.A., Groden, E., Haynes, D.L., and Edens, T.C. 1989. Some aspects of the biology of a predaceous anthomyiid fly *Coenosia tigrina*. Great Lakes Entomologist, **22:** 11-18.

Duchesne, P., Étienne, C., and Bernatchez, L. 2006. PERM: a computer program to detect structuring factors in social units. Molecular Ecology Notes, **6:** 965-967.

Edye-Rowntree, J., Ayotte, B., Bazlik, E., Bilenduke, M., Brandson, L., Bussell, M., Campbell, C., Chartier, B., Daley, D., Fitzpatrick, P., Hickes, G., Hunter, D., Ingebrigtson, M., Lawrie, G., Macri, M., McEwan, G., Morand, M., Paddock, C., Spence, M., Welburn, E., Bukowsky, R., Goodyear, M., M'Lot, M., Oakes, J., and Riewe, R. 2006. Resident's perspectives on the Churchill River. Aboriginal Issues Press, University of Manitoba, Winnipeg, Canada.

Elberling, H., and Olesen, J.M. 1999. The structure of a high latitude plant-flower visitor system: the dominance of flies. Ecography, **23:** 314-323.

Elias, M., Hill, R.I., Willmott, K.R., Dasmahapatra, K.K., Brower, A.V.Z., Mallet, J., and Jiggins, C.D. 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. Proceedings of the Royal Society B: Biological Sciences, **274:** 2881-2889.

Eliasson, K. 2004. New waste transfer station for Churchill [online]. Hudson Bay Post, May issue. Available from http://www.polarbearalley.com/churchill-dump.html [accessed 30 October 2010].

Ellison, A.M., Record, S., Arguello, A., and Gotelli N.J. 2007. Rapid inventory of the ant assemblage in a temperate hardwood forest: species composition and assessment of sampling methods. Environmental Entomology, **36:** 766-775.

Environment Canada 2010. Adjusted and homogenized Canadian climate data (AHCCD) [online]. Available from http://ec.gc.ca/dccha-ahccd [accessed 10 July 2010].

Environmental Systems Research Institute (ESRI) 2009. ArcMap 9.2 [CD-Rom]. ESRI, Redlands, USA.

Everett, J.T., and Fitzharris, B.B. 1998. The Arctic and Antarctic. *In* The regional impacts of climate change: an assessment of vulnerability. *Edited by* R.T. Watson, M.C. Zinyowera and R.H. Moss. Cambridge University Press, Cambridge, UK. pp 85-103.

Falkingham, J., Melling, H., and Wilson, K. 2002. Shipping in the Canadian Arctic: other possible climate change scenarios [online]. Weathering Change: Newsletter of the Northern Climate Exchange, fall: 4-5. Available from http://www.taiga.net/nce/resources/newsletters/NCE_Newsletter_Fall2002.pdf [accessed 10 April 2008].

Favret, C., and DeWalt, R.E. 2002. Comparing the Ephemeroptera and Plecoptera specimen databases at the Illinois Natural History Survey and using them to document changes in the Illinois fauna. Annals of the Entomological Society of America, **95:** 35-40.

Feldhamer, G.A., Thompson, B.C. and Chapman, J.A. 2003. Wild mammals of North America: biology, management, and conservation, 2nd ed. Johns Hopkins University Press, Baltimore, USA.

Fernández-Triana, J., Smith, M.A., Boudreault, C., Goulet, H., Hebert, P.D.N., Smith, A.C., and Roughley, R. 2011. A poorly known high-latitude parasitoid wasp community: unexpected diversity and dramatic changes through time. PLoS ONE, **6:** 1-8.

Ferrar, P. 1987. A guide to the breeding habits and immature stages of Diptera Cyclorrhapha. Entomonograph 8, Part 1.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, **3:** 294-299.

Foottit, R.G., and Adler, P.H. 2009. Insect biodiversity: science and society. Wiley - Blackwell, Hoboken, USA.

Frankham, R., Ballou, J.D., and Briscoe, D.A. 2004. A primer of conservation genetics. Cambridge University Press, Cambridge, UK.

Freeman, T.N. 1956. A historical account of insect collecting in northern Canada. Proceedings of the Tenth International Congress of Entomology, **1:** 613-617.

Freeman, T.N. 1959. The Canadian Northern Insect Survey, 1947-1957. Polar Record, **9:** 299-307.

Freeman, T.N., and Twinn, C.R. 1948. Present trends and future needs of entomological research in Northern Canada. Arctic, **7:** 275-283.

Gaston, K.J. 2000. Global patterns in biodiversity. Nature, **405:** 220-227.

Gaston, K.J., and Spicer, J.I. 2004. Biodiversity: an introduction, second edition. Blackwell Publishing, Malden, USA.

Giberson, D.J. 2005. Mayflies and muscids: update on the "insects of the Arctic" project. Newsletter of the Biological Survey of Canada (Terrestrial Arthropods), **24:** 56-57.

Gilioli, G., Baumgartner, J., and Vacante, V. 2005. Temperature influences on functional response of *Coenosia attenuata* (Diptera: Muscidae) individuals. Journal of Economic Entomology, **98:** 1524-1530.

Girschner, E. 1894. Beitrag zur systematik der Musciden. Berliner Entomologische Zeitschrift, **38:** 297-312.

Google Earth (Version 5.1) 2010 [online]. Available from http://www.google.com/earth/index.html [accessed 10 May 2010].

Gotelli, N.J. 2004. A taxonomic wish-list for community ecology. Philosophical Transactions of the Royal Society B: Biological Sciences, **359:** 585-597.

Gotelli, N.J., and Colwell, R.K. 2001. Quantifying biodiversity: procedures and pitfalls in the measurements and comparison of species richness. Ecology Letters, **4:** 379-391.

Gotelli, N.J., and Entsminger, G.L. 2010. EcoSim: null models software for ecology, version 7 [online]. Acquired Intelligence Inc. & Kesey-Bear. Available from http://garyentsminger.com/ecosim/index.htm [accessed April 2011].

Goulson, D., Derwent, L.C., Hanley, M.E., Dunn, D.W., and Abolins, S.R. 2005. Predicting calyptrate fly populations from the weather, and probable consequences of climate change. Journal of Applied Ecology, **42:** 795-804.

Goulson, D., Hughes, W.O.H., Chapman, J.W. 1999. Fly populations associated with landfill and composting sites used for household refuse disposal. Bulletin of Entomological Research, **89:** 493-498.

Graham, C.H., Ferrier, S., Huettman, F., Moritz, C., and Peterson, A.T. 2004. New development in museum-based informatics and applications in biodiversity analysis. Trends in Ecology and Evolution, **19:** 497-503.

Greely, A.W. 1888. International polar expedition. Report of the proceedings of the United States expedition to Lady Franklin Bay, Grinnell Land. Gouvernment Printing Office, Washington.

Greenberg, B. 1971. Flies and Disease. Princeton University Press, New Jersey.

Greg, L. 2008. Expanding horizons for Canada's only Arctic port [online]. Available from http://www.wd.gc.ca/images/content/10195a-eng.pdf [accessed 22 December 2008].

Grégoire Taillefer, A., and Wheeler, T.A. 2010. Effect of drainage ditches on Brachycera (Diptera) diversity in a southern Quebec peatland. The Canadian Entomologist, **142:** 160-172.

Griffiths, G.C.D 1972. The phylogenetic classification of the Diptera Cyclorrhapha with special reference of the male postabdomen. Junk Publisher, The Hague.

Grixti, J.C., and Packer, L. 2006. Changes in the bee fauna (Hymenoptera: Apoidea) of an old field site in Ontario, revisited after 34 years. The Canadian Entomologist, **138:** 147-164.

Hajibabaei, M., deWaard, J.R., Ivanova, N.V., Ratnasingham, S., Dooh, R.T., Kirk, S.L., Mackie, P.M., and Hebert, P.D.N. 2005. Critical factors for assembling a high volume of DNA barcodes. Philosophical Transactions of the Royal Society B: Biological Sciences, **360:** 1959-1967.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W., and Hebert, P.D.N. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Science USA, **103:** 968-971.

Hajibabaei, M., Singer, G.A., Hebert, P.D., and Hickey, D.A. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genetics, **23:** 167-172.

Hall, B.G. 2004. Phylogenetic trees made easy, a how-to manual. Sinauer Associates, Inc., Massachusetts, USA.

Hanec, W. 1956. A study of the environmental factors affecting the dispersion of house flies (*Musca domestica* L.) in a dairy community near Fort Whyte, Manitoba. The Canadian Entomologist, **88:** 270-272.

Hansen, J., Ruedy, R., Sato, M., and Lo, K. 2010. Global surface temperature change. Reviews of Geophysics, **48:** RG4004. doi: 10.1029/2010RG000345.

Harper, F., and Harper, P.P. 1981. Northern Canadian mayflies (Insecta: Ephemeroptera) records and descriptions. Canadian Journal of Zoology, **59:** 1784-1789.

Haufe, W.O., and Burgess, L. 1956. Development of *Aedes* (Diptera: Culicidae) at Fort Churchill, Manitoba, and prediction of dates of emergence. Ecology, **37:** 500-519.

Hebert, P.D.N., Cywinska, A., Ball, S.L., and deWaard, J.R. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences, **270:** 313-322.

Hebert, P.D.N., deWaard, J.R., and Landry, J.-F. 2009. DNA barcodes for 1/1000 of the animal kingdom. Biology Letters, **6:** 359-362.

Hebert, P.D.N., and Gregory, T.R. 2005. The promise of DNA barcoding for taxonomy. Systematic Biology, **54:** 852-859.

Hebert, P.D.N., Penton, E.H., Burns, J., Janzen, D.H., and Hallwachs, W. 2004a. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly, *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of USA, **101:** 14812-14817.

Hebert, P.D.N., Ratnasingham, S., and deWaard, J.R. 2003b. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society B: Biological Sciences, **270:** 96-99.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S., and Francis, C.M. 2004b. Identification of birds through DNA barcoding. PLoS Biology, **2:** 1657-1663.

Hennig, W. 1955-1964. Muscidae. *In* Die fliegen der Palaearktischen region 63b **7:** 1-1110. *Edited by* E. Lindner. Stuttgart, Germany.

Hennig, W. 1965. Vorarbeiten zu einem phylogenetischen system der Muscidae (Diptera: Cyclorrhapha). Stuttgarter Beiträge zur Naturkunde, **141:** 1-100.

Henriksen, K.L. 1937. Insects collected on the Fifth Thule expedition. Report of the Fifth Thule Expedition 1921-24. Gyldendal, Denmark.

Herting, B. 1957. Das weibliche postabdomen der calyptraten fliegen (Diptera) und sein merkmalswert für die systematik der gruppe. Zeitschrift für Morphologie und Ökologie der Tiere, **45:** 429-461.

Hickling, R., Roy, D.B., Hill, J.K., Fox, R., and Thomas, C.D. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. Global Change Biology, **12:** 450-455.

Hicks, S.D. 1955. Natural history survey of Coppermine, Northwest Territories, 1951. Canadian Field Naturalist, **69:** 162-166.

Hill, J.K., Thomas, C.D., and Huntley, B. 1999. Climate and habitat availability determine 20th century changes in a butterfly's range margin. Proceedings of the Royal Society B: Biological Sciences, **266:** 1197-1206.

Hodkinson, I.D. 1978. The psyllids (Homoptera: Psyllidae) of Alaska. Systematic Entomology, **3:** 333-360.

Hogg, I.D., and Hebert, P.D.N. 2004. Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using DNA barcodes. Canadian Journal of Zoology, **82:** 749-754.

Hogsette, J.A., Farkas, R., and Coler, R.R. 2002. Development of *Hydrotaea aenescens* (Diptera: Muscidae) in manure of unweaned dairy calves and lactating cows. Journal of Economic Entomology, **95:** 527-530.

Høye, T.T., and Forchhammer, M.C. 2008. The influence of weather conditions on the activity of high-arctic arthropods inferred from long-term observations. BMC Ecology, **8:** 1-7.

Høye, T.T., Post, E., Meltofte, H., Schmidt, N.M., and Forchhammer, M.C. 2007. Rapid advancement of spring in the High Arctic. Current Biology, **17:** 449-451.

Huckett, H.C. 1932. The North American species of the genus *Limnophora* Robineau-Desvoidy with descriptions of new species (Muscidae: Diptera). Journal of the New York Entomological Society, **40:** 25-76, 107-158, 279-339.

Huckett, H.C. 1934a. Revision of the North American species belonging to the genus *Coenosia* Meigen and related genera (Diptera: Muscidae). Part I. The subgenera *Neodexiopsis*, *Coenosia*, *Hoplogaster* and related genera *Allognota*, *Bithoracochaeta* and *Schoenomyza*. Transactions of the American Entomological Society, **60:** 57-119.

Huckett, H.C. 1934b. Revision of the North American species belonging to the genus *Coenosia* Meigen and related genera. (Diptera: Muscidae). Part II. The subgenus *Limosia* (*Coenosia* of authors). Transactions of the American Entomological Society, **60:** 133-198.

Huckett, H.C. 1934c. Notes on Francis Walker's type specimens of North American anthomyiid flies in the British Museum (Diptera: Muscidae). The Canadian Entomologist, **66:** 132-140.

Huckett, H.C. 1936. A revision of connectant forms between coenosian and limnophorine genera occurring in North America (Diptera: Muscidae). Journal of the New York Entomological Society, **44:** 187-223.

Huckett, H.C. 1954. A review of the North American species belonging to the genus *Hydrotaea* Robineau-Desvoidy (Diptera: Muscidae). Annals of the Entomological Society of America, **47:** 316-342.

Huckett, H.C. 1964. Arctic Muscidae from the Cape Thompson region of Alaska (Diptera). Bulletin of Brooklyn Entomological Society, **59:** 46-50.

Huckett, H.C. 1965a.The Muscidae of Northern Canada, Alaska, and Greenland (Diptera). Memoirs of the Entomological Society of Canada, **42:** 3-369.

Huckett, H.C. 1965b. The Muscidae. *In* A Catalog of the Diptera of America North of Mexico. *Edited by* A. Stone, C.W. Sabrosky, W.W. Wirth, R.H. Foote, and J.T.P. Coulson. U.S. Department of Agriculture, Handbook no. 276.

Huckett, H.C., and Vockeroth, J.R. 1987. Muscidae. *In* Manual of Nearctic Diptera, volume 2. *Edited by* J.F. McAlpine. Research Branch, Agriculture Canada, Ottawa, Canada. pp. 1115-1131.

Intergovernmental Panel on Climate Change (IPCC) 1990. Climate change, the IPCC scientific assessment. Cambridge University Press, Cambridge, UK.

Intergovernmental Panel on Climate Change (IPCC) 2007. Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate change. Cambridge University Press, Cambridge, UK.

International Barcode of Life Project 2008. Microplate and data submission package [online]. Available from http://ibol.org/wp-content/uploads/2010/07/Sample_Submission_Package-Microplate-7-101.pdf [accessed March 2011].

Ivanova, N.V., deWaard, J.R., and Hebert, P.D.N. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes, **6:** 998-1002.

Ivanova, N.V., deWaard, J.R., and Hebert, P.D.N. 2007. CCDB protocols, glass fiber plate DNA extraction [online]. Available from http://www.dnabarcoding.ca/CCDB_DOCS/CCDB_DNA_Extraction.pdf [accessed 15 April 2011].

Ivanova, N.V., and Grainger, C.M. 2007a. CCDB protocols, COI amplification [online]. Available from http://www.dnabarcoding.ca/CCDB_DOCS/CCDB_Amplification.pdf [accessed 15 April 2011].

Ivanova, N.V., and Grainger, C.M. 2007b. CCDB protocols, sequencing [online]. Available from http://www.dnabarcoding.ca/CCDB_DOCS/CCDB_Sequencing.pdf [accessed 15 April 2011].

Jacoby, G.C., and Darrigo, R. 1989. Reconstructed northern hemisphere annual temperature since 1671 based on high-latitude tree-ring data from North America. Climatic Change, **14:** 39-59.

Janzen, D.H., Hajibabaei, M., Burns, J.M., Hallwachs, W., Remigio, E.D., and Hebert, P.D.N. 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. Philosophical Transactions of the Royal Society B: Biological Sciences, **360:** 1835-1845.

Janzen, D.H., Wachs, W.H., Blandin, P., Burns, J.M., Cadiou, J.M., Chacon, I.S., Daprey, T., Deans, A.R., Epstein, M.E., Espinoza, B., Franclemont, J.G., Haber, W.A., Hajibabaei, M., Hall, J.P.W., Hebert, P.D.N, Gauld, I.D., Harvey, D.J. Hausmann, A., Kitching, I.J., Lafontaine, D., Landry, J.-F., Lemaire, C., Miller, J.Y., Miller, J.S., Miller, L., Miller, S.E., Montero, J., Munroe, E., Green, S.R., Ratnasingham, S., Rawlins, J.E., Robbins, R.K., Rodriguez, J.J., Rougerie, R., Sharkey, M.J., Smith, M.A., Solis, M.A., Sullivan, J.B., Thiaucourt, P., Wahl, D.B., Weller, S.J., Whitfield, J.B., Willmott, K.R., Wood, D.M., Woodely, N.E., and Wilson, J.E. 2009. Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. Molecular Ecology Resources, **9:** 1-26.

Jones, S.R., and Kunz, S.E. 1997. Importance of supercooling points in the overwintering of the horn fly and stable fly (Diptera: Muscidae). Journal of Medical Entomology, **34:** 426-429.

Karl, O. 1928. Zweiflügler oder Diptera II. Muscidae. *In* Die tierwelt Deutschlands 13. *Edited by* F. Dahl. R.G. Fisher Verlag, Jena, Germany.

Karl, T.R., Knight, R.W., Easterling, D.R., and Quayle, R.G. 1996. Indices of climate change for the United States. Bulletin of the American Meteorological Society, **77:** 279-292.

Keiper, J.B., Walton, W.E., Foote, B.A. 2002. Biology and ecology of higher Diptera from freshwater wetlands. Annual Review of Entomology, **47:** 207-232.

Kim, K.C., and McPherson, B. 1993. Insect pests and evolution. *In* Evolution of insect pests: patterns of variation. *Edited by* K.C. Kim and B. McPherson. John Wiley and Sons, New York, USA. pp. 3-25.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, **16:** 11-120.

Knowles, L.L., and Carstens, B.C. 2007. Delimiting species without monophyletic gene trees. Systematic Biology, **56:** 887-895.

Krebs, C.J. 2008. Ecology: the experimental analysis of distribution and abundance, 6th edition. Benjamin Cummings, San Francisco, USA.

Kutty, S.N., Pape, T., Pont, A., Wiegmann, B.M., and Meier, R. 2008. The Muscoidea (Diptera: Calyptratae) are paraphyletic: evidence from four mitochondrial and four nuclear genes. Molecular Phylogenetics and Evolution, **49:** 639-652.

Larson, B.M.H., Kevan, P.G., and Inouye, D.W. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. The Canadian Entomologist, **133:** 439-465.

Lee, J.E. 1991. Principles of insects at low temperature tolerance. *In* Insects at low temperature. *Edited by* R.E. Lee and D.L. Denlinger. Chapman and Hall, New York, USA. pp. 17-45.

Leong, J.M., and Thorp, R.W. 1999. Colour-coded sampling: the pan trap colour preferences of oligolectic and nonoligolectic bees associated with a vernal pool plant. Ecological Entomology, **24:** 329-335.

Lévêque, C., and Mounolou, J.-C. 2003. Biodiversity. John Wiley and Sons, Chichester, UK.

Levesque, C.M., and J.F. Burger 1982. Insects (Diptera: Hymenoptera) associated with *Minuarta groenlandica* (Caryophyllacaea) on Mount Washington, New Hampshire, USA, and their possible role as pollinators. Arctic and Alpine Research, **14:** 117-124.

Lewis, T., and Taylor, L.R. 1965. Diurnal periodicity of flight by insects. Transactions of the Royal Entomological Society of London, **116:** 393-479.

Li, Y., Zhou, X., Feng, G., Hu, H., Niu, L., Hebert, P.D.N., and Huang, D. 2010. COI and ITS sequences delimit species reveal cryptic taxa and host specificity of fig-associated *Sycophila* (Hymenoptera: Eurytomidae). Molecular Ecology Resources, **10:** 31-40.

Liebherr, J.K., and Song, H. 2002. Distinct ground beetle (Coleoptera: Carabidae) assemblages within a New York state wetland complex. Journal of the New York Entomological Society, **110:** 127-141.

Longino, J.T., Coddington, J., and Colwell, R.K. 2002. The ant fauna of a tropical rain forest: estimating species richness three different ways. Ecology, **83:** 689-702.

Lukhtanov, V.A., Sourakov, A., Zakharov, E.V., and Hebert, P.D.N. 2009. DNA barcoding Central Asian butterflies: increasing geographical dimension does not significantly reduce the success of species identification. Molecular Ecology Resources, **9:** 1302-1310.

Mac Nally, R.M., Fleishman. E.P., Bulluck, L., and Betrus, C.J. 2004. Comparative influence of spatial scale on beta diversity within regional assemblages of birds and butterflies. Journal of Biogeography, **31:** 917-929.

Maclaurin, J., and Sterelny, K. 2008. What is biodiversity? The University of Chicago Press, Chigaco, USA.

Maclean, S.F.J., and Hodkinson, I.D. 1980. Distribution of psyllids Homoptera (Homoptera: Psylloidea) in Arctic and Subarctic, Alaska, USA. Arctic and Alpine Research, **12:** 369-376.

Maddison, W. P., and Maddison, D.R. 2010. Mesquite: a modular system for evolutionary analysis [online]. Version 2.73. Available from http://mesquiteproject.org/mesquite/mesquite.html [accessed April 2011].

Magnacca, K.N., and Brown, M.J. 2010. Mitochondrial heteroplasmy and DNA barcoding in Hawaiian *Hylaeus* (*Nesoprosopis*) bees (Hymenoptera: Colletidae). BMC Evolutionary Biology, **10:** 174.

Magurran, A.E. 2004. Measuring Biological Diversity. Blackwell Science, Ltd., Malden, USA.

Malaise, R. 1937. A new insect-trap. Entomologisk Tidskrift, **58:** 148-160.

Malloch, J.R. 1918. Diptera from southwestern United States. Part IV. Anthomyiidae. Transactions of the American Entomological Society, **44:** 263-319.

Malloch, J.R. 1919. Report of the Canadian Arctic Expedition 1913-18, volume 3. Insects, Part C, Diptera, 60c-90c.

Malloch, J.R. 1920. Descriptions of new North American Anthomyiidae (Diptera). Transactions of the American Entomological Society, **46:** 133-196.

Malloch, J.R. 1923. Flies of the anthomyiid genus *Phaonia* Robineau-Desvoidy and related genera, known to occur in North America. Transactions of the American Entomological Society, **48:** 227-282.

Malloch, J.R. 1935. Exotic Muscaridae (Diptera). Annals and Magazine of Natural History, **10:** 562-572.

Marquez, J.G., Cummings, M.A., and Krafsur, E.S. 2007. Phylogeography of stable fly (Diptera: Muscidae) estimated by diversity at ribosomal 16S and cytochrome oxidase I mitochondrial genes. Journal of Medical Entomology, **44:** 998-1008.

Marshall, S., Anderson, R.S., Roughley, R.E., Behan-Pelletier, V., and Danks, H.V. 1994. Terrestrial arthropod biodiversity: planning a study and recommended sampling techniques. Supplement, Bulletin of the Entomological Society of Canada, **26:** 1-33.

Martin, J.E.H. 1977. The insects and arachnids of Canada, part 1: collecting, preparing and preserving insects, mites, and spiders. Biosystematics Research Institute Canada, Research Branch Department of Agriculture, Ottawa, Canada.

McAlpine, J.F. 1965. Observations on anthophilous Diptera at Lake Hazen, Ellesmere Island. Canadian Field Naturalist, **79:** 247-252.

McAlpine, J.F. 1979. Diptera. *In* Canada and its insect fauna. *Edited by* H.V. Danks. Memoir of the Entomological Society of Canada, **108:** 387.

McAlpine, J.F. 1989. Phylogeny and classification of the Muscomorpha. *In* Manual of Nearctic Diptera. *Edited by* J.F. McAlpine and D.M. Wood. Monograph 32, Research Branch, Department of Agriculture Canada, Ottawa, Canada. pp. 1333-1581.

McCafferty, W.P. 1985. The Ephemeroptera of Alaska. Proceedings of the Entomological Society of Washington, **87:** 381-386.

McClure, H.E. 1937. Barren land bugs. Beaver, **267:** 16-21.

McClure, H.E. 1943. Aspection in the biotic communities of the Churchill area, Manitoba. Ecological Monographs, **13:** 1-35.

McCorquodale, D.B. 2001. New records and notes on previously reported species of Cerambycidae (Coleoptera) for Ontario and Canada. Proceedings of the Entomological Society of Ontario, **132:** 3-13.

McLintock, J., and Depner, K.R. 1954. A review of the life-history and habits of the horn fly, *Siphona irritans* (L.) (Diptera: Muscidae). The Canadian Entomologist, **86:** 20-32.

Meir, R., Shiyang, K., Vaiday, G., and Ng, P.K.L. 2006. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. Systematic Biology, **55:** 715-728.

Menéndez, R., Gonzalez Megias, A., Hill, J.K., Braschler, B., Willis, S.G., Cillingham, Y., Fox, R., Roy, D.B., and Thomas, C.D. 2006. Species richness changes lag behind climate change. Proceedings of the Royal Society B: Biological Sciences, **273:** 1465-1470.

Meusnier, I., Singer, G.A.C., Landry, J.-F., Hickey, D.A., Hebert, P.D.N., and Hajibabaei, M. 2008. A universal DNA mini-barcode for biodiversity analysis. BMC Genomics, **9:** 214-217.

Michelsen, V. 1991. Revision of the aberrant new world genus *Coenosopsia* (Diptera: Anthomyiidae), with a discussion of anthomyiid relationships. Systematic Entomology, **16:** 85-104.

Michelsen, V. 2006. Annotated catalogue of the Anthomyiidae, Fanniidae, Muscidae and Scathophagidae (Diptera: Muscoidea) of Greenland. Steenstrupia, **29:** 105-126.

Microsoft Excel 2007 [CD-Rom]. Microsoft, Redmond, USA.

Miller, S.E. 2007. DNA barcoding and the renaissance of taxonomy. Proceedings of the National Academy of Sciences USA, **104:** 4775-4776.

Monaghan, M.T., Balke, M., Pons, J., and Vogler, A.P. 2006. Beyond barcodes: complex DNA taxonomy of a South Pacific Island radiation. Proceedings of the Royal Society B: Biological Sciences, **273:** 887-893.

Moritz, C., and Cicero, C. 2004. DNA barcoding: promises and pitfalls. PLoS Biology, **2:** 1529-1531.

Morris, D.E., and Cloutier, C. 1987. Biology of the predator fly *Coenosia tigrina* (Fab.) (Diptera: Anthomyiidae)-reproduction, development, and larval feeding on earthworms in the laboratory. The Canadian Entomologist, **119:** 381-393.

Muller, S.W. 1945. Permafrost or permanently frozen ground and related engineering problems, second edition. United States Geological Survey Special Report Strategic Engineering Study No. 62.

Nagy, Z.T., Breman, F.C., and Dall'Asta, H. 2010. DNA barcoding of museum specimens of Lymantriidae preserved in the Royal Museum for Central Africa. Entomologica Romanica, **15:** 11-16.

Nei, M., and Kumar, S. 2000. Molecular evolution and phylogenetics. Oxford University Press, New York, USA.

Nelson, L.A., Wallman, J.F., and Dowton, M. 2008. Identification of forensically important *Chrysomya* (Diptera: Calliphoridae) species using the second ribosomal internal transcribed spacer (ITS2). Forensic Science International, **177:** 238-247.

Newbold, T. 2010. Applications and limitations of museum data for conservation and ecology, with particular attention to species distribution models. Progress in Physical Geography, **34:** 3-22.

Newton, S.T., Fast, H., and Henley, T. 2002. Sustainable development for Canada's Arctic and Subarctic communities: a backcasing approach to Churchill, Manitoba. Arctic, **55:** 281-290.

Nice, C.C., Gompert, Z., Forister, M.L., and Fordyce, J.A. 2009. An unseen foe in arthropod conservation efforts: the case of *Wolbachia* infections in the Karner blue butterfly. Biological Conservation, **142:** 3137-3146.

Niemelä, J, 1997. Invertebrates and boreal forest management. Conservation Biology, **11:** 601-610.

Overpeck, J., Hughen, K., Hardy, D., Bradley, R., Case, R., Douglas, M., Finney, B., Gajewski, K., Jacoby, G., Jennings, A., Lamoureux, S., Lasca, A., Macdonald, G., Moore, J., Retelle, M., Smith, S., Wolfe, A., and Zielinski, G. 1997. Arctic environmental changes of the last four centuries. Science, **278:** 1251-1256.

Pandellé, L. 1898. Études sur les Muscidae de France, IIIe partie. Revue d'Entomologie, **23:** 1-41.

Pandellé, L. 1904. Catalogue des Muscidae de France. Revue d'Entomologie, **17:** 1-32.

Parmesan, C. 1996. Climate and species' range. Nature, **382:** 765-766.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution and Systematics, **37:** 637-69.

Parmesan, C., Ryrholm, N., Stefanescus, C., Hill, J.K., Thomas, C.D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W.J., Thomas, J.A., and Warrens, M. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature, **399:** 579-583.

Patenaud, A. 2007. Diversity, composition and seasonality of wild bees (Hymenoptera: Apidoidae) in a northern mixed-grass prairies preserve. M.Sc. Thesis. University of Manitoba, Winnipeg, Manitoba, Canada.

Pattengale, N.D., Alipour, M., Bininda-Emonds, O.R.P., Moret, B.M.E., and Stamatakis, A. 2009. Research in computational molecular biology. Lecture Notes in Computer Science, **5541:**184-200.

Payton, M.E., Greenstone, M.H., and Schenker, N. 2003. Overlapping confidence intervals or standard error intervals: what do they mean in terms of statistical significance? Journal of Insect Science, **3:** 34.

Perovich, D., Meier, W., Maslanik, J., and Richter-Menge, J. 2010. Sea ice cover [online]. *In* Arctic report card 2010. *Edited by* J. Richter-Menge and J.E. Overland. Available from http://www.arctic.noaa.gov/reportcard/ArcticReportCard_full_report.pdf [accessed 30 October 2010].

Peters, R.L. 1992. Diversity in the face of climate change. *In* Global warming and biological diversity. *Edited by* R.L. Peters and T.E. Lovejoy. Yale University Press, New Haven, USA. pp.16-29.

Pielou, E.C. 1975. Ecological Diversity. Wiley-Interscience Publication, Halifax.

Piercey-Normore, M.D. 2005. Lichens from the Hudson Bay Lowlands: northeastern coastal regions of Wapusk National Park in Manitoba. Canadian Journal of Botany, **83:** 1029-1038.

Pollard, E., Moss, D., and Yates, T.J. 1995. Population trend of common British butterflies at monitored sites. Journal of Applied Ecology, **32:** 9-16.

Pollard, E., and Yates, T.J. 1993. Monitoring butterflies for ecology and conservation. Chapman & Hall, London, UK.

Ponder, W. 1999. Using museum collection data to assist in biodiversity assessment. *In* The other 99%. The conservation and biodiversity of invertebrates. *Edited by* W. Ponder and D. Lunney. The Royal Zoological Society of New South Wales, Mosman, Australia.

Ponder, W., Carter, G.A., Flemons, P., and Chapman, R.R. 2001. Evaluation of museum collection data for use in biodiversity assessment. Conservation Biology, **15:** 648-657.

Pont, A.C. 1984. A revision of the Fanniidae and Muscidae (Diptera) described by Fallén. Entomologica Scandinavica, **15:** 277-297.

Pont, A.C. 1986. Family Muscidae. *In* Catalogue of Palaearctic Diptera, volume 11: Scathophagidae-Hypodermatidae. *Edited by* Á. Soós and L. Papp. Akadémiai Kiadó, Budapest, Hungary. pp. 55-215.

Pont, A.C. 1993. Observations on anthophilous Muscidae and other Diptera (Insecta) in Abisko National Park, Sweden. Journal of Natural History, **27:** 631-643.

Pont, A.C. 2011. The Muscidae described by J.W. Zetterstedt (Insecta: Diptera). Zootaxa, **2852:** 1-83.

Pöyry, J., Luoto, M., Heikkinen, R.K., Kuussaari, M., and Saarinen, K. 2009. Species traits explain recent range shifts of Finnish butterflies. Global Change Biology, **15:** 732-743.

Quarterman, K.D., Kilpatrick, J.W., and Mathis, W. 1954. Fly dispersal in a rural area near Savannah, Geogia. Journal of Economical Entomology, **47:** 405-412.

Ratnasingham, S., and Hebert, P.D.N. 2007. BOLD: the barcode of life data system (www.barcodinglife.org). Molecular Ecology Notes, **7:** 355-367.

Resh, V.H 1976. Changes in the caddis-fly fauna of Lake Erie, Ohio, and of the Rock River, Illinois, over a fifty year period of environmental deterioration. Proceedings of the First International Symposium in Trichoptera, Lutz am See, Austria, 1974. Dr. W. Junk, The Hague, Netherlands. pp.167-170.

Riegert, P.W. 1985. The heritage lecture: some aspects of a limited history of northern insect studies. Bulletin of the Entomological Society of Canada, **17:** 90-96.

Rivera, J., and Currie, D. 2009. Identification of Nearctic black flies using DNA barcodes (Diptera: Simuliidae). Molecular Ecology Resources, **9:** 224-236.

Roback, S.S. 1951. A classification of the muscoid calyptrate Diptera. Annals of the Entomological Society of America, **44:** 327-361.

Robinson, E.A., Blagoev, G.A., Hebert, P.D.N., and Adamowicz, S.J. 2009. Prospects for using DNA barcoding to identify spiders in species-rich genera. ZooKeys, **16:** 27-46.

Rodriguez-Trelles, F., and Rodriguez, M.A. 1998. Rapid micro-evolution and loss of chromosomal diversity in *Drosophila* in response to climate warming. Evolutionary Ecology, **12:** 829-383.

Rossi, M.N., and Godoy, W.A.C. 2006. Prey choice by *Nesticodes rufipes* (Araneae: Theridiidae) on *Musca domestica* (Diptera: Muscidae) and *Dermestes ater* (Coleoptera: Dermestidae). The Journal of Arachnology, **34:** 186-193.

Roughley, R., and Alperyn, M. 2009. Common and historical collecting localities within Canada [online]. Biological Survey of Canada, Ottawa. Available from http://www.biology.ualberta.ca/bsc/english/locality.htm [accessed 30 October 2010].

Roy, D.B., Rothery, P., Moss, D., Pollard, E., and Thomas, J. 2001. Butterfly numbers and weather: predicting historical trends in abundance and the future effects of climate change. Journal of Animal Ecology, **70:** 201-217.

Samways, M.J., McGoech, M.A., and New, T.R. 2010. Insect conservation, a handbook of approaches and methods. Oxford University Press, New York.

Savage, J. 2002. Cleaning up the world: dipteran decomposers. Biodiversity, **3:** 12-16.

Savage, J. 2003. Revision of the genus *Thricops* Rondani (Diptera: Muscidae). Insect Systematics and Evolution, **supplement 61:** 3-143.

Savage, J., and Wheeler, T. 2004. Phylogeny of the Azeliini (Diptera: Muscidae). Studia Dipterologica, **11:** 259-299.

Savage, J., Wheeler, T.A., Moores, A.M.A., and Grégoire Taillefer, A. 2011. Effects of habitat size, vegetation cover, and surrounding land use on Diptera diversity in temperate Nearctic bogs. Wetlands, **31:** 101-112.

Savage, J., Wheeler, T.A., and Wiegmann, B.M. 2004. Phylogenetic analysis of the genus *Thricops* Rondani (Diptera: Muscidae) based on molecular and morphological characters. Systematic Entomology, **29:** 395-414.

Sax, D.F., and Gaines, S.D. 2003. Species diversity: from global decreases to local increases. Trends in Ecology and Evolution, **18:** 561-566.

Schmidt, N.M., Berg, T.B., and Meltofte, H. 2010. Biobasis, conceptual design and sampling procedure of the biological monitoring programme within Zackenberg Basic, 13th Edition. National Environment Research Institute, Aarhus University, Denmark.

Schoof, H.F., and Siverly, R.E. 1954a. Multiple release studies on the dispersion of *Musca domestica* at Phoenix, Arizona. Journal of Economic Entomology, **47:** 830-838.

Schoof, H.F., and Siverly, R.E. 1954b. Urban fly dispersion studies with special reference to movement pattern of *Musca domestica*. American Journal of Tropical Medicine and Hygiene, **3:** 539-547.

Schuehli, G.S.E., de Carvalho, C.J.B, and Wiegmann, B.M. 2004. Regarding the taxonomic status of *Ophyra* Robineau-Desvoidy (Diptera: Muscidae): a molecular approach. Zootaxa, **712:** 1-12.

Schuehli, G.S.E., de Carvalho, C.J.B, and Wiegmann, B.M. 2007. Molecular phylogenetics of the Muscidae (Diptera: Calyptratae): new ideas in a congruence context. Invertebrate Systematics, **21:** 263-278.

Scudder, G.G.E. 2009. The importance of insects. *In* Insect biodiversity: science and society. *Edited by* R.G. Foottit and P.H. Adler. Blackwell Publishing, Oxford, UK. pp. 7-32.

Secretariat of the Convention on Biological Diversity. 2005. Handbook of the Convention on Biological Diversity including its Cartagena Protocol on biosafety, 3rd edition. Montreal, Canada.

Séguy, E. 1937. Diptera, family Muscidae. *In* Genera insectorum. *Edited by* P. Wytsman, Desmet-Verteneuil, Bruxelles, Belgique.

Shaffer, H.B, Fisher, R.N., and Davidson, C. 1998. The role of natural history collections in documenting species declines. Trends in Ecology and Evolution, **13:** 27-30.

Sheffield, C.S., Hebert, P.D.N., Kevan, P.G., and Packer, L. 2009. DNA barcoding a regional bee (Hymenoptera: Apoidea) fauna and its potential for ecological studies. Molecular Ecology Resources, **9:** 196-207.

Shelford, V.E., and Twomey, A.C. 1941. Tundra animal communities in the vicinity of Churchill, Manitoba. Ecology, **22:** 47-69.

Singer, G., and Hajibabaei, M. 2009. iBarcode.org: web-based molecular biodiversity analysis. BMC Bioinformatics, **10:** S14.

Skidmore, P. 1973. Notes on the biology of Palearctic Muscids. Entomologist, **106:** 25-29.

Skidmore, P. 1985. The biology of the Muscidae of the world. Junk Publishers, Dordrecht, Netherlands.

Smith, M.A., Fernández-Triana, J., Roughley, R., and Hebert, P.D.N. 2009. DNA barcode accumulation curves for understudied taxa and areas. Molecular Ecology Resources, **9:** 208-216.

Smith, M.A., and Fisher, B.L. 2009. Invasions, DNA barcodes, and rapid biodiversity assessment using ants of Mauritius. Frontiers in Zoology, **6:** 31.

Smith, M.A., Rodriguez, J.J., Whitfield, J.B., Deans, A.R., Janzen, D.H., Hallwachs, W., and Hebert, P.D.N. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. PNAS, **105:** 12359-12364.

Smith, M.A., Wood, D.M., Janzen, D.H., Hallwachs, W., and Hebert, P.D.N. 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera: Tachinidae) are not all generalists. PNAS, **104:** 4967-4972.

Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W. and Hebert, P.D.N. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). PNAS, **103:** 3657-3662.

Snow, W.A. 1891. The moose fly - a new *Haematobia*. The Canadian Entomologist, **23:** 87-89.

Snyder, F.M. 1940. Review of the genus *Myospila* Rondani with descriptions of new species (Diptera: Muscidae). American Museum Novitates, **1087:** 1-10.

Snyder, F.M. 1949a. Nearctic *Helina* Robineau-Desvoidy (Diptera: Muscidae). American Museum of Natural History, **94:** 112-159.

Snyder, F.M. 1949b. Review of Nearctic *Mydaea*, *sensu stricto*, and *Xenomydaea* (Diptera: Muscidae). American Museum Novitates, **1401:** 1-38.

Snyder, F.M. 1954. A review of Nearctic *Lispe* Latreille (Diptera: Muscidae). American Museum Novitates, **1675:** 1-40.

Southwood, T.R.E. 1978. The components of diversity. *In* Diversity of insect faunas. *Edited by* L.A. Mound and N. Waloff. Blackwell Scientific Publications, New York, USA. pp. 19-40.

Sperling, F.A.H., and Roe, A.D. 2009. Molecular dimensions of insect taxonomy. *In* Insect biodiversity: science and society. *Edited by* R.G. Foottit and P.H. Adler. Blackwell Publishing LTD, Chichester, UK. pp. 397-416.

Stafford, C.K. 2008. Fly management handbook, a guide to biology, dispersal, and management of the house fly and related flies for farmers, municipalities, and public health officials. Bulletin of the Connecticut Agricultural Experiment Station, **1013:** 1-40.

Statistics Canada. 2006. Community profiles: Churchill, Manitoba (town) [online]. Available from http://www12.statcan.ca/census-recensement/2006 [accessed 27 January 2008].

Stein, P. 1916. Die Anthomyiden Europas. Tabellen zur bestimmung der gattungen und aller mirbekannten arten, nebst mehr oder weniger ausfürhrlichen beschreibungen. Archiv für Naturgeschichte, **81:** 1-224.

Stein, P. 1919. Die Anthomyiden gattungen der welt, analytisch bearbeitet, nebst einem kritischsystematischen verzeichnis aller aussereuropäischen arten. Archiv für Naturgeschichte., **83:** 85-178.

Stendel, M., Christensen, J.H., and Petersen, D. 2008. Arctic climate and climate change with a focus on Greenland. Advances in Ecological Research, **40:** 13-43.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods [online]. Molecular Biology and Evolution, doi:10.1093/molbev/msr121: 1-9. Available from http://kumarlab.net/pdf_new/TamuraKumar11.pdf [accessed 10 June 2011].

Taylor, A. 1993. Churchill chapter, 1931 [online]. Manitoba History, **25.** Available from http://www.mhs.mb.ca/docs/mb_history/25/churchill1931.shtml [accessed 6 September 2011].

Taylor, D.B., Berkebile, D.R., and Scholl, P.J. 2007. Stable fly population dynamics in eastern Nebraska in relation to climatic variables. Journal of Medical Entomology, **44:** 765-771.

Taylor, P., and Westwood, R. 2008. Extended range and flight period of the common buckeye butterfly in southern Manitoba. Blue Jay, **66:** 107-11.

Thomas, G., and Jespersen, J.B. 1994. Non-biting Muscidae and control methods. Revue Scientifique et Technique, **13:** 1159-1173.

Thuiller, W. 2007. Climate change and the ecologist. Nature, **448:** 550-552.

Totland, O. 1993. Pollination in alpine Norway: flowering phenology, insect visitor, and visitation rates in two plant communities. Canadian Journal of Botany, **71:** 1072-1079.

Townes, H. 1972. A light-weighted Malaise trap. Entomological News, **83:** 239-247.

Travis, J.M.J. 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. Proceedings of the Royal Society B: Biological Sciences, **270:** 467-473.

Tulio de Oliveira, M., de Azeredo-Espin, A.N.L., and Lessinger, A.C. 2005. Evolutionary and structural analysis of the cytochrome *c* oxidase subunit I (COI) gene from *Haematobia irritans, Stomoxys calcitrans* and *Musca domestica* (Diptera: Muscidae) mitochondrial DNA. DNA Sequence, **16:** 156-160.

Underwood, A.J., and Chapman, M.G. 1999. Problems and practical solutions for quantitative assessment of biodiversity of invertebrates in coastal habitats. *In* The other 99%. The conservation and biodiversity of invertebrates. *Edited by* W. Ponder and D. Lunney. Royal Zoological Society of New South Wales, Mosman, Australia. pp. 19-25.

UNEP/GRID-Arendal. 2005. Definitions of the Arctic [online]. Available from http://maps.grida.no/go/graphic/definitions_of_the_arctic [accessed 10 October 2010].

Van Houdt, J.K.J., Breman, F.C., Virgilio, M., and De Meyer, M. 2010. Recovering full DNA barcodes from natural history collections of tephritid fruitflies (Diptera: Tephritidae) using mini barcodes. Molecular Ecology Resources, **10:** 459-465.

Verral, G.H. 1891. Note at the meeting of 14 October 1891. Bulletin bimensuel de la Société d'Entomologie de la France, **15:** cxxxiii-cxxxiv.

Virgilio, M., Backeljau, T., Nevado, B., and De Meyer, M. 2010. Comparative performances of DNA barcoding across insect orders. BMC Bioinformatics, **11:** 206-215.

Vockeroth, J.R. 1979. Muscidae. *In* Canada and its insect fauna. *Edited by* H.V. Danks. Memoirs of the Entomological Society of Canada 108, Ottawa, Canada. pp. 416.

Vockeroth, J.R. 2002. Exploring the diversity of flies (Diptera). Biodiversity, **3:** 3-5.

Vodden, C. 1992. No Stone Unturned: The First 150 years of the Geological Survey of Canada [online]. Available from http://gsc.nrcan.gc.ca/hist/150_e.php [accessed 13 October 2011].

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., and Hebert, P.D.N. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences, **1462:** 1-11.

Webb, J.E. 1956. Observations on some filth flies in the vicinity of Fort Churchill, Manitoba, Canada, 1953-54. Journal of Economic Entomology, **49:** 595-600.

Weber, N.A. 1950. A survey of the insects and related arthropods of arctic Alaska. Transactions of the American Entomological Society, **76:** 147-206.

Weber, N.A. 1954. Arctic Alaskan Diptera. Proceedings of the Entomological Society of Washington, **56:** 86-91.

Werner, D., and Pont, A.C. 2003. Dipteran predators of simuliid blackflies: a worldwide review. Medical and Veterinary Entomology, **17:** 115-132.

Werner, D., and Pont, A.C. 2006. New results on Diptera predators in the blackfly plague areas of Central Europe and the Caucasus. Acta Entomologica Serbica, **supplement:** 131-140.

Westwood, R., and Blair, D. 2010. Effect of regional climate warming on the phenology of butterflies in boreal forests in Manitoba, Canada. Environmental Entomology, **39:** 1122-1133.

Wheeler, T.A., Huber, J.T., and Currie, D.C. 2001. Label data standards for terrestrial arthropods [online]. Biological Survey of Canada (Terrestrial Arthropods), Ottawa. Available from http://www.biology.ualberta.ca/bsc/briefs/brlabelstandards.htm [accessed May 2011.]

White, P.J.T., and Kerr, J.T. 2006. Contrasting spatial and temporal global change impacts on butterfly species richness during the 20th century. Ecography, **29:** 908-918.

White, P.J.T., and Kerr, J.T. 2007. Human impacts on environment-diversity relationships: evidence for biotic homogenization from butterfly species richness patterns. Global Ecology and Biogeography, **16:** 290-299.

Whitworth, T.L., Dawson, R.D., Magalon, H. and Baudry, E. 2007. DNA barcoding cannot reliably identified species of the blowfly genus *Protocalliphora* (Diptera: Calliphoridae). Proceedings of the Royal Society B: Biological Sciences, **274:** 1731-1739.

Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A., and Losos, E. 1998. Quantifying threats to imperiled species in the United States. Bioscience, **48:** 607-615.

Wilson, R.J., Gutierrez, D., Gutierrez, J., and Monserrat, V.J. 2007. An elevational shift in butterfly species richness and composition accompanying recent climate change. Global Change Biology, **13:** 1873-1887.

Woodward, S.L. 2003. Biomes of earth, terrestrial, aquatic and human-dominated. Greenwood Press, Wesport, USA.

Zhou, X., Jacobus, L.M., DeWalt, R.E., Adamowicz, S.J., and Hebert, P.D.N. 2010. Ephemeroptera, Plecoptera, and Trichoptera fauna of Churchill (Manitoba, Canada): insights into biodiversity patterns from DNA barcoding. The Journal of the North American Benthological Society, **29:** 814-837.

Zhou, X., Robinson, J.L., Geraci, C.J., Parker, C.R., Flint Jr, O.S., Etnier, D.A., Ruiter, D., DeWalt, R.E., Jacobus, L.M., and Hebert, P.D.N. 2011. Accelerated construction of a regional DNA-barcode reference library: caddisflies (Trichoptera) in the Great Smoky Mountains National Park. Journal of the North American Benthological Society, **30:** 131-162.

Zumpt, F. 1973. The stomoxyine biting flies of the world (Diptera: Muscidae), taxonomy, biology, economic importance and control measures. Gustav Fisher Verlag, Stuttgart, Germany.

Zych, M. 2007. On flower visitors and true pollinators: the case of protandrous *Heracleum sphondylium* L. (Hymenoptera: Apiaceae). Plant Systematics and Evolution, **263:** 159-179.

APPENDICES

Appendix A. Ecological requirements of muscid genera from Churchill (Snow 1891; McAlpine 1965; Skidmore 1973, 1985; Burger and Anderson 1974; Arntfield 1975; Danks 1981; Levesque and Burger 1982; Ferrar 1987; Morris and Cloutier 1987; Drummond *et al.* 1989; Pont 1993; Totland 1993; Thomas and Jespersen 1994; Larson *et al*. 2001; Werner and Pont 2003, 2006; Gilioli *et al*. 2005; Zych 2007).

Genus	Adult habitat, feeding habits and special behaviours	Larval habitat (breeding media), feeding habits and special behaviours	Thermal preference/tolerance	Medical, veterinary, economical & ecological roles
Coenosia Meigen	In wet areas. Predators of culicids, simuliids & chironomids and insects inhabiting herb layers. Visit flowers.	Rich humid substrates, tree cavities, excrement, parts of plants infested by other fly larvae. Predators of fly larvae & earthworms.	Few at high altitude or latitude.	Control of populations of some biting flies.
Drymeia Meigen	Abundant on tundra, mountains & flowering areas. Visit flowers.	Under moss, in humus & some in excrement. Obligate predators.	N/A	Pollinators in sub-arctic and arctic ecosystems.
Graphomya R.-D.	Anthophilous. Feed on nectar and pollen. Also coprophagous to saprophagous.	Liquid or semi-liquid rotting substrates. Predators of syrphids and ptychopterids.	N/A	Pollinators. No medical or veterinary importance.

Appendix A. (continued)

Genus	Adult habitat, feeding habits and special behaviours	Larval habitat (breeding media), feeding habits and special behaviours	Thermal preference/tolerance	Medical, veterinary, economical & ecological importance
Haematobia Le Peletier et Serville	Haematophagous	Breed in dung. Pupate in the soil.	Overwinter in pupal stage. Active from late spring to fall.	The fly does not seem to affect the health of the moose.
Hebecnema Schnabl	Frequent at bushy areas, females abundant at breeding sites. Feed on honeydew, flowers & excrement. Some are sweat flies. Males stay on foliage & form swarms.	Excrement, decaying plants & animal matter, moss and humus. Carnivorous.	Overwinter as larva or pupa. Life cycle takes a minimum of 3-4 weeks.	None of such importance.
Helina R.- D.	Ubiquitous. Anthophiles, also attracted to sunlit foliage & excrement. Some indoor species.	Under moss, in humus, decaying plants & animal matter, excrement, sap runs, rotten tree holes, birds/Hymenoptera nests, in burrows. Saprophagous, some coprophagous.	Overwinter as larva (and as adult in Britain).	Some are pest households.

Appendix A. (continued)

Genus	Adult habitat, feeding habits and special behaviours	Larval habitat (breeding media), feeding habits and special behaviours	Thermal preference/tolerance	Medical, veterinary, economical & ecological importance
Hydrotaea R.-D.	Ubiquitous. Associated with trees. Sweat flies, saprophagous, also feed on honeydew. Hover in small swarms.	Decaying plants & animals, excrement, bird nests, burrows, moss & humus. Saprophagous & predaceous.	N/A	Transmission of pathogens to humans and other animals.
Limnophora R.-D.	In wet areas. Predators of culicids, simuliids & chironomids. Visit flowers.	Excrement, decaying plant & animal matter, aquatic larva inhabiting running water. Predaceous.	N/A	Predation on some biting flies.
Limnospila Schnabl	Predaceous.	N/A	N/A	N/A
Lispe Latreille	Shores of rivers, fens & marshes, adjacent vegetation to breeding sites. Predators of culicids, simuliids & chironomids.	Rich humid substrates, wet sand or mud with organic content, algal matter, some halobionts (saline areas). Predaceous.	*L. canadensis* is more tolerant of extreme cold than other *Lispe* spp. Overwinter as larva.	Control of populations of some biting flies.

Appendix A. (continued)

Genus	Adult habitat, feeding habits and special behaviours	Larval habitat (breeding media), feeding habits and special behaviours	Thermal preference/tolerance	Medical, veterinary, economical & ecological importance
Lispocephala (R.-D.)	In wet areas. Predator of culicids, simuliids & chironomids.	Aquatic, some species in running water, some hiding in moss. Predaceous.	N/A	N/A
Lophosceles Ringdahl	Some attracted to dung. Coprophagous.	N/A	N/A	N/A
Morellia R.- D.	Sweat flies, saprophagous, coprophagous, some also feed on pollen and/or nectar.	Excrement of ungulates. Coprophagous.	Some tolerant of more extreme cold condition, e.g., *M. podagrica*.	N/A
Musca Linnaeus	Synanthropic. Sweat flies, saprophagous, coprophagous.	Excrement, decaying plants & animal matter. Saprophagous.	Optimum temperature 20-25 ºC. Minimum survival temperature -10 ºC.	Many impacts, such as transportation of pathogens from carrion, garbage & dung to human food.

Appendix A. (continued)

Genus	Adult habitat, feeding habits and special behaviours	Larval habitat (breeding media), feeding habits and special behaviours	Thermal preference/tolerance	Medical, veterinary, economical & ecological importance
Muscina R.- D.	Ubiquitous.	Decaying plants & animal matter, excrement. Coprophagous, predaceous.	N/A	Transmission of pathogens to human. Some spread by commerce.
Mydaea R.- D. (including *Opsolasia* Coquillett)	Feed on flowers (pollen and/or nectar) & honeydew.	Excrements, rotting fungi (wide range of species) & wood. Saprophagous, predaceous.	N/A	N/A
Myospila Rondani	Feed on flowers.	Excrement. Predators of other Diptera.	N/A	N/A
Phaonia R.- D.	Up to the northern edge of birch forest, not in open tundra. Feed on honeydew, rarely on excrement. Anthophilous, attracted to tree trunks & foliage.	Wide range of media, (excrement rarely used), water holes in old trees, litter, compost, rotten wood & fungi. Pupate in rotten wood above level of water. Carnivorous.	N/A	Important pollinators with *Spilogona* & *Thricops*.

Appendix A. (continued)

Genus	Adult habitat, feeding habits and special behaviours	Larval habitat (breeding media), feeding habits and special behaviours	Thermal preference/tolerance	Medical, veterinary, economical & ecological importance
Potamia	Anthophilous	Human faeces, rotten wood, nests of birds and social insects, liquid substrates in garbage and fungi. Facultative carnivore.	Overwinter as larva.	Pollinators
Pseudocoenosia Stein	N/A	Aquatic/sub-aquatic. In wet sphagnum or moss.	Overwinter as larva.	None of such importance.
Schoenomyza Haliday	N/A	Excrement, under moss & in humus.	N/A	None of such importance.
Spilogona Schnabl	Tundra & mountains. Some of the most successful muscids of the tundra. Also: wet or drained fertile habitats, littoral zone (also coastal). Predaceous, anthophilous (visiting a wide range of flowers).	Range of habitats: dry, aquatic, damp soil, moss cushions, shallow and temporary pools with emergent vegetation, some halobionts (saline areas), on shores of reservoirs. Saprophagous, predaceous. Pupate in sand.	Tolerant of cold weather, the only genus breeding as far as the eightieth parallel.	The most important pollinators in Sub-arctic and Arctic along with *Thricops* and *Phaonia*.

Appendix A. (concluded)

Genus	Adult habitat, feeding habits and special behaviours	Larval habitat (breeding media), feeding habits and special behaviours	Thermal preference/tolerance	Medical, veterinary, economical & ecological importance
Stomoxys Geoffroy	Haematophagous	Manure, decaying organic matter.	Optimum temperature for eggs to hatch: 23-30 ºC. Adults prefer temperature over 12 ºC.	Transports pathogens; nuisance.
Thricops Rondani	Abundant on tundra, mountains & flowering areas. Anthophilous sun-loving flies, rest on foliage & rocks.	Under moss, in humus, some in burrows. Saprophagous, predaceous.	N/A	Important pollinators in arctic ecosystems with *Spilogona* and *Phaonia*.

Appendix B. Permanent (1-9) and occasional (10-35) sites visited in the 2007 survey of Muscidae of Churchill (Manitoba). See Figure 3.1 for the position of each site on the map of Churchill.

Site	Latitude, longitude	Site name/description	Site	Latitude, longitude	Site name/description
1	58.746, -94.134	Beech Bay	19	58.737, -93.819	CNSC (Research Center)
2	58.618, -93.828	Burned Area	20	58.760, -94.086	Dryas heath
3	58.705, -94.054	**Farnworth Lake**	21	58.753, -93.951	**Eastern creek (a)**
4	58.754,-93.998	Launch Road	22	58.759, -93.952	**Eastern creek (b)**
5	58.677, -94.144	Goose Creek Road	23	58.758, -94.062	**Fort Churchill**
6	58.626, -94.230	Pump House	24	58.756, -94.087	Garbage depot
7	58.730, -93.780	Ramsay Creek	25	58.776, -93.736	Gordon Point (a)
8	58.752, -93.910	Twin Golf Balls	26	58.791, -93.751	Gordon Point (b)
9	58.632, -93.788	Twin Lakes	27	58.759, -93.967	Gun range
10	58.759, -93.972	Side of Goose Crk Rd	28	58.733, -94.111	Historical site: Dene Village
11	58.738, -94.058	Near airport	29	58.760, -93.931	Intertidal area
12	58.746, -94.114	Akudlik Marsh	30	58.756, -94.027	Old dump
13	58.759, -94.175	Beach on mouth of Churchill River	31	58.619, -93.828	Regenerating burned area
14	58.765, -93.932	Beach on Hudson Bay	32	58.734, -94.108	Rotting grain pile near railroad
15	58.632, -94.219	Between marina and pump house	33	58.633, -93.835	Spruce-lichen woody area
16	58.738, -94.114	Biological waste depot	34	58.769, -94.174	**Town of Churchill**
17	58.761, -93.898	Bird Cove: coastal bluff (a)	35	58.675, -94.167	Weir on Churchill River
18	58.763, -93.867	Bird Cove: coastal bluff (b)			

Note: Boldface type denotes a site known to be visited by previous collectors

Appendix C. North American and European collections from which specimens and information regarding holdings of Muscidae from Churchill (Manitoba) were received.

Collection	Holdings
Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, ON, Canada	2559
National Museum of Natural History, Smithsonian Institution, Washington, DC, USA	141
American Museum of Natural History, New York, NY, USA	52
Field Museum of Natural History, Chicago, IL, USA	51
California Academy of Sciences, San Francisco, CA, USA	39
University of Guelph, Guelph, ON, Canada	28
The Natural History Museum, London, England	6
Royal Ontario Museum, Toronto, ON, Canada	2
Maurice T. James Entomological Collection, Washington State University, Pullman, WA, USA	2
The Manitoba Museum, Winnipeg, MB, Canada	1
J.B. Wallis/R.E Roughley Museum of Entomology, University of Manitoba, Winnipeg, MB, Canada	0
Lyman Entomological Museum, McGill University, Ste-Anne-de-Bellevue, QC, Canada	0
Oxford University Museum of Natural History, Oxford, England	0
Cornell University Insect Collection, Ithaca, NY, USA	0
Royal Saskatchewan Museum, Regina, SK, Canada	0
Snow Entomological Museum, Lawrence, University of Kansas, KS, USA	0
C.A. Triplehorn Insect Collection, Ohio State University, Columbus, OH, USA	0
Carnegie Museum of Natural History, Pittsburgh, PA, USA	0
Essig Museum of Entomology, University of California, Berkeley, CA, USA	0
E.H. Strickland Entomological Museum, University of Alberta, Edmonton, AB, Canada	0
University of Massachusetts, Amherst, MA, USA	0
Swedish Museum of Natural History, Stockholm, Sweden	0

Appendix D. Species and specimen numbers by collecting method for the 2007 survey of Muscidae of Churchill (Manitoba) (M: Malaise trap; P: pan trap; S(p): net sweeping at permanent sites; S(o): net sweeping at occasional sites) and for all species found in pre-1965 museum material and/or listed in the literature for Churchill (Manitoba).

Species	Number of specimens				
	2007 inventory				Pre-1965
	M	P	S(p)	S(o)	
Coenosia atritibia Ringdahl	1	36	0	3	55
Coenosia comita (Huckett)	613	17	8	4	34
Coenosia conforma Huckett	96	56	3	0	2
Coenosia demoralis Huckett	47	2	0	0	2
Coenosia flaviseta Huckett	4	0	0	0	6
***Coenosia frisoni* Malloch**	2	0	0	0	0
Coenosia longimaculata Stein	159	12	1	2	8
Coenosia minor Huckett	175	0	0	0	2
Coenosia mollicula (Fallén)	73	11	1	0	0
Coenosia nigrescens Stein	3	9	0	0	3
Coenosia nigricoxa Stein	102	38	5	19	73
Coenosia octopunctata (Zetterstedt)	246	27	4	5	182
Coenosia pumila (Fallén)	4	1	0	0	101
***Coenosia remissa* Huckett**	2	0	0	0	0
Coenosia tarsata Huckett ♂	0	0	0	1	14
Coenosia tarsata/ C. verralli Collin ♀	27	7	6	22	32
Coenosia triseta Stein	279	6	3	6	8
Coenosia verralli ♂	5	0	0	0	5
***Drymeia groenlandica* (Lundbeck)**	1	0	0	0	0
***Drymeia pribilofensis* Malloch**	0	0	1	20	9*
Drymeia quadrisetosa Malloch	7	109	0	0	39
Drymeia segnis (Holmgren)	0	0	0	0	N/A
Graphomya idessa Walker	6	1	0	1	8
Graphomya minuta Arntfield	0	0	0	1	2
Graphomya transitionis Arntfield	2	0	0	1	2
Hebecnema vespertina Fallén	0	0	0	0	2
***Haematobia alcis* (Snow)**	1	0	0	0	0
Helina annosa (Zetterstedt)	0	1	0	0	N/A
Helina cinerella (van der Wulp)	10	0	2	2	1*
Helina evecta (Harris)	284	66	12	2	36
Helina flavisquama (Zetterstedt)	279	34	6	4	66
***Helina fulvisquama* (Zetterstedt)**	63	6	2	0	0
***Helina humilis* (Stein)**	2	1	0	0	0
Helina laxifrons (Zetterstedt)	8	1	0	2	4
Helina longicornis (Zetterstedt)	2	0	0	0	10

Appendix D. (continued)

Species	Number of specimens				
	2007 inventory				Pre-1965
	M	P	S(p)	S(o)	
Helina luteisquama (Zetterstedt)	2	2	0	0	11
Helina maculipennis (Zetterstedt)	6	1	0	0	0
***Helina marguerita* Snyder**	1	3	0	0	0
***Helina nigribasis* Malloch**	4	4	0	0	0
Helina obscurata (Meigen)	55	8	4	5	4
Helina reversio (Harris)	80	31	42	46	12
***Helina spinosa* (Walker)**	0	0	1	0	0
Helina squalens (Zetterstedt)	5	3	0	0	16
Helina subvittata (Séguy)	1	0	0	0	1
***Hydrotaea aenescens* (Wiedemann)**	0	0	0	10	0
Hydrotaea anxia (Zetterstedt)	2	2	6	67	29
***Hydrotaea cristata* Malloch**	0	0	0	7	0
Hydrotaea floccosa (Fallén)	0	0	1	0	0
***Hydrotaea pilitibia* Stein**	3	0	0	1	0
***Hydrotaea ringdahli* Stein**	1	0	0	0	0
Hydrotaea scambus (Zetterstedt)	2	0	0	1	0
Limnophora discreta Stein	6	2	0	1	1
Limnophora nigripes (R.-D.) ♂	197	10	0	0	2
Limnophora nigripes/ *L. rotundata* Collin ♀	129	24	2	2	28
***Limnophora rotundata* ♂**	425	20	1	0	3*
Limnophora uniseta Stein	160	96	8	5	429
Limnophora sp. 1	44	39	4	2	0
Limnospila albifrons Zetterstedt	15	1	0	0	32
Lispe canadensis Snyder	1	0	0	1	82
Lispe cotidiana Snyder	15	0	0	0	2
Lispe frigida Erichson	0	0	0	8	N/A
Lispe johnsoni Aldrich	0	0	0	0	1
Lispe salina Aldrich	26	0	2	0	8
Lispe tentaculata (DeGeer)	26	4	0	3	50
Lispe uliginosa Fallén	20	1	0	0	12
Lispocephala alma (Meigen)	168	14	5	1	72
Lispocephala erythrocera (R.-D.)	32	1	2	6	71
***Lispocephala tinctinervis* Malloch**	1	0	0	0	0
***Lispocephala varians* Malloch**	1	0	0	0	8*
Lophosceles cinereiventris Zetterstedt	16	0	0	0	15
Lophosceles frenatus Holmgren	13	0	0	0	2
***Lophosceles impar* (Zetterstedt)**	2	0	0	0	0
Lophosceles minimus Malloch	0	0	0	0	2
Morellia podagrica Loew	0	0	1	0	1
Musca domestica Linnaeus	0	0	0	6	8
Muscina levida (Harris)	24	41	0	3	53
***Mydaea affinis* Meade**	8	13	0	0	1*
***Mydaea furtiva* Stein**	12	1	0	0	0
***Mydaea obscurella* Malloch**	21	21	0	0	0

Appendix D. (continued)

Species	Number of specimens				
	2007 inventory				Pre-1965
	M	P	S(p)	S(o)	
***Mydaea occidentalis* Malloch**	12	5	0	0	0
Mydaea palpalis Stein	3	0	0	0	6
***Mydaea pseudonubila* Huckett**	2	2	0	0	0
Myospila meditabunda Fabricius	22	3	0	1	1
Opsolasia orichalcea (Zetterstedt)	11	2	0	1	1
Phaonia alpicola (Zetterstedt)	0	1	0	1	1
***Phaonia apicalis* Stein**	2	0	0	0	0
***Phaonia atrocyanea* Ringdahl**	4	0	0	0	0
Phaonia consobrina (Zetterstedt) ♂	32	27	0	1	2
Phaonia consobrina/ P. rugia (Walker) ♀	24	28	0	5	4
Phaonia errans (Meigen)	244	35	0	0	3
***Phaonia inenarrabilis* Huckett**	1	0	0	0	0
Phaonia monticola Malloch	1	0	0	0	1
Phaonia protuberans Malloch	36	20	2	0	0
Phaonia rugia ♂	0	1	0	0	1
Phaonia savonoskii Malloch	61	143	0	9	12
Phaonia serva (Meigen)	5	3	0	1	0
Phaonia subfuscinervis (Zetterstedt)	0	0	1	0	8
Potamia littoralis R.-D.	2	0	0	0	2
***Pseudocoenosia brevicauda* Huckett**	2	0	0	3	18*
Pseudocoenosia fletcheri (Malloch)	0	0	0	10	7
Pseudocoenosia solitaria (Zetterstedt)	3	0	1	1	13
Schoenomyza dorsalis Loew ♂	3	0	0	0	11
Schoenomyza dorsalis/ S. litorella (Fallén) ♀	28	5	1	1	21
Schoenomyza litorella ♂	6	0	0	1	1
Spilogona acuticornis (Malloch)	0	0	0	0	N/A
Spilogona aenea Huckett	35	6	1	0	6
Spilogona aerea (Fallén)	583	31	32	35	237
Spilogona albisquama Ringdahl	22	13	1	1	3
Spilogona arctica (Zetterstedt)	10	1	3	0	46
Spilogona arenosa (Ringdahl)	0	0	0	0	4
Spilogona atrisquamula Hennig ♂	18	4	0	0	11
Spilogona atrisquamula/ S. pusilla Huckett ♀	13	1	0	2	9
Spilogona bifimbriata Huckett	1	0	0	0	3*
Spilogona brevicornis (Malloch)	0	0	0	0	1
Spilogona calcaria Huckett	4	0	0	0	1
Spilogona churchillensis Huckett	12	0	1	1	76
***Spilogona confluens* Huckett**	2	0	0	0	0
Spilogona contractifrons (Zetterstedt)	725	99	45	35	117
Spilogona deflorata (Holmgren)	132	28	1	3	27
Spilogona fatima Huckett	24	1	4	0	1
***Spilogona firmidisetosa* Huckett**	28	6	0	2	1*
***Spilogona flavinervis* Huckett**	4	0	0	0	0
***Spilogona forticula* Huckett**	133	2	2	1	0

Appendix D. (continued)

Species	Number of specimens				
	2007 inventory				Pre-1965
	M	P	S(p)	S(o)	
***Spilogona genualis* Huckett**	1	0	0	1	0
***Spilogona griseola* Collin**	44	3	0	1	0
Spilogona imitatrix (Malloch)	223	4	2	41	56
***Spilogona incerta* Huckett**	1	0	0	0	0
Spilogona infuscata Huckett	0	0	0	1	41
Spilogona leucogaster (Zetterstedt)	19	1	1	2	79
Spilogona malaisei (Ringdahl)	1	2	1	0	28
Spilogona melanosoma Huckett	3	0	0	5	15
Spilogona micans (Ringdahl)	11	2	0	1	2
Spilogona monacantha Collin	0	0	0	0	1
Spilogona mydaeinaformis Huckett	0	0	0	0	1
***Spilogona narina* (Walker)**	2	0	0	0	0
Spilogona norvegica Ringdahl	0	0	0	0	2
Spilogona novemaculata (Zetterstedt)	25	2	2	2	6
***Spilogona nutaka* Huckett**	0	0	0	0	10*
Spilogona obscura Malloch	0	0	0	0	1
***Spilogona obscuripennis* (Stein)**	35	2	0	0	5*
Spilogona opaqua Schnabl	38	1	1	0	11
Spilogona pacifica (Meigen)	7	0	0	0	N/A
Spilogona pseudodispar (Frey)	0	0	0	0	4
Spilogona pusilla ♂	7	0	0	0	N/A
Spilogona quinquelineata (Zetterstedt)	0	0	0	0	1
***Spilogona reflecta* Huckett**	10	0	0	0	0
Spilogona setinervis Huckett	44	0	0	1	28
***Spilogona setipes* Huckett**	1	0	0	0	0
Spilogona sororcula (Zetterstedt)	77	1	1	2	1
Spilogona surda (Zetterstedt)	2	0	0	1	18
Spilogona suspecta (Malloch)	40	5	6	1	1
***Spilogona tornensis* (Ringdahl)**	1	0	0	0	0
Spilogona trigonata (Zetterstedt)	32	2	1	1	3
***Spilogona trigonifera* (Zetterstedt)**	1	0	0	0	0
Spilogona trilineata Huckett	13	0	1	0	6
***Spilogona tundrae* (Schnabl)**	0	0	0	0	4*
Spilogona zaitzevi (Schnabl)	1	0	0	0	2
Spilogona sp.1	2	0	0	0	0
Spilogona sp.2	1	0	0	0	0
Spilogona sp.3	1	0	0	0	0
Spilogona sp.4	1	0	0	0	0
Spilogona sp.5	1	0	0	0	0
Spilogona sp.6	1	0	0	0	0
Spilogona sp.7	0	0	1	0	0
Spilogona sp.8	1	0	0	0	0
Spilogona sp.9	1	0	1	0	0

Appendix D. (concluded)

Species	Number of specimens				
	2007 inventory				Pre-1965
	M	P	S(p)	S(o)	
Spilogona sp.10	3	0	0	0	0
Spilogona sp.11	1	0	0	0	0
Stomoxys calcitrans Linnaeus	2	0	0	0	0
Thricops albibasalis (Zetterstedt)	26	6	1	0	0
Thricops diaphanus (Wiedemann)	3	0	0	1	0
Thricops hirtulus (Zetterstedt)	23	22	4	20	166
Thricops innocuus (Zetterstedt)	924	17	6	9	19
Thricops septentrionalis (Stein) ♂	94	1	1	3	10
Thricops septentrionalis/T. spiniger (Stein) ♀	166	13	9	0	42
Thricops spiniger ♂	45	0	3	0	0
Total	**8212**	**1334**	**270**	**478**	**2881**

Note: Specimen numbers for species with undistinguishable females reported separately for males and pooled for females.

Boldface type denotes a new distribution record for the province of Manitoba; N/A denotes a species listed in the literature but for which no specimens were found in collections.

* Specimens discovered in museum collections belonging to taxa not listed in the literature.

Appendix E. Names and collecting localities of 1226 specimens of Muscidae with Sample ID and GenBank accession number of 976 COI sequences. Province listed for Canadian localities only (AB=Alberta, MB=Manitoba, NL= Newfoundland and Labrador, NT=Northwest Territories, ON= Ontario, QC=Quebec, and SK=Saskatchewan), non-Canadian localities listed only by country. Sequences can be retrieved from the Barcode of Life data system (Sample ID) (www.bolsystems.org) and Genbank (accession number) (www.ncbi.nlm.nih.gov/genbank). Specimens in boldface type were not included in data analysis as their processing for DNA amplification and sequencing was still pending at the time of thesis submission. Specimens identified with an asterisk (*) are those that failed to amplify successfully.

Species	Collecting locality	Sample ID	Accession number
Coenosia atritibia Ringdahl	Churchill, MB	BUIC-CHU0600	HM891644
Coenosia atritibia Ringdahl	Churchill, MB	BUIC-CHU0601	HM891645
Coenosia atritibia Ringdahl	Churchill, MB	BUIC-CHU0603	HM891647
Coenosia comita (Huckett)	Churchill, MB	JBWM0319540	N/A
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0054	HM388852
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0055	HM388853
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0319	HM389045
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0337	HM389058
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0338	HM389059
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0339	HM389060
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0340	HM389061
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0341	HM389062
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0342	HM389063
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0343	HM389064
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0344	HM389065
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0345	HM389066
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0346	HM389067
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0347	HM389068
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0348	HM389069
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0349	HM389070
Coenosia comita (Huckett)	Churchill, MB	BUIC-CHU0899	HM891894
Coenosia conforma Huckett	Churchill, MB	JBWM0319553	N/A
Coenosia conforma Huckett	Churchill, MB	BUIC-CHU0043	HQ991811
Coenosia conforma Huckett	Churchill, MB	BUIC-CHU0044	HQ991812
Coenosia conforma Huckett	Churchill, MB	BUIC-CHU0353	HM389072
Coenosia conforma Huckett	Churchill, MB	BUIC-CHU0354	HQ991842
Coenosia conforma Huckett	Churchill, MB	BUIC-CHU0798	HM891806

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Coenosia conforma Huckett*	Churchill, MB	BUIC-CHU0604	N/A
Coenosia conforma Huckett*	Churchill, MB	BUIC-CHU0605	N/A
***Coenosia demoralis* Huckett**	Churchill, MB	BUIC-CHU0994	N/A
***Coenosia demoralis* Huckett**	Churchill, MB	BUIC-CHU0995	N/A
Coenosia demoralis Huckett	Churchill, MB	JBWM0234404	N/A
Coenosia demoralis Huckett*	Churchill, MB	BUIC-CHU0606	N/A
Coenosia demoralis Huckett*	Churchill, MB	BUIC-CHU0607	N/A
Coenosia demoralis Huckett*	Churchill, MB	BUIC-CHU0608	N/A
Coenosia demoralis Huckett*	Churchill, MB	BUIC-CHU0051	N/A
Coenosia demoralis Huckett*	Churchill, MB	BUIC-CHU0358	N/A
***Coenosia flaviseta* Huckett**	Churchill, MB	BUIC-CHU1028	N/A
Coenosia flaviseta Huckett*	Churchill, MB	BUIC-CHU0609	N/A
Coenosia flaviseta Huckett*	Churchill, MB	BUIC-CHU0610	N/A
Coenosia frisoni Malloch	Churchill, MB	BUIC-CHU0611	HM891648
Coenosia frisoni Malloch	Churchill, MB	BUIC-CHU0616	HM891653
Coenosia longimaculata Stein	Churchill, MB	JBWM0234395	N/A
Coenosia longimaculata Stein	Churchill, MB	JBWM0234398	N/A
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0302	HM389034
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0303	HM389035
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0306	HM389037
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0307	HM389038
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0308	HM389039
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0309	HM389040
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0312	HM389041
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0613	HM891650
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0614	HM891651
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0615	HM891652
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0801	HM891808
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0802	HM891809
Coenosia longimaculata Stein	Churchill, MB	BUIC-CHU0804	HM891810
Coenosia longimaculata Stein*	Churchill, MB	BUIC-CHU0617	N/A
Coenosia longimaculata Stein*	Churchill, MB	BUIC-CHU0799	N/A
Coenosia longimaculata Stein*	Churchill, MB	BUIC-CHU0313	N/A
Coenosia longimaculata Stein*	Churchill, MB	BUIC-CHU0314	N/A
Coenosia longimaculata Stein*	Churchill, MB	BUIC-CHU0803	N/A
Coenosia minor Huckett*	Churchill, MB	BUIC-CHU0621	N/A
Coenosia minor Huckett	Churchill, MB	BUIC-CHU0045	HQ991813
Coenosia minor Huckett	Churchill, MB	BUIC-CHU0046	HQ991814
Coenosia minor Huckett	Churchill, MB	BUIC-CHU0618	HM891654
Coenosia minor Huckett	Churchill, MB	BUIC-CHU0619	HM891655
Coenosia minor Huckett	Churchill, MB	BUIC-CHU0620	HM891656
Coenosia mollicula (Fallén)	Churchill, MB	BUIC-CHU0047	HM388849
Coenosia mollicula (Fallén)	Churchill, MB	BUIC-CHU0049	HM388850
Coenosia mollicula (Fallén)	Churchill, MB	BUIC-CHU0050	HM388851

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Coenosia mollicula (Fallén)*	Churchill, MB	BUIC-CHU0622	N/A
Coenosia mollicula (Fallén)*	Churchill, MB	BUIC-CHU0048	N/A
Coenosia nigrescens Stein	Churchill, MB	JBWM0319562	N/A
Coenosia nigrescens Stein	Churchill, MB	BUIC-CHU0037	HM388847
Coenosia nigrescens Stein	Churchill, MB	BUIC-CHU0038	HM388848
Coenosia nigrescens Stein	Churchill, MB	BUIC-CHU0357	HM389073
Coenosia nigricoxa Stein	Churchill, MB	JBWM0319563	N/A
Coenosia nigricoxa Stein	Churchill, MB	JBWM0234380	N/A
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0062	HQ991816
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0316	HM389042
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0317	HM389043
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0318	HM389044
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0320	HQ991839
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0321	HM389046
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0322	HM389047
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0323	HQ991840
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0324	HQ991841
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0325	HM389048
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0327	HM389050
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0329	HM389052
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0360	HM389075
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0602	HM891646
Coenosia nigricoxa Stein	Churchill, MB	BUIC-CHU0623	HM891657
Coenosia nigricoxa Stein	Churchill, MB	CHU05-FLY-127	N/A
Coenosia nigricoxa Stein	Churchill, MB	CHU05-FLY-318	N/A
Coenosia nigricoxa Stein	Churchill, MB	CHU05-FLY-331	N/A
Coenosia nigricoxa Stein	Churchill, MB	CHU05-FLY-336	N/A
Coenosia nigricoxa Stein	Churchill, MB	CHU05-FLY-422	N/A
Coenosia nigricoxa Stein	Churchill, MB	CHU05-FLY-423	N/A
Coenosia nigricoxa Stein	Churchill, MB	CHU05-FLY-465	N/A
Coenosia octopunctata (Zetterstedt)	Churchill, MB	JBWM0319532	N/A
Coenosia octopunctata (Zetterstedt)	Churchill, MB	JBWM0319555	N/A
Coenosia octopunctata (Zetterstedt)	Churchill, MB	BUIC-CHU0057	HM388855
Coenosia octopunctata (Zetterstedt)	Churchill, MB	BUIC-CHU0058	HM388856
Coenosia octopunctata (Zetterstedt)	Churchill, MB	BUIC-CHU0061	HQ991815
Coenosia octopunctata (Zetterstedt)	Churchill, MB	BUIC-CHU0350	HM389071
Coenosia octopunctata (Zetterstedt)	Churchill, MB	BUIC-CHU0351	HM389247

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Coenosia octopunctata (Zetterstedt)	Churchill, MB	BUIC-CHU0352	HM389248
Coenosia pumila (Fallén)	Churchill, MB	BUIC-CHU0612	HM891649
Coenosia pumila (Fallén)	Churchill, MB	BUIC-CHU0627	HM891660
Coenosia pumila (Fallén)	Churchill, MB	BUIC-CHU0806	HM891811
Coenosia pumila (Fallén)	Churchill, MB	BUIC-CHU0807	HM891812
Coenosia pumila (Fallén)*	Churchill, MB	BUIC-CHU0805	N/A
Coenosia remissa Huckett*	Churchill, MB	BUIC-CHU0624	N/A
Coenosia remissa Huckett	Churchill, MB	BUIC-CHU0625	HM891658
Coenosia sp.*	Churchill, MB	BUIC-CHU0800	N/A
Coenosia sp.*	Churchill, MB	BUIC-CHU0052	N/A
Coenosia sp.*	Churchill, MB	BUIC-CHU0053	N/A
Coenosia sp.*	Churchill, MB	BUIC-CHU0056	N/A
Coenosia sp.*	Churchill, MB	BUIC-CHU0059	N/A
Coenosia sp.*	Churchill, MB	BUIC-CHU0060	N/A
Coenosia sp.*	Churchill, MB	BUIC-CHU0063	N/A
Coenosia sp.*	Churchill, MB	BUIC-CHU0297	N/A
Coenosia sp.*	Churchill, MB	BUIC-CHU0305	N/A
Coenosia sp. 1	Churchill, MB	BUIC-CHU0626	HM891659
Coenosia tarsata Huckett	Churchill, MB	BUIC-CHU0359	HM389074
Coenosia triseta Stein	Churchill, MB	JBWM0319537	N/A
Coenosia triseta Stein	Churchill, MB	BUIC-CHU0067	HQ991817
Coenosia triseta Stein	Churchill, MB	BUIC-CHU0068	HM388859
Coenosia triseta Stein	Churchill, MB	BUIC-CHU0299	HQ991838
Coenosia triseta Stein	Churchill, MB	BUIC-CHU0629	HM891661
Coenosia triseta Stein	Churchill, MB	CHU05-FLY-119	N/A
Coenosia triseta Stein	Churchill, MB	CHU05-FLY-120	N/A
Coenosia triseta Stein	Churchill, MB	CHU05-FLY-138	N/A
Coenosia triseta Stein*	Churchill, MB	BUIC-CHU0064	N/A
Coenosia triseta Stein*	Churchill, MB	BUIC-CHU0065	N/A
Coenosia triseta Stein*	Churchill, MB	BUIC-CHU0066	N/A
Coenosia verralli Collin*	Churchill, MB	BUIC-CHU0552	N/A
Coenosia verralli Collin*	Churchill, MB	BUIC-CHU0628	N/A
Coenosia verralli Collin	Churchill, MB	BUIC-CHU0304	HM389036
Coenosia verralli Collin	Churchill, MB	BUIC-CHU0326	HM389049
Coenosia verralli Collin	Churchill, MB	BUIC-CHU0328	HM389051
Coenosia verralli Collin	Churchill, MB	BUIC-CHU0330	HM389053
Coenosia verralli Collin	Churchill, MB	BUIC-CHU0331	HM389054
Coenosia verralli Collin	Churchill, MB	BUIC-CHU0332	HM389055
Coenosia verralli Collin	Churchill, MB	BUIC-CHU0333	HM389056
Coenosia verralli Collin	Churchill, MB	BUIC-CHU0334	HM389057
Drymeia groenlandica (Lundbeck)	Churchill, MB	BUIC-CHU0903	HM891898
Drymeia groenlandica (Lundbeck)	Churchill, MB	BUIC-CHU0904	HM891899
Drymeia pribilofensis Malloch	Churchill, MB	BUIC-CHU0095	HM388879

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Drymeia pribilofensis Malloch	Churchill, MB	BUIC-CHU0096	HM388880
Drymeia pribilofensis Malloch	Churchill, MB	BUIC-CHU0097	HM388881
Drymeia pribilofensis Malloch	Churchill, MB	BUIC-CHU0098	HM388882
Drymeia pribilofensis Malloch	Churchill, MB	BUIC-CHU0099	HM388883
Drymeia pribilofensis Malloch	Churchill, MB	BUIC-CHU0100	HM388884
Drymeia pribilofensis Malloch	Churchill, MB	BUIC-CHU0631	HM891663
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0101	HM388885
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0102	HM388886
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0103	HM388887
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0104	HM388888
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0105	HM388889
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0106	HM388890
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0107	HM388891
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0108	HM388892
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0109	HM388893
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0110	HM388894
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0111	HM388895
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0112	HM388896
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0113	HM388897
Drymeia quadrisetosa Malloch	Churchill, MB	BUIC-CHU0114	HM388898
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0972	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0973	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0974	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0975	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0976	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0977	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0978	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0979	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0980	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0981	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0982	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0983	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0984	N/A
***Graphomya idessa* Walker**	Churchill, MB	BUIC-CHU0985	N/A
***Graphomya minuta* Arntfield**	Churchill, MB	BUIC-CHU0989	N/A
***Graphomya* sp.**	Churchill, MB	BUIC-CHU0990	N/A
***Graphomya* sp.**	Churchill, MB	BUIC-CHU0991	N/A
***Graphomya* sp.**	Churchill, MB	BUIC-CHU0992	N/A
***Graphomya* sp.**	Churchill, MB	BUIC-CHU0993	N/A
***Graphomya transitionis* Arntfield**	Churchill, MB	BUIC-CHU0986	N/A
***Graphomya transitionis* Arntfield**	Churchill, MB	BUIC-CHU0987	N/A
***Graphomya transitionis* Arntfield**	Churchill, MB	BUIC-CHU0988	N/A
***Haematobia alcis* (Snow)**	Gaspésie, QC	BUIC-CHU1054	N/A
Haematobia alcis (Snow)	Churchill, MB	BUIC-CHU0553	HM389222

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Haematobia alcis (Snow)	Churchill, MB	BUIC-CHU0554	HM389223
***Helina annosa* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1045	N/A
***Helina annosa* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1046	N/A
Helina annosa (Zetterstedt)	Churchill, MB	JBWM0319514	N/A
Helina cinerella (van der Wulp)	Churchill, MB	BUIC-CHU0632	HM891664
Helina cinerella (van der Wulp)	Churchill, MB	BUIC-CHU0633	HM891665
Helina cinerella (van der Wulp)	Churchill, MB	BUIC-CHU0634	HM891666
Helina cinerella (van der Wulp)	Churchill, MB	BUIC-CHU0635	HM891667
Helina cinerella (van der Wulp)	Churchill, MB	BUIC-CHU0652	HM891683
Helina cinerella (van der Wulp)	Churchill, MB	BUIC-CHU0900	HM891895
***Helina evecta* (Harris)**	Dorchester-on-Thames, England	BUIC-CHU1022	N/A
***Helina evecta* (Harris)**	Gaspésie, QC	BUIC-CHU1047	N/A
***Helina evecta* (Harris)**	Gaspésie, QC	BUIC-CHU1048	N/A
Helina evecta (Harris)	Churchill, MB	JBWM0319518	N/A
Helina evecta (Harris)	Churchill, MB	JBWM0319519	N/A
Helina evecta (Harris)	Churchill, MB	JBWM0319531	N/A
Helina evecta (Harris)	Churchill, MB	JBWM0319533	N/A
Helina evecta (Harris)	Churchill, MB	JBWM0319544	N/A
Helina evecta (Harris)	Churchill, MB	JBWM0319549	N/A
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0011	HM388822
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0021	HM388831
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0022	HM388832
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0023	HM388833
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0024	HM388834
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0025	HM388835
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0026	HM388836
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0027	HM388837
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0028	HM388838
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0029	HM388839
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0030	HM388840
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0031	HM388841
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0032	HM388842
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0033	HM388843
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0034	HM388844
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0035	HM388845
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0036	HM388846
Helina evecta (Harris)	Churchill, MB	BUIC-CHU0638	HM891670
Helina evecta (Harris)	Churchill, MB	CHU05-FLY-166	N/A
Helina evecta (Harris)	Churchill, MB	CHU05-FLY-169	N/A
Helina evecta (Harris)	Churchill, MB	CHU05-FLY-170	N/A
Helina evecta (Harris)	Churchill, MB	CHU05-FLY-175	N/A
Helina evecta (Harris)	Churchill, MB	CHU05-FLY-260	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Helina flavisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0005	HM388816
Helina flavisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0006	HM388817
Helina flavisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0007	HM388818
Helina flavisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0008	HM388819
Helina flavisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0009	HM388820
Helina flavisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0010	HM388821
Helina flavisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0012	HM388823
Helina flavisquama (Zetterstedt)	Churchill, MB	CHU05-FLY-167	N/A
Helina flavisquama (Zetterstedt)	Churchill, MB	CHU05-FLY-290.1	N/A
Helina flavisquama (Zetterstedt)	Churchill, MB	CHU06-FLY-070.1	N/A
***Helina fulvisquama* (Zetterstedt)**	Churchill, MB	BUIC-CHU1024	N/A
***Helina fulvisquama* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1044	N/A
Helina fulvisquama (Zetterstedt)	Churchill, MB	JBWM0234384	N/A
Helina fulvisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0013	HM388824
Helina fulvisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0015	HM388825
Helina fulvisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0643	HM891675
Helina fulvisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0644	HM891676
Helina fulvisquama (Zetterstedt)	Churchill, MB	BUIC-CHU0645	HM891677
Helina fulvisquama (Zetterstedt)*	Churchill, MB	BUIC-CHU0014	N/A
Helina humilis (Stein)	Churchill, MB	BUIC-CHU0894	HM891890
Helina humilis (Stein)	Churchill, MB	BUIC-CHU0895	HM891891
Helina humilis (Stein)	Churchill, MB	BUIC-CHU0896	HM891892
***Helina laxifrons* (Zetterstedt)**	Churchill, MB	BUIC-CHU1023	N/A
Helina laxifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0636	HM891668
Helina laxifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0637	HM891669
Helina laxifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0639	HM891671
Helina longicornis (Zetterstedt)	Churchill, MB	BUIC-CHU0641	HM891673
Helina longicornis (Zetterstedt)	Churchill, MB	BUIC-CHU0642	HM891674
Helina maculipennis (Zetterstedt)	Churchill, MB	JBWM0319534	N/A
Helina maculipennis (Zetterstedt)	Churchill, MB	BUIC-CHU0646	HM891678
Helina maculipennis (Zetterstedt)	Churchill, MB	BUIC-CHU0647	HM891679
***Helina maculipennis* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1049	N/A
Helina marguerita Snyder	Churchill, MB	JBWM0234397	N/A
Helina marguerita Snyder	Churchill, MB	BUIC-CHU0019	HM388829
Helina marguerita Snyder	Churchill, MB	BUIC-CHU0020	HM388830
Helina marguerita Snyder	Churchill, MB	BUIC-CHU0650	HM891681
Helina nigribasis Malloch	Churchill, MB	BUIC-CHU0810	HM891815
Helina nigribasis Malloch	Churchill, MB	BUIC-CHU0811	HM891816
Helina nigribasis Malloch	Churchill, MB	BUIC-CHU0812	HM891817
Helina nigribasis Malloch	Churchill, MB	BUIC-CHU0905	HM891900
Helina nigribasis Malloch	Churchill, MB	BUIC-CHU0906	HM891901
Helina nigribasis Malloch	Churchill, MB	BUIC-CHU0907	HM891902

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Helina nigribasis Malloch	Churchill, MB	BUIC-CHU0908	HM891903
Helina nigribasis Malloch	Churchill, MB	BUIC-CHU0909	HM891904
Helina obscurata (Meigen)	Churchill, MB	BUIC-CHU0016	HM388826
Helina obscurata (Meigen)	Churchill, MB	BUIC-CHU0017	HM388827
Helina obscurata (Meigen)	Churchill, MB	BUIC-CHU0018	HM388828
Helina obscurata (Meigen)	Churchill, MB	BUIC-CHU0648	HM891680
Helina obscurata (Meigen)	Churchill, MB	CHU05-FLY-160	N/A
Helina obscurata (Meigen)*	Churchill, MB	BUIC-CHU0649	N/A
Helina reversio (Harris)	Churchill, MB	JBWM0319508	N/A
Helina reversio (Harris)	Churchill, MB	JBWM0319527	N/A
Helina reversio (Harris)	Churchill, MB	BUIC-CHU0001	HM388812
Helina reversio (Harris)	Churchill, MB	BUIC-CHU0002	HM388813
Helina reversio (Harris)	Churchill, MB	BUIC-CHU0003	HM388814
Helina reversio (Harris)	Churchill, MB	BUIC-CHU0004	HM388815
Helina spinosa (Walker)	Churchill, MB	BUIC-CHU0653	HM891684
Helina spinosa (Walker)	Churchill, MB	CHU05-FLY-719	N/A
Helina squalens (Zetterstedt)	Churchill, MB	07WNP-10867	N/A
Helina squalens (Zetterstedt)	Churchill, MB	BUIC-CHU0660	HM891691
Helina squalens (Zetterstedt)	Churchill, MB	BUIC-CHU0661	HM891692
Helina squalens (Zetterstedt)	Churchill, MB	BUIC-CHU0662	HM891693
Helina squalens (Zetterstedt)	Churchill, MB	BUIC-CHU0663	HM891694
Helina subvittata (Seguy)	Churchill, MB	BUIC-CHU0651	HM891682
Hydrotaea aenescens (Wiedemann)	Churchill, MB	BUIC-CHU0069	HM388860
Hydrotaea aenescens (Wiedemann)	Churchill, MB	BUIC-CHU0070	HQ991818
Hydrotaea aenescens (Wiedemann)*	Churchill, MB	BUIC-CHU0664	N/A
Hydrotaea aenescens (Wiedemann)*	Churchill, MB	BUIC-CHU0665	N/A
Hydrotaea aenescens (Wiedemann)*	Churchill, MB	BUIC-CHU0071	N/A
Hydrotaea aenescens (Wiedemann)*	Churchill, MB	BUIC-CHU0072	N/A
Hydrotaea anxia (Zetterstedt)	Jebrenjokk, Sweden	BUIC-CHU0966	HM891631
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0073	HM388861
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0074	HM388862
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0077	HM388863
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0078	HM388864
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0079	HM388865
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0080	HM388866
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0081	HM388867
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0082	HM388868
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0083	HM388869
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0084	HM388870

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0666	HM891695
Hydrotaea anxia (Zetterstedt)	Churchill, MB	BUIC-CHU0667	HM891696
Hydrotaea cristata Malloch	Churchill, MB	JBWM0319500	N/A
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0085	HM388871
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0087	HM388873
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0088	HM388874
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0091	HM388875
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0092	HM388876
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0093	HM388877
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0094	HM388878
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0654	HM891685
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0655	HM891686
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0814	HM891819
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0816	HM891820
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0817	HM891821
Hydrotaea cristata Malloch	Churchill, MB	BUIC-CHU0818	HM891822
Hydrotaea cristata Malloch*	Churchill, MB	BUIC-CHU0075	N/A
Hydrotaea cristata Malloch*	Churchill, MB	BUIC-CHU0076	N/A
Hydrotaea floccosa (Fallèn)	Goring-On-Thames, England	BUIC-CHU0963	N/A
Hydrotaea floccosa (Fallèn)	Gaspésie, QC	BUIC-CHU0955	HM891626
Hydrotaea floccosa (Fallèn)	Churchill, MB	BUIC-CHU0656	HM891687
Hydrotaea pilitibia Stein	Churchill, MB	BUIC-CHU0658	HM891689
Hydrotaea pilitibia Stein	Churchill, MB	BUIC-CHU0659	HM891690
Hydrotaea pilitibia Stein	Churchill, MB	BUIC-CHU0901	HM891896
Hydrotaea pilitibia Stein	Churchill, MB	BUIC-CHU0902	HM891897
Hydrotaea ringdalhi Stein	Churchill, MB	BUIC-CHU0657	HM891688
***Hydrotaea scambus* (Zetterstedt)**	Churchill, MB	BUIC-CHU1016	N/A
Hydrotaea scambus (Zetterstedt)	Lake Vuolep, Sweden	BUIC-CHU0962	N/A
Hydrotaea sp.*	Churchill, MB	BUIC-CHU0089	N/A
Hydrotaea sp.*	Churchill, MB	BUIC-CHU0090	N/A
Hydrotaea sp.*	Churchill, MB	BUIC-CHU0815	N/A
Hydrotaea sp. 1	Churchill, MB	BUIC-CHU0086	HM388872
Limnophora discreta Stein	Orilllia, ON	BUIC-CHU0465	N/A
Limnophora discreta Stein	Simcoe County, ON	BUIC-CHU0466	N/A
Limnophora discreta Stein	Gaspésie, QC	BUIC-CHU0468	N/A
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0455	HM389158
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0456	HM389159
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0457	HM389160
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0458	HM389161
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0482	HQ991843

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0484	HM389171
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0485	HQ991844
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0534	HQ991850
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0537	HM389207
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0538	HM389208
Limnophora discreta Stein	Churchill, MB	BUIC-CHU0549	HM389219
Limnophora discreta Stein	Churchill, MB	JBWM0234403	N/A
Limnophora discreta Stein*	Churchill, MB	BUIC-CHU0482	N/A
Limnophora discreta Stein*	Churchill, MB	BUIC-CHU0485	N/A
Limnophora discreta Stein*	Churchill, MB	BUIC-CHU0534	N/A
Limnophora incrassata Malloch	Mont Rainier, United States of America	BUIC-CHU0470	N/A
Limnophora narona Walker	Bermuda	BUIC-CHU0459	N/A
Limnophora narona Walker	Bermuda	BUIC-CHU0461	N/A
Limnophora narona Walker	Bermuda	BUIC-CHU0462	N/A
Limnophora narona Walker	Bermuda	BUIC-CHU0463	N/A
Limnophora narona Walker	N/A	BUIC-CHU0460	N/A
Limnophora nigripes (R.-D.)	N/A	BUIC-CHU0497	N/A
Limnophora nigripes (R.-D.)	Abisko, Sweden	BUIC-CHU0499	N/A
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0500	HM389176
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0501	HM389177
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0502	HQ991846
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0503	HM389178
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0504	HM389179
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0505	HM389180
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0506	HQ991847
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0507	HM389181
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0515	HM389187
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0516	HM389188
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0519	HM389190
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0520	HM389191
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0521	HM389192
Limnophora nigripes (R.-D.)	Churchill, MB	BUIC-CHU0522	HM389193
Limnophora nigripes (R.-D.)*	Churchill, MB	BUIC-CHU0498	N/A
Limnophora nigripes (R.-D.)*	Churchill, MB	BUIC-CHU0502	N/A
Limnophora nigripes (R.-D.)*	Churchill, MB	BUIC-CHU0523	N/A
Limnophora nigripes (R.-D.)*	Churchill, MB	BUIC-CHU0506	N/A
Limnophora rotundata Collin	Gaspésie, QC	BUIC-CHU0494	N/A
Limnophora rotundata Collin	Abisko, Sweden	BUIC-CHU0496	N/A
Limnophora rotundata Collin	Churchill, MB	JBWM0319556	N/A
Limnophora rotundata Collin	Churchill, MB	JBWM0319560	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Limnophora rotundata Collin	Churchill, MB	JBWM0319564	N/A
Limnophora rotundata Collin	Churchill, MB	JBWM0234378	N/A
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0487	HM389172
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0488	HM389173
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0489	HM389174
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0490	HM389175
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0491	HQ991845
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0508	HM389182
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0509	HM389183
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0510	HQ991848
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0512	HM389184
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0513	HM389185
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0514	HM389186
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0518	HM389189
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0524	HM389195
Limnophora rotundata Collin	Churchill, MB	BUIC-CHU0550	HM389220
Limnophora rotundata Collin*	Churchill, MB	BUIC-CHU0491	N/A
Limnophora rotundata Collin*	Churchill, MB	BUIC-CHU0492	N/A
Limnophora rotundata Collin*	Churchill, MB	BUIC-CHU0493	N/A
Limnophora rotundata Collin*	Churchill, MB	BUIC-CHU0495	N/A
Limnophora rotundata Collin*	Churchill, MB	BUIC-CHU0510	N/A
Limnophora rotundata Collin*	Churchill, MB	BUIC-CHU0511	N/A
Limnophora sp.*	Churchill, MB	BUIC-CHU0481	N/A
Limnophora sp.*	Churchill, MB	BUIC-CHU0486	N/A
Limnophora sp. 1	Churchill, MB	JBWM0234389	N/A
Limnophora sp. 1	Churchill, MB	JBWM0234394	N/A
Limnophora sp. 1	Churchill, MB	BUIC-CHU0471	HM389162
Limnophora sp. 1	Churchill, MB	BUIC-CHU0472	HM389163
Limnophora sp. 1	Churchill, MB	BUIC-CHU0473	HM389164
Limnophora sp. 1	Churchill, MB	BUIC-CHU0475	HM389165
Limnophora sp. 1	Churchill, MB	BUIC-CHU0476	HM389166
Limnophora sp. 1	Churchill, MB	BUIC-CHU0477	HM389167
Limnophora sp. 1	Churchill, MB	BUIC-CHU0478	HM389168
Limnophora sp. 1	Churchill, MB	BUIC-CHU0479	HM389169
Limnophora sp. 1	Churchill, MB	BUIC-CHU0517	HQ991849
Limnophora sp. 1	Churchill, MB	BUIC-CHU0530	HM389201
Limnophora sp. 1	Churchill, MB	BUIC-CHU0531	HM389202
Limnophora sp. 1	Churchill, MB	BUIC-CHU0532	HM389203
Limnophora sp. 1	Churchill, MB	BUIC-CHU0533	HM389204
Limnophora sp. 1	Churchill, MB	BUIC-CHU0536	HM389206
Limnophora sp. 1	Churchill, MB	BUIC-CHU0539	HM389209
Limnophora sp. 1*	Churchill, MB	BUIC-CHU0449	N/A
Limnophora sp. 1*	Churchill, MB	BUIC-CHU0474	N/A
Limnophora sp. 1*	Churchill, MB	BUIC-CHU0542	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Limnophora sp. 1*	Churchill, MB	BUIC-CHU0517	N/A
Limnophora sp. 1	Churchill, MB	BUIC-CHU0540	HM389210
Limnophora sp. 1	Churchill, MB	BUIC-CHU0541	HM389211
Limnophora sp. 1	Churchill, MB	BUIC-CHU0543	HM389213
Limnophora sp. 1	Churchill, MB	BUIC-CHU0546	HM389216
Limnophora sp. 1	Churchill, MB	BUIC-CHU0547	HM389217
Limnophora sp. 1	Churchill, MB	BUIC-CHU0548	HM389218
Limnophora sp. 1	Churchill, MB	CHU05-FLY-213	N/A
Limnophora sp. 1	Churchill, MB	CHU06-FLY-024.1	N/A
***Limnophora triangula* (Fallén)**	Dublin Forest, Czech Republic	BUIC-CHU0996	N/A
Limnophora uniseta Stein	Churchill, MB	JBWM0319517	N/A
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0450	HM389155
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0451	HM389156
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0454	HM389157
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0480	HM389170
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0525	HM389196
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0526	HM389197
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0527	HM389198
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0528	HM389199
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0529	HM389200
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0535	HM389205
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0544	HM389214
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0545	HM389215
Limnophora uniseta Stein	Churchill, MB	BUIC-CHU0551	HQ991851
Limnophora uniseta Stein*	Churchill, MB	BUIC-CHU0452	N/A
Limnophora uniseta Stein*	Churchill, MB	BUIC-CHU0453	N/A
Limnophora uniseta Stein*	Churchill, MB	BUIC-CHU0551	N/A
Limnospila albifrons Zetterstedt	Churchill, MB	BUIC-CHU0669	HM891698
Limnospila albifrons Zetterstedt	Churchill, MB	BUIC-CHU0670	HM891699
Limnospila albifrons Zetterstedt	Churchill, MB	BUIC-CHU0671	HM891700
Limnospila albifrons Zetterstedt	Churchill, MB	BUIC-CHU0672	HM891701
Limnospila albifrons Zetterstedt	Churchill, MB	CHU05-FLY-692	N/A
Limnospila albifrons Zetterstedt	Churchill, MB	CHU05-FLY-740	N/A
Limnospila albifrons Zetterstedt	Churchill, MB	CHU05-FLY-757	N/A
Limnospila albifrons Zetterstedt	Churchill, MB	CHU05-FLY-512.1	N/A
Lispe canadensis Snyder	Churchill, MB	BUIC-CHU0674	HM891702
Lispe canadensis Snyder*	Churchill, MB	BUIC-CHU0673	N/A
Lispe cotidiana Snyder	Churchill, MB	JBWM0234390	N/A
Lispe cotidiana Snyder	Churchill, MB	BUIC-CHU0117	HM388901
Lispe cotidiana Snyder	Churchill, MB	BUIC-CHU0118	HM388902

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Lispe cotidiana Snyder	Churchill, MB	BUIC-CHU0119	HM388903
Lispe cotidiana Snyder	Churchill, MB	CHU05-FLY-203	N/A
Lispe cotidiana Snyder	Churchill, MB	CHU05-FLY-220	N/A
Lispe frigida Erichson	Churchill, MB	BUIC-CHU0131	HM388915
Lispe frigida Erichson	Churchill, MB	BUIC-CHU0132	HM388916
Lispe frigida Erichson	Churchill, MB	BUIC-CHU0133	HM388917
Lispe frigida Erichson	Churchill, MB	BUIC-CHU0134	HM388918
Lispe frigida Erichson	Churchill, MB	BUIC-CHU0135	HM388919
Lispe frigida Erichson	Churchill, MB	CHU05-FLY-129	N/A
Lispe salina Aldrich	Churchill, MB	JBWM0319520	N/A
Lispe salina Aldrich	Churchill, MB	BUIC-CHU0126	HM388910
Lispe salina Aldrich	Churchill, MB	BUIC-CHU0127	HM388911
Lispe salina Aldrich	Churchill, MB	BUIC-CHU0128	HM388912
Lispe salina Aldrich	Churchill, MB	BUIC-CHU0129	HM388913
Lispe salina Aldrich	Churchill, MB	BUIC-CHU0130	HM388914
Lispe salina Aldrich*	Churchill, MB	BUIC-CHU0675	N/A
Lispe tentaculata (DeGeer)	Churchill, MB	JBWM0319546	N/A
Lispe tentaculata (DeGeer)	Churchill, MB	BUIC-CHU0115	HM388899
Lispe tentaculata (DeGeer)	Churchill, MB	BUIC-CHU0116	HM388900
Lispe tentaculata (DeGeer)	Churchill, MB	BUIC-CHU0175	HM388951
Lispe tentaculata (DeGeer)	Churchill, MB	CHU06-FLY-003	N/A
Lispe tentaculata (DeGeer)	Churchill, MB	CHU06-FLY-026	N/A
Lispe tentaculata (DeGeer)	Churchill, MB	CHU06-FLY-002.1	N/A
Lispe uliginosa Fallén	Churchill, MB	BUIC-CHU0120	HM388904
Lispe uliginosa Fallén	Churchill, MB	BUIC-CHU0121	HM388905
Lispe uliginosa Fallén	Churchill, MB	BUIC-CHU0122	HM388906
Lispe uliginosa Fallén	Churchill, MB	BUIC-CHU0123	HM388907
Lispe uliginosa Fallén	Churchill, MB	BUIC-CHU0124	HM388908
Lispe uliginosa Fallén	Churchill, MB	BUIC-CHU0125	HM388909
Lispe uliginosa Fallén	Churchill, MB	CHU05-FLY-277.1	N/A
***Lispocephala alma* (Meigen)**	Churchill, MB	BUIC-CHU1001	N/A
***Lispocephala alma* (Meigen)**	Churchill, MB	BUIC-CHU1002	N/A
***Lispocephala alma* (Meigen)**	Churchill, MB	BUIC-CHU1003	N/A
Lispocephala alma (Meigen)	Churchill, MB	JBWM0319530	N/A
Lispocephala alma (Meigen)	Churchill, MB	JBWM0319557	N/A
Lispocephala alma (Meigen)	Churchill, MB	JBWM0319559	N/A
Lispocephala alma (Meigen)	Churchill, MB	JBWM0234376	N/A
Lispocephala alma (Meigen)	Churchill, MB	JBWM0234401	N/A
Lispocephala erythrocera (R.-D.)	Churchill, MB	BUIC-CHU0953	HM891624
Lispocephala erythrocera (R.-D.)	Churchill, MB	BUIC-CHU0592	HM891638
Lispocephala erythrocera (R.-D.)	Churchill, MB	BUIC-CHU0594	HM891639
Lispocephala erythrocera (R.-D.)	Churchill, MB	BUIC-CHU0595	HM891640

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Lispocephala erythrocera (R.-D.)	Churchill, MB	BUIC-CHU0596	HM891641
Lispocephala erythrocera (R.-D.)	Churchill, MB	BUIC-CHU0599	HM891643
Lispocephala erythrocera (R.-D.)	Churchill, MB	BUIC-CHU0809	HM891814
Lispocephala erythrocera (R.-D.)*	Churchill, MB	BUIC-CHU0593	N/A
Lispocephala sp.*	Churchill, MB	JBWM0319502	N/A
***Lispocephala tinctinervis* Malloch**	Gaspésie, QC	BUIC-CHU1029	N/A
***Lispocephala tinctinervis* Malloch**	Gaspésie, QC	BUIC-CHU1030	N/A
Lispocephala tinctinervis Malloch	Churchill, MB	BUIC-CHU0597	HM891642
***Lispocephala varians* Malloch**	Churchill, MB	BUIC-CHU1000	N/A
***Lispocephala varians* Malloch**	Churchill, MB	BUIC-CHU0598	N/A
*Lispocephala varians**	Churchill, MB	BUIC-CHU0952	N/A
*Lispocephala varians**	Churchill, MB	BUIC-CHU0598	N/A
Lophosceles cinereiventris Zetterstedt	Gaspésie, QC	BUIC-CHU0950	N/A
Lophosceles cinereiventris Zetterstedt	Gaspésie, QC	BUIC-CHU0951	HM891623
Lophosceles cinereiventris Zetterstedt	Churchill, MB	BUIC-CHU0571	HM389234
Lophosceles cinereiventris Zetterstedt	Churchill, MB	BUIC-CHU0572	HM389235
Lophosceles cinereiventris Zetterstedt	Churchill, MB	BUIC-CHU0676	HM891703
Lophosceles cinereiventris Zetterstedt	Churchill, MB	BUIC-CHU0677	HM891704
Lophosceles cinereiventris Zetterstedt	Churchill, MB	BUIC-CHU0679	HM891706
Lophosceles frenatus Holmgren	Churchill, MB	BUIC-CHU0566	HM389229
Lophosceles frenatus Holmgren	Churchill, MB	BUIC-CHU0567	HM389230
Lophosceles frenatus Holmgren	Churchill, MB	BUIC-CHU0568	HM389231
Lophosceles frenatus Holmgren	Churchill, MB	BUIC-CHU0569	HM389232
Lophosceles frenatus Holmgren	Churchill, MB	BUIC-CHU0573	HM389236
Lophosceles frenatus Holmgren	Churchill, MB	BUIC-CHU0897	HQ991857
Lophosceles impar (Zetterstedt)	Jebrenjokk , Sweden	BUIC-CHU0959	HM891629
Lophosceles impar (Zetterstedt)	Churchill, MB	BUIC-CHU0570	HM389233
Lophosceles impar (Zetterstedt)	Churchill, MB	BUIC-CHU0678	HM891705
***Morellia podagrica* Loew**	Gaspésie, QC	BUIC-CHU1055	N/A
Morellia podagrica Loew	Gaspésie, QC	BUIC-CHU0954	HM891625
Morellia podagrica Loew	Churchill, MB	BUIC-CHU0416	HM389125
Musca domestica Linnaeus	Churchill, MB	BUIC-CHU0575	HM389238
Musca domestica Linnaeus	Churchill, MB	BUIC-CHU0576	HM389239
Musca domestica Linnaeus	Churchill, MB	BUIC-CHU0577	HM389240
Musca domestica Linnaeus*	Churchill, MB	BUIC-CHU0574	N/A
*Muscidae**	Churchill, MB	JBWM0319535	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
*Muscidae**	Churchill, MB	JBWM0319548	N/A
Muscina flukei Snyder	Churchill, MB	CHU06-FLY-033.1	N/A
***Muscina flukei* Snyder**	Churchill, MB	BUIC-CHU1004	N/A
***Muscina flukei* Snyder**	Churchill, MB	BUIC-CHU1005	N/A
***Muscina flukei* Snyder**	Churchill, MB	BUIC-CHU1006	N/A
***Muscina flukei* Snyder**	Gaspésie, QC	BUIC-CHU1007	N/A
***Muscina levida* (Harris)**	Waldsleversdor, Germany	BUIC-CHU1021	N/A
***Muscina levida* (Harris)**	Churchill, MB	JBWM0319515	N/A
Muscina levida (Harris)	Churchill, MB	JBWM0319547	N/A
Muscina levida (Harris)	Churchill, MB	JBWM0234386	N/A
Muscina levida (Harris)	Churchill, MB	BUIC-CHU0559	HM389227
Muscina levida (Harris)	Churchill, MB	BUIC-CHU0562	HQ991853
Muscina levida (Harris)	Churchill, MB	BUIC-CHU0564	HQ991854
Muscina levida (Harris)	Churchill, MB	BUIC-CHU0681	HM891707
Muscina levida (Harris)	Churchill, MB	CHU05-FLY-168	N/A
Muscina levida (Harris)	Churchill, MB	CHU05-FLY-187	N/A
Muscina levida (Harris)	Churchill, MB	CHU06-FLY-034.1	N/A
Muscina levida (Harris)*	Churchill, MB	BUIC-CHU0560	N/A
Muscina levida (Harris)*	Churchill, MB	BUIC-CHU0561	N/A
Muscina levida (Harris)*	Churchill, MB	BUIC-CHU0562	N/A
Muscina levida (Harris)*	Churchill, MB	BUIC-CHU0563	N/A
Muscina levida (Harris)*	Churchill, MB	BUIC-CHU0564	N/A
Muscina levida (Harris)*	Churchill, MB	BUIC-CHU0680	N/A
Mydaea affinis Meade	Churchill, MB	BUIC-CHU0161	HM388937
Mydaea affinis Meade	Churchill, MB	BUIC-CHU0162	HM388938
Mydaea affinis Meade	Churchill, MB	BUIC-CHU0682	HM891708
Mydaea affinis Meade	Churchill, MB	BUIC-CHU0683	HM891709
***Mydaea furtiva* Stein**	Churchill, MB	BUIC-CHU1008	N/A
***Mydaea furtiva* Stein**	Churchill, MB	BUIC-CHU1009	N/A
***Mydaea furtiva* Stein**	Churchill, MB	BUIC-CHU1010	N/A
***Mydaea furtiva* Stein**	Churchill, MB	BUIC-CHU1011	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0687	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0688	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0689	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0690	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0152	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0153	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0154	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0155	N/A
Mydaea furtiva Stein*	Churchill, MB	BUIC-CHU0156	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Mydaea obscurella Malloch	Churchill, MB	JBWM0319513	N/A
Mydaea obscurella Malloch	Gaspésie, QC	BUIC-CHU0949	HM891622
Mydaea obscurella Malloch	Churchill, MB	BUIC-CHU0167	HM388943
Mydaea obscurella Malloch	Churchill, MB	BUIC-CHU0168	HM388944
Mydaea obscurella Malloch	Churchill, MB	BUIC-CHU0169	HM388945
Mydaea obscurella Malloch	Churchill, MB	BUIC-CHU0170	HM388946
Mydaea obscurella Malloch	Churchill, MB	BUIC-CHU0171	HM388947
Mydaea obscurella Malloch	Churchill, MB	BUIC-CHU0172	HM388948
Mydaea obscurella Malloch	Churchill, MB	BUIC-CHU0173	HM388949
Mydaea obscurella Malloch	Churchill, MB	BUIC-CHU0174	HM388950
Mydaea occidentalis Malloch	Churchill, MB	BUIC-CHU0163	HM388939
Mydaea occidentalis Malloch	Churchill, MB	BUIC-CHU0164	HM388940
Mydaea occidentalis Malloch	Churchill, MB	BUIC-CHU0165	HM388941
Mydaea occidentalis Malloch	Churchill, MB	BUIC-CHU0166	HM388942
***Mydaea palpalis* Stein**	Gaspésie, QC	BUIC-CHU1056	N/A
Mydaea palpalis Stein	Churchill, MB	BUIC-CHU0684	HM891710
Mydaea palpalis Stein	Churchill, MB	BUIC-CHU0686	HM891712
Mydaea pseudonubila Huckett	Gaspésie, QC	BUIC-CHU0971	N/A
Mydaea pseudonubila Huckett	Churchill, MB	JBWM0234387	N/A
Mydaea pseudonubila Huckett	Churchill, MB	BUIC-CHU0150	HM388931
Mydaea pseudonubila Huckett	Churchill, MB	BUIC-CHU0151	HM388932
Mydaea pseudonubila Huckett	Churchill, MB	BUIC-CHU0783	HM891792
Mydaea pseudonubila Huckett*	Churchill, MB	BUIC-CHU0784	N/A
Myospila meditabunda Fabricius	Churchill, MB	BUIC-CHU0555	HM389224
Myospila meditabunda Fabricius	Churchill, MB	BUIC-CHU0556	HM389225
Myospila meditabunda Fabricius	Churchill, MB	BUIC-CHU0557	HM389226
Myospila meditabunda Fabricius	Churchill, MB	BUIC-CHU0558	HQ991852
Myospila meditabunda Fabricius	Churchill, MB	BUIC-CHU0691	HM891713
Myospila meditabunda Fabricius*	Churchill, MB	BUIC-CHU0558	N/A
Opsolasia orichalcea (Zetterstedt)	Churchill, MB	BUIC-CHU0157	HM388933
Opsolasia orichalcea (Zetterstedt)	Churchill, MB	BUIC-CHU0158	HM388934
Opsolasia orichalcea (Zetterstedt)	Churchill, MB	BUIC-CHU0159	HM388935
Opsolasia orichalcea (Zetterstedt)	Churchill, MB	BUIC-CHU0160	HM388936
Opsolasia orichalcea (Zetterstedt)	Churchill, MB	BUIC-CHU0685	HM891711
Phaonia alpicola (Zetterstedt)	Churchill, MB	BUIC-CHU0692	HM891714
Phaonia alpicola (Zetterstedt)	Churchill, MB	BUIC-CHU0693	HM891715
Phaonia apicalis Stein	Churchill, MB	BUIC-CHU0274	HM389018
Phaonia apicalis Stein	Churchill, MB	BUIC-CHU0275	HQ991835
Phaonia atrocyanea Ringdahl	Churchill, MB	BUIC-CHU0256	HM389005
Phaonia atrocyanea Ringdahl	Churchill, MB	BUIC-CHU0257	HM389006
Phaonia atrocyanea Ringdahl	Churchill, MB	BUIC-CHU0694	HM891716
Phaonia consobrina (Zetterstedt)	Churchill, MB	JBWM0319507	N/A
Phaonia consobrina (Zetterstedt)	Churchill, MB	BUIC-CHU0258	HM389007
Phaonia consobrina (Zetterstedt)	Churchill, MB	BUIC-CHU0261	HM389010

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Phaonia consobrina (Zetterstedt)	Churchill, MB	BUIC-CHU0695	HM891717
Phaonia errans (Meigen)	Churchill, MB	JBWM0234396	N/A
Phaonia errans (Meigen)	Churchill, MB	BUIC-CHU0238	HM388991
Phaonia errans (Meigen)	Churchill, MB	BUIC-CHU0239	HQ991830
Phaonia errans (Meigen)	Churchill, MB	BUIC-CHU0240	HM388992
Phaonia errans (Meigen)	Churchill, MB	BUIC-CHU0241	HM388993
Phaonia errans (Meigen)	Churchill, MB	BUIC-CHU0242	HQ991831
Phaonia errans (Meigen)	Churchill, MB	BUIC-CHU0276	HQ991836
Phaonia errans (Meigen)*	Churchill, MB	BUIC-CHU0237	N/A
Phaonia inenarrabilis Huckett	Churchill, MB	BUIC-CHU0260	HM389009
***Phaonia inenarrabilis* Huckett**	Gaspésie, QC	BUIC-CHU1031	N/A
***Phaonia inenarrabilis* Huckett**	Gaspésie, QC	BUIC-CHU1032	N/A
***Phaonia inenarrabilis* Huckett**	Gaspésie, QC	BUIC-CHU1033	N/A
Phaonia luteva (Walker)	Churchill, MB	JBWM0319538	N/A
Phaonia luteva (Walker)	Churchill, MB	BUIC-CHU0266	HQ991834
Phaonia luteva (Walker)	Churchill, MB	BUIC-CHU0269	HM389013
Phaonia luteva (Walker)	Churchill, MB	BUIC-CHU0270	HM389014
Phaonia luteva (Walker)	Churchill, MB	BUIC-CHU0277	HQ991837
Phaonia luteva (Walker)	Churchill, MB	BUIC-CHU0280	HM389019
Phaonia luteva (Walker)*	Churchill, MB	BUIC-CHU0267	N/A
Phaonia luteva (Walker)*	Churchill, MB	BUIC-CHU0268	N/A
Phaonia luteva (Walker)*	Churchill, MB	BUIC-CHU0278	N/A
Phaonia luteva (Walker)*	Churchill, MB	BUIC-CHU0279	N/A
Phaonia monticola Malloch	Churchill, MB	BUIC-CHU0259	HM389008
Phaonia protuberans Malloch	Churchill, MB	JBWM0234385	N/A
Phaonia protuberans Malloch	Churchill, MB	BUIC-CHU0272	HM389016
Phaonia protuberans Malloch	Churchill, MB	BUIC-CHU0273	HM389017
Phaonia protuberans Malloch	Churchill, MB	BUIC-CHU0301	HM389033
Phaonia protuberans Malloch*	Churchill, MB	BUIC-CHU0300	N/A
***Phaonia rugia* (Walker)**	Gaspésie, QC	BUIC-CHU1039	N/A
***Phaonia rugia* (Walker)**	Gaspésie, QC	BUIC-CHU1040	N/A
***Phaonia rugia* (Walker)**	Gaspésie, QC	BUIC-CHU1041	N/A
Phaonia rugia (Walker)	Churchill, MB	BUIC-CHU0262	HM389011
Phaonia savonoskii Malloch	Churchill, MB	JBWM0319511	N/A
Phaonia savonoskii Malloch	Churchill, MB	JBWM0234377	N/A
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0244	HM388995
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0245	HM388996
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0246	HM388997
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0247	HM388998
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0248	HM388999
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0249	HM389000
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0250	HM389001
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0251	HM389002
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0252	HM389003

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0253	HM389004
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0254	HQ991832
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0255	HQ991833
Phaonia savonoskii Malloch	Churchill, MB	BUIC-CHU0265	HM389012
***Phaonia serva* (Meigen)**	Gaspésie, QC	BUIC-CHU1042	N/A
***Phaonia serva* (Meigen)**	Gaspésie, QC	BUIC-CHU1043	N/A
Phaonia serva (Meigen)	Churchill, MB	BUIC-CHU0243	HM388994
Phaonia serva (Meigen)	Churchill, MB	BUIC-CHU0298	HM389032
Phaonia serva (Meigen)*	Churchill, MB	BUIC-CHU0696	N/A
Phaonia serva (Meigen)*	Churchill, MB	BUIC-CHU0697	N/A
Phaonia subfuscinervis (Zetterstedt)	Churchill, MB	JBWM0319510	N/A
Phaonia subfuscinervis (Zetterstedt)	Churchill, MB	JBWM0319512	N/A
Phaonia subfuscinervis (Zetterstedt)	Churchill, MB	BUIC-CHU0640	HM891672
Potamia littoralis R.-D.	Gaspésie, QC	BUIC-CHU0960	N/A
Potamia littoralis R.-D.	Churchill, MB	BUIC-CHU0271	HM389015
Potamia littoralis R.-D.	Churchill, MB	BUIC-CHU0808	HM891813
Potamia littoralis R.-D.	Churchill, MB	BUIC-CHU0891	HM891887
Pseudocoenosia brevicauda Huckett	Churchill, MB	BUIC-CHU0699	HM891719
Pseudocoenosia brevicauda Huckett	Churchill, MB	BUIC-CHU0700	HM891720
*Pseudocoenosia brevicauda**	Churchill, MB	BUIC-CHU0698	N/A
Pseudocoenosia fletcheri (Malloch)	Banff, AB	BUIC-CHU0296	N/A
Pseudocoenosia fletcheri (Malloch)	Churchill, MB	JBWM0319503	N/A
Pseudocoenosia fletcheri (Malloch)	Churchill, MB	JBWM0319504	N/A
Pseudocoenosia fletcheri (Malloch)	Churchill, MB	BUIC-CHU0295	HM389031
Pseudocoenosia fletcheri (Malloch)	Churchill, MB	BUIC-CHU0701	HM891721
Pseudocoenosia fletcheri (Malloch)	Churchill, MB	BUIC-CHU0702	HM891722
Pseudocoenosia fletcheri (Malloch)	Churchill, MB	BUIC-CHU0703	HM891723
***Pseudocoenosia solitaria* (Zetterstedt)**	Lapplaeger , Sweden	BUIC-CHU0998	N/A
***Pseudocoenosia solitaria* (Zetterstedt)**	Lapplaeger , Sweden	BUIC-CHU0999	N/A
Pseudocoenosia solitaria (Zetterstedt)	Churchill, MB	BUIC-CHU0291	HM389029
Pseudocoenosia solitaria (Zetterstedt)	Churchill, MB	BUIC-CHU0704	HM891724
Pseudocoenosia solitaria (Zetterstedt)	Churchill, MB	BUIC-CHU0705	HM891725
Pseudocoenosia solitaria (Zetterstedt)	Churchill, MB	BUIC-CHU0706	HM891726

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Pseudocoenosia solitaria (Zetterstedt)	Churchill, MB	BUIC-CHU0707	HM891727
Schoenomyza dorsalis Loew	Swift Current, SK	BUIC-CHU0136	N/A
Schoenomyza dorsalis Loew	Swift Current, SK	BUIC-CHU0137	N/A
Schoenomyza dorsalis Loew	Churchill, MB	BUIC-CHU0144	HM388925
Schoenomyza dorsalis Loew	Churchill, MB	BUIC-CHU0145	HM388926
Schoenomyza dorsalis Loew	Churchill, MB	BUIC-CHU0146	HM388927
Schoenomyza dorsalis Loew	Churchill, MB	CHU06-COL-184	N/A
Schoenomyza litorella (Fallén)	Quieux , France	BUIC-CHU0961	N/A
Schoenomyza litorella (Fallén)	Gaspésie, QC	BUIC-CHU0139	N/A
***Schoenomyza litorella* (Fallén)**	Lapplaeger , Sweden	BUIC-CHU0997	N/A
Schoenomyza litorella (Fallén)	Churchill, MB	CHU05-FLI-560	N/A
Schoenomyza litorella (Fallén)	Churchill, MB	CHU05-FLI-710	N/A
Schoenomyza litorella (Fallén)	Churchill, MB	BUIC-CHU0138	HM388920
Schoenomyza litorella (Fallén)	Churchill, MB	BUIC-CHU0140	HM388921
Schoenomyza litorella (Fallén)	Churchill, MB	BUIC-CHU0141	HM388922
Schoenomyza litorella (Fallén)	Churchill, MB	BUIC-CHU0142	HM388923
Schoenomyza litorella (Fallén)	Churchill, MB	BUIC-CHU0143	HM388924
Schoenomyza litorella (Fallén)	Churchill, MB	BUIC-CHU0147	HM388928
Schoenomyza litorella (Fallén)	Churchill, MB	BUIC-CHU0148	HM388929
Schoenomyza litorella (Fallén)	Churchill, MB	BUIC-CHU0149	HM388930
Schoenomyza litorella (Fallén)	Churchill, MB	CHU05-FLY-371	N/A
Schoenomyza litorella (Fallén)	Churchill, MB	CHU05-FLY-432	N/A
Schoenomyza litorella (Fallén)	Churchill, MB	CHU05-FLY-439	N/A
Schoenomyza litorella (Fallén)	Churchill, MB	CHU05-FLY-451	N/A
Schoenomyza sp.*	Churchill, MB	BUIC-CHU0708	N/A
Schoenomyza sp.*	Churchill, MB	BUIC-CHU0709	N/A
Schoenomyza sp.*	Churchill, MB	BUIC-CHU0710	N/A
Spilogona aenea Huckett	Churchill, MB	JBWM0319536	N/A
Spilogona aenea Huckett	Churchill, MB	BUIC-CHU0711	HM891728
Spilogona aenea Huckett	Churchill, MB	BUIC-CHU0712	HM891729
Spilogona aenea Huckett	Churchill, MB	BUIC-CHU0713	HM891730
Spilogona aenea Huckett	Churchill, MB	BUIC-CHU0714	HM891731
Spilogona aenea Huckett	Churchill, MB	BUIC-CHU0844	HM891841
Spilogona aerea (Fallén)	Churchill, MB	JBWM0319551	N/A
Spilogona aerea (Fallén)	Churchill, MB	BUIC-CHU0420	HM389128
Spilogona aerea (Fallén)	Churchill, MB	BUIC-CHU0431	HM389138
Spilogona aerea (Fallén)	Churchill, MB	BUIC-CHU0432	HM389139
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-391	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-403	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-424	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-466	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-688	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-689	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-690	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-691	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-694	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-695	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-697	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-699	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-700	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-703	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-704	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-705	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-745	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-749	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-750	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-753	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-754	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-756	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-759	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-763	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-764	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-770	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-771	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-776	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-777	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-778	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-779	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-121.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-686.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-693.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-702.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-706.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-718.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-720.1	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-748.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-755.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-758.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-765.1	N/A
Spilogona aerea (Fallén)	Churchill, MB	CHU05-FLY-768.1	N/A
***Spilogona albisquama* Ringdahl**	Churchill, MB	BUIC-CHU1012	N/A
***Spilogona albisquama* Ringdahl**	Churchill, MB	BUIC-CHU1013	N/A
***Spilogona albisquama* Ringdahl**	Churchill, MB	BUIC-CHU1014	N/A
***Spilogona albisquama* Ringdahl**	Churchill, MB	BUIC-CHU1015	N/A
Spilogona alticola (Malloch)	NT	BUIC-CHU0379	N/A
Spilogona arctica (Zetterstedt)	Gaspésie, QC	BUIC-CHU0920	N/A
Spilogona arctica (Zetterstedt)	Churchill, MB	JBWM0319516	N/A
Spilogona arctica (Zetterstedt)	Churchill, MB	JBWM0319524	N/A
Spilogona arctica (Zetterstedt)	Churchill, MB	JBWM0234382	N/A
Spilogona arctica (Zetterstedt)	Churchill, MB	JBWM0234400	N/A
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0281	HM389020
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0283	HM389022
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0289	HM389028
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0361	HM389076
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0365	HM389080
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0376	HM389089
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0377	HM389090
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0831	HM891830
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0841	HM891838
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0845	HM891842
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0851	HM891848
Spilogona arctica (Zetterstedt)	Churchill, MB	BUIC-CHU0872	HM891868
Spilogona arctica (Zetterstedt)	Gaspésie, QC	BUIC-CHU0921	HM891915
Spilogona arctica (Zetterstedt)	Gaspésie, QC	BUIC-CHU0922	HM891916
Spilogona arctica (Zetterstedt)	Gaspésie, QC	BUIC-CHU0923	HM891917
***Spilogona arctica* (Zetterstedt)**	Abisko, Sweden	BUIC-CHU1020	N/A
***Spilogona arctica* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1036	N/A
***Spilogona arctica* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1037	N/A
***Spilogona arctica* (Zetterstedt)**	Abisko, Sweden	BUIC-CHU1038	N/A
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0380	HM389091
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0384	HM389095
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0398	HM389109

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0399	HM389110
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0400	HM389111
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0402	HM389113
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0444	HM389151
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0445	HM389152
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0446	HM389153
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0715	HM891732
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0716	HM891733
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0719	HM891736
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0720	HM891737
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0722	HM891739
Spilogona atrisquamula Hennig	Churchill, MB	BUIC-CHU0911	HM891906
Spilogona atrisquamula Hennig	Churchill, MB	CHU05-FLY-438	N/A
Spilogona bifimbriata Huckett	Churchill, MB	BUIC-CHU0725	HM891742
Spilogona bifimbriata Huckett	Churchill, MB	BUIC-CHU0726	HM891743
***Spilogona calcaria* Huckett**	Churchill, MB	BUIC-CHU1025	N/A
Spilogona calcaria Huckett*	Churchill, MB	BUIC-CHU0727	N/A
Spilogona calcaria Huckett*	Churchill, MB	BUIC-CHU0728	N/A
Spilogona calcaria Huckett*	Churchill, MB	BUIC-CHU0729	N/A
Spilogona calcaria Huckett*	Churchill, MB	BUIC-CHU0833	N/A
Spilogona churchillensis Huckett	Churchill, MB	BUIC-CHU0440	HM389147
Spilogona churchillensis Huckett	Churchill, MB	BUIC-CHU0441	HM389148
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-711	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-712	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-713	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-714	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-715	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-717	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-730	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-761	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-159.1	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-716.1	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-721.1	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-722.1	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-724.1	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-732.1	N/A
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-773.1	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona churchillensis Huckett	Churchill, MB	CHU05-FLY-775.1	N/A
Spilogona confluens Huckett	Churchill, MB	BUIC-CHU0401	HM389112
Spilogona confluens Huckett	Churchill, MB	BUIC-CHU0724	HM891741
Spilogona contractifrons (Zetterstedt)	Finse, Norway	BUIC-CHU0378	N/A
Spilogona contractifrons (Zetterstedt)	Churchill, MB	JBWM0319509	N/A
Spilogona contractifrons (Zetterstedt)	Churchill, MB	JBWM0319521	N/A
Spilogona contractifrons (Zetterstedt)	Churchill, MB	JBWM0319526	N/A
Spilogona contractifrons (Zetterstedt)	Churchill, MB	JBWM0319529	N/A
Spilogona contractifrons (Zetterstedt)	Churchill, MB	JBWM0319552	N/A
Spilogona contractifrons (Zetterstedt)	Churchill, MB	JBWM0319561	N/A
Spilogona contractifrons (Zetterstedt)	Churchill, MB	JBWM0234399	N/A
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0286	HM389025
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0287	HM389026
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0362	HM389077
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0363	HM389078
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0364	HM389079
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0366	HM389081
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0367	HM389082
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0368	HM389083
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0371	HM389084
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0372	HM389085
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0373	HM389086
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0375	HM389088
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0786	HM891794

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0842	HM891839
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0859	HM891856
Spilogona contractifrons (Zetterstedt)	Churchill, MB	BUIC-CHU0860	HM891857
Spilogona contractifrons (Zetterstedt)	Churchill, MB	CHU05-FLY-118	N/A
***Spilogona contractifrons* (Zetterstedt)**	Lapplaeger, Sweden	BUIC-CHU1017	N/A
***Spilogona contractifrons* (Zetterstedt)**	Jebrenjokk, Sweden	BUIC-CHU1018	N/A
***Spilogona contractifrons* (Zetterstedt)**	Abiskojokk , Sweden	BUIC-CHU1019	N/A
***Spilogona contractifrons* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1034	N/A
***Spilogona contractifrons* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1035	N/A
Spilogona contractifrons (Zetterstedt)*	Churchill, MB	BUIC-CHU0369	N/A
Spilogona contractifrons (Zetterstedt)*	Churchill, MB	BUIC-CHU0370	N/A
Spilogona deflorata (Holmgren)	Churchill, MB	BUIC-CHU0733	HM891747
Spilogona deflorata (Holmgren)	Churchill, MB	BUIC-CHU0734	HM891748
Spilogona deflorata (Holmgren)	Churchill, MB	BUIC-CHU0735	HM891749
Spilogona deflorata (Holmgren)	Churchill, MB	BUIC-CHU0736	HM891750
Spilogona deflorata (Holmgren)	Churchill, MB	BUIC-CHU0820	HM891824
Spilogona deflorata (Holmgren)	Churchill, MB	BUIC-CHU0916	HM891911
Spilogona deflorata (Holmgren)	Churchill, MB	CHU05-FLY-453	N/A
Spilogona deflorata (Holmgren)	Churchill, MB	CHU05-FLY-707	N/A
Spilogona fatima Huckett	Churchill, MB	BUIC-CHU0434	HM389141
Spilogona fatima Huckett	Churchill, MB	BUIC-CHU0435	HM389142
Spilogona fatima Huckett	Churchill, MB	BUIC-CHU0737	HM891751
Spilogona fatima Huckett	Churchill, MB	BUIC-CHU0740	HM891753
Spilogona fatima Huckett*	Churchill, MB	BUIC-CHU0738	N/A
Spilogona firmidisetosa Huckett	Churchill, MB	BUIC-CHU0717	HM891734
Spilogona firmidisetosa Huckett	Churchill, MB	BUIC-CHU0718	HM891735
Spilogona firmidisetosa Huckett	Churchill, MB	BUIC-CHU0741	HM891754
Spilogona firmidisetosa Huckett	Churchill, MB	BUIC-CHU0743	HM891755
Spilogona firmidisetosa Huckett	Churchill, MB	BUIC-CHU0744	HM891756
Spilogona firmidisetosa Huckett	Churchill, MB	BUIC-CHU0917	HM891912
Spilogona firmidisetosa Huckett	Churchill, MB	BUIC-CHU0918	HM891913
Spilogona firmidisetosa Huckett	Churchill, MB	BUIC-CHU0919	HM891914
Spilogona firmidisetosa Huckett*	Churchill, MB	BUIC-CHU0742	N/A
Spilogona flavinervis Huckett*	Churchill, MB	BUIC-CHU0745	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona flavinervis Huckett*	Churchill, MB	BUIC-CHU0746	N/A
Spilogona forticula Huckett	Churchill, MB	JBWM0319522	N/A
Spilogona forticula Huckett	Churchill, MB	JBWM0319550	N/A
Spilogona forticula Huckett	Churchill, MB	BUIC-CHU0285	HM389024
Spilogona forticula Huckett	Churchill, MB	BUIC-CHU0288	HM389027
Spilogona forticula Huckett	Churchill, MB	BUIC-CHU0404	HM389114
Spilogona forticula Huckett	Churchill, MB	BUIC-CHU0830	HM891829
Spilogona forticula Huckett	Churchill, MB	BUIC-CHU0832	HM891831
Spilogona forticula Huckett	Churchill, MB	BUIC-CHU0915	HM891910
Spilogona forticula Huckett*	Churchill, MB	BUIC-CHU0403	N/A
Spilogona genualis Huckett	Churchill, MB	BUIC-CHU0747	HM891757
Spilogona genualis Huckett	Churchill, MB	BUIC-CHU0748	HM891758
***Spilogona gibsoni* Malloch**	Gaspésie, QC	BUIC-CHU1059	N/A
***Spilogona gibsoni* Malloch**	Gaspésie, QC	BUIC-CHU1060	N/A
Spilogona griseola Collin	Churchill, MB	JBWM0319554	N/A
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0381	HM389092
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0382	HM389093
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0383	HM389094
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0385	HM389096
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0386	HM389097
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0387	HM389098
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0388	HM389099
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0389	HM389100
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0390	HM389101
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0391	HM389102
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0392	HM389103
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0393	HM389104
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0394	HM389105
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0749	HM891759
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0819	HM891823
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0846	HM891843
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0847	HM891844
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0848	HM891845
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0849	HM891846
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0850	HM891847
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0852	HM891849
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0853	HM891850
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0854	HM891851
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0855	HM891852
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0856	HM891853
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0857	HM891854
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0858	HM891855
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0861	HM891858
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0862	HM891859

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0863	HM891860
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0864	HM891861
Spilogona griseola Collin	Churchill, MB	BUIC- CHU0866	HM891862
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0868	HM891864
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0869	HM891865
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0870	HM891866
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0873	HM891869
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0874	HM891870
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0875	HM891871
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0876	HM891872
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0877	HM891873
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0878	HM891874
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0879	HM891875
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0880	HM891876
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0881	HM891877
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0882	HM891878
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0883	HM891879
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0884	HM891880
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0885	HM891881
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0886	HM891882
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0887	HM891883
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0888	HM891884
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0889	HM891885
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0890	HM891886
Spilogona griseola Collin	Churchill, MB	BUIC-CHU0910	HM891905
Spilogona imitatrix (Malloch)	Goose Bay, NL	BUIC-CHU0415	N/A
Spilogona imitatrix (Malloch)	Churchill, MB	BUIC-CHU0412	HM389122
Spilogona imitatrix (Malloch)	Churchill, MB	BUIC-CHU0413	HM389123
Spilogona imitatrix (Malloch)	Churchill, MB	BUIC-CHU0414	HM389124
Spilogona imitatrix (Malloch)	Churchill, MB	BUIC-CHU0424	HM389132
Spilogona imitatrix (Malloch)	Churchill, MB	BUIC-CHU0425	HM389133
Spilogona incerta Huckett	Churchill, MB	BUIC-CHU0750	HM891760
Spilogona infuscata Huckett	Churchill, MB	BUIC-CHU0730	HM891744
Spilogona infuscata Huckett	Churchill, MB	BUIC-CHU0751	HM891761
Spilogona leucogaster (Zetterstedt)	Churchill, MB	BUIC-CHU0436	HM389143
Spilogona leucogaster (Zetterstedt)	Churchill, MB	BUIC-CHU0437	HM389144
Spilogona leucogaster (Zetterstedt)	Churchill, MB	BUIC-CHU0438	HM389145
Spilogona leucogaster (Zetterstedt)	Churchill, MB	BUIC-CHU0439	HM389146
***Spilogona magnipunctata* Malloch**	Gaspésie, QC	BUIC-CHU1057	N/A
***Spilogona magnipunctata* Malloch**	Gaspésie, QC	BUIC-CHU1058	N/A
Spilogona malaisei (Ringdahl)	Churchill, MB	BUIC-CHU0753	HM891763
Spilogona malaisei (Ringdahl)	Churchill, MB	BUIC-CHU0754	HM891764
Spilogona melanosoma Huckett	Churchill, MB	JBWM0234402	N/A

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona melanosoma Huckett	Churchill, MB	BUIC-CHU0442	HM389149
Spilogona melanosoma Huckett	Churchill, MB	BUIC-CHU0443	HM389150
Spilogona melanosoma Huckett*	Churchill, MB	BUIC-CHU0828	N/A
Spilogona micans (Ringdahl)	Churchill, MB	BUIC-CHU0755	HM891765
Spilogona micans (Ringdahl)	Churchill, MB	BUIC-CHU0756	HM891766
Spilogona micans (Ringdahl)	Churchill, MB	BUIC-CHU0757	HM891767
***Spilogona monacantha* Collin**	Gaspésie, QC	BUIC-CHU1050	N/A
***Spilogona monacantha* Collin**	Gaspésie, QC	BUIC-CHU1051	N/A
Spilogona narina (Walker)	Churchill, MB	BUIC-CHU0759	HM891768
Spilogona narina (Walker)*	Churchill, MB	BUIC-CHU0758	N/A
Spilogona novemaculata (Zetterstedt)	Churchill, MB	BUIC-CHU0433	HM389140
Spilogona novemaculata (Zetterstedt)	Churchill, MB	BUIC-CHU0739	HM891752
Spilogona obscuripennis (Stein)	Churchill, MB	BUIC-CHU0731	HM891745
Spilogona obscuripennis (Stein)	Churchill, MB	BUIC-CHU0732	HM891746
Spilogona obscuripennis (Stein)	Churchill, MB	BUIC-CHU0760	HM891769
Spilogona obscuripennis (Stein)	Churchill, MB	BUIC-CHU0761	HM891770
Spilogona obscuripennis (Stein)	Churchill, MB	BUIC-CHU0762	HM891771
Spilogona obscuripennis (Stein)	Churchill, MB	CHU06-FLY-102.1	N/A
Spilogona opaca Schnabl	Churchill, MB	BUIC-CHU0395	HM389106
Spilogona opaca Schnabl	Churchill, MB	BUIC-CHU0396	HM389107
Spilogona opaca Schnabl	Churchill, MB	BUIC-CHU0764	HM891773
***Spilogona opaca* Schnabl**	Churchill, MB	BUIC-CHU1026	N/A
Spilogona pacifica (Meigen)	Churchill, MB	JBWM0319542	N/A
Spilogona pacifica (Meigen)	Churchill, MB	JBWM0319543	N/A
Spilogona pacifica (Meigen)	Churchill, MB	JBWM0234388	N/A
Spilogona pacifica (Meigen)	Churchill, MB	BUIC-CHU0422	HM389130
Spilogona pacifica (Meigen)	Churchill, MB	BUIC-CHU0428	HM389135
Spilogona pacifica (Meigen)	Churchill, MB	BUIC-CHU0836	HM891833
Spilogona pacifica (Meigen)	Churchill, MB	BUIC-CHU0837	HM891834
Spilogona pacifica (Meigen)	Churchill, MB	BUIC-CHU0838	HM891835
Spilogona pacifica (Meigen)	Churchill, MB	BUIC-CHU0839	HM891836
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0406	HM389116
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0407	HM389117
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0408	HM389118
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0421	HM389129
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0423	HM389131
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0721	HM891738
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0723	HM891740
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0763	HM891772
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0770	HM891779
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0771	HM891780

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0821	HM891825
Spilogona pusilla Huckett	Churchill, MB	BUIC-CHU0822	HM891826
Spilogona reflecta Huckett	Churchill, MB	BUIC-CHU0767	HM891776
Spilogona reflecta Huckett	Churchill, MB	BUIC-CHU0768	HM891777
Spilogona reflecta Huckett	Churchill, MB	BUIC-CHU0769	HM891778
***Spilogona reflecta* Huckett**	Churchill, MB	BUIC-CHU1027	N/A
Spilogona reflecta Huckett*	Churchill, MB	BUIC-CHU0766	N/A
Spilogona setinervis Huckett	Churchill, MB	BUIC-CHU0765	HM891774
Spilogona setinervis Huckett	Churchill, MB	BUIC-CHU0772	HM891781
Spilogona setinervis Huckett	Churchill, MB	BUIC-CHU0774	HM891783
Spilogona setinervis Huckett	Churchill, MB	BUIC-CHU0775	HM891784
Spilogona setipes Huckett	Churchill, MB	BUIC-CHU0776	HM891785
Spilogona sororcula (Zetterstedt)	Gaspésie, QC	BUIC-CHU0419	N/A
Spilogona sororcula (Zetterstedt)	Churchill, MB	JBWM0319528	N/A
Spilogona sororcula (Zetterstedt)	Churchill, MB	BUIC-CHU0417	HM389126
Spilogona sororcula (Zetterstedt)	Churchill, MB	BUIC-CHU0418	HM389127
Spilogona sororcula (Zetterstedt)	Churchill, MB	BUIC-CHU0429	HM389136
Spilogona sororcula (Zetterstedt)	Churchill, MB	BUIC-CHU0430	HM389137
Spilogona sororcula (Zetterstedt)	Churchill, MB	CHU05-FLY-134	N/A
Spilogona sp.*	Churchill, MB	BUIC-CHU0834	N/A
Spilogona sp.*	Churchill, MB	BUIC-CHU0865	N/A
***Spilogona* sp. 1**	Churchill, MB	BUIC-CHU823	N/A
***Spilogona* sp. 1**	Churchill, MB	BUIC-CHU824	N/A
***Spilogona* sp. 1**	Churchill, MB	BUIC-CHU826	N/A
***Spilogona* sp. 1**	Churchill, MB	BUIC-CHU827	N/A
Spilogona sp. 2	Churchill, MB	BUIC-CHU0284	HM389023
Spilogona sp. 3	Churchill, MB	BUIC-CHU0405	HM389115
Spilogona sp. 4	Churchill, MB	BUIC-CHU0773	HM891782
Spilogona sp. 5	Churchill, MB	BUIC-CHU0292	HM389030
Spilogona sp. 6	Churchill, MB	BUIC-CHU0867	HM891863
Spilogona sp. 7	Churchill, MB	BUIC-CHU0840	HM891837
Spilogona sp. 8	Churchill, MB	BUIC-CHU0825	HM891827
Spilogona sp. 9	Churchill, MB	BUIC-CHU0835	HM891832
Spilogona sp. 9	Churchill, MB	BUIC-CHU0843	HM891840
Spilogona sp. 10	Churchill, MB	BUIC-CHU0892	HM891888
Spilogona sp. 10	Churchill, MB	BUIC-CHU0893	HM891889
Spilogona sp. 10	Churchill, MB	BUIC-CHU0898	HM891893
Spilogona sp. 11	Churchill, MB	BUIC-CHU0789	HM891797
Spilogona sp. 12	Gaspésie, QC	BUIC-CHU0927	HM891601
Spilogona sp. 12	Churchill, MB	BUIC-CHU0282	HM389021
Spilogona sp. 12	Churchill, MB	BUIC-CHU0374	HM389087
Spilogona sp. 12	Churchill, MB	BUIC-CHU0871	HM891867
Spilogona sp. 12	Gaspésie, QC	BUIC-CHU0924	HM891918
Spilogona sp. 12	Gaspésie, QC	BUIC-CHU0925	HM891919

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona sp. 12	Gaspésie, QC	BUIC-CHU0926	HM891920
Spilogona surda (Zetterstedt)	Churchill, MB	JBWM0319501	N/A
Spilogona surda (Zetterstedt)	Churchill, MB	BUIC-CHU0777	HM891786
Spilogona surda (Zetterstedt)	Churchill, MB	BUIC-CHU0778	HM891787
Spilogona surda (Zetterstedt)	Churchill, MB	BUIC-CHU0779	HM891788
Spilogona surda (Zetterstedt)	Churchill, MB	BUIC-CHU0780	HM891789
Spilogona surda (Zetterstedt)	Churchill, MB	CHU05-FLY-193	N/A
Spilogona surda (Zetterstedt)	Churchill, MB	CHU05-FLY-726	N/A
Spilogona surda (Zetterstedt)	Churchill, MB	CHU06-FLY-089.1	N/A
Spilogona suspecta (Malloch)	Churchill, MB	JBWM0319558	N/A
Spilogona suspecta (Malloch)	Gaspésie, QC	BUIC-CHU0947	HM891620
Spilogona suspecta (Malloch)	Gaspésie, QC	BUIC-CHU0948	HM891621
Spilogona suspecta (Malloch)	Churchill, MB	BUIC-CHU0397	HM389108
Spilogona suspecta (Malloch)	Churchill, MB	BUIC-CHU0409	HM389119
Spilogona suspecta (Malloch)	Churchill, MB	BUIC-CHU0410	HM389120
Spilogona suspecta (Malloch)	Churchill, MB	BUIC-CHU0411	HM389121
Spilogona suspecta (Malloch)	Churchill, MB	CHU05-FLY-136	N/A
Spilogona suspecta (Malloch)	Churchill, MB	CHU05-FLY-301.1	N/A
Spilogona suspecta (Malloch)	Churchill, MB	CHU06-FLY-004.1	N/A
Spilogona suspecta (Malloch)	Churchill, MB	CHU06-FLY-095.1	N/A
Spilogona suspecta (Malloch)	Churchill, MB	CHU06-FLY-096.1	N/A
Spilogona tornensis (Ringdahl)	Mt Slattatjakka, Sweden	BUIC-CHU0958	N/A
Spilogona tornensis (Ringdahl)	Churchill, MB	BUIC-CHU0782	HM891791
Spilogona trigonata (Zetterstedt)	Churchill, MB	JBWM0319505	N/A
Spilogona trigonata (Zetterstedt)	Churchill, MB	JBWM0319541	N/A
Spilogona trigonata (Zetterstedt)	Churchill, MB	JBWM0319545	N/A
Spilogona trigonata (Zetterstedt)	Churchill, MB	BUIC-CHU0785	HM891793
Spilogona trigonata (Zetterstedt)	Churchill, MB	BUIC-CHU0787	HM891795
Spilogona trigonata (Zetterstedt)	Churchill, MB	BUIC-CHU0788	HM891796
Spilogona trigonata (Zetterstedt)	Churchill, MB	BUIC-CHU0912	HM891907
Spilogona trigonata (Zetterstedt)	Churchill, MB	BUIC-CHU0913	HM891908
Spilogona trigonata (Zetterstedt)	Churchill, MB	BUIC-CHU0914	HM891909
***Spilogona trigonata* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1052	N/A
***Spilogona trigonata* (Zetterstedt)**	Gaspésie, QC	BUIC-CHU1053	N/A
Spilogona trigonata (Zetterstedt)*	Churchill, MB	BUIC-CHU0791	N/A
Spilogona trigonifera (Zetterstedt)	Gaspésie, QC	BUIC-CHU0929	N/A
Spilogona trigonifera (Zetterstedt)	Gaspésie, QC	BUIC-CHU0928	HM891602

Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Spilogona trigonifera (Zetterstedt)	Gaspésie, QC	BUIC-CHU0930	HM891603
Spilogona trilineata Huckett	Churchill, MB	BUIC-CHU0427	HM389134
Spilogona trilineata Huckett	Churchill, MB	BUIC-CHU0790	HM891798
Spilogona trilineata Huckett	Churchill, MB	BUIC-CHU0792	HM891800
Spilogona trilineata Huckett	Churchill, MB	BUIC-CHU0793	HM891801
Spilogona trilineata Huckett	Churchill, MB	CHU06-FLY-014	N/A
Spilogona zaitzevi (Schnabl)	Churchill, MB	BUIC-CHU0752	HM891762
*Spilogona zaitzevi (*Schnabl)	Churchill, MB	BUIC-CHU0829	HM891828
Stomoxys calcitrans Linnaeus	Marmaris, Turkey	BUIC-CHU0964	N/A
Stomoxys calcitrans Linnaeus	Churchill, MB	BUIC-CHU0565	HM389228
Stomoxys calcitrans Linnaeus	Churchill, MB	BUIC-CHU0781	HM891790
Thricops albibasalis (Zetterstedt)	Gaspésie, QC	BUIC-CHU0943	N/A
Thricops albibasalis (Zetterstedt)	Churchill, MB	JBWM0319525	N/A
Thricops albibasalis (Zetterstedt)	Churchill, MB	JBWM0319539	N/A
Thricops albibasalis (Zetterstedt)	Churchill, MB	JBWM0234379	N/A
Thricops albibasalis (Zetterstedt)	Gaspésie, QC	BUIC-CHU0944	HM891617
Thricops albibasalis (Zetterstedt)	Churchill, MB	BUIC-CHU0191	HM388966
Thricops albibasalis (Zetterstedt)	Churchill, MB	BUIC- CHU0192	HM388967
Thricops albibasalis (Zetterstedt)	Churchill, MB	BUIC-CHU0193	HM388968
Thricops albibasalis (Zetterstedt)	Churchill, MB	BUIC-CHU0194	HM388969
Thricops albibasalis (Zetterstedt)	Churchill, MB	BUIC-CHU0195	HM388970
Thricops albibasalis (Zetterstedt)	Churchill, MB	BUIC-CHU0797	HM891805
Thricops diaphanus (Wiedemann)	Churchill, MB	BUIC-CHU0794	HM891802
Thricops diaphanus (Wiedemann)	Churchill, MB	BUIC-CHU0795	HM891803
Thricops diaphanus (Wiedemann)	Churchill, MB	BUIC-CHU0796	HM891804
Thricops diaphanus (Wiedemann)	Gaspésie, QC	BUIC-CHU0970	HM891635
Thricops hirtulus (Zetterstedt)	Churchill, MB	BUIC-CHU0184	HM388959
Thricops hirtulus (Zetterstedt)	Churchill, MB	BUIC-CHU0185	HM388960
Thricops hirtulus (Zetterstedt)	Churchill, MB	BUIC-CHU0186	HM388961
Thricops hirtulus (Zetterstedt)	Churchill, MB	BUIC-CHU0187	HM388962
Thricops hirtulus (Zetterstedt)	Churchill, MB	BUIC-CHU0188	HM388963
Thricops hirtulus (Zetterstedt)	Churchill, MB	BUIC-CHU0189	HM388964
Thricops hirtulus (Zetterstedt)	Churchill, MB	BUIC-CHU0190	HM388965
Thricops hirtulus (Zetterstedt)	Churchill, MB	BUIC-CHU0668	HM891697
Thricops hirtulus (Zetterstedt)	Churchill, MB	CHU05-FLY-198	N/A
Thricops innocuus (Zetterstedt)	Churchill, MB	JBWM0319523	N/A
Thricops innocuus (Zetterstedt)	Churchill, MB	JBWM0234391	N/A
Thricops innocuus (Zetterstedt)	Churchill, MB	JBWM0234392	N/A
Thricops innocuus (Zetterstedt)	Churchill, MB	JBWM0234393	N/A
Thricops innocuus (Zetterstedt)	Gaspésie, QC	BUIC-CHU0941	HM891614
Thricops innocuus (Zetterstedt)	Gaspésie, QC	BUIC-CHU0942	HM891615
Thricops innocuus (Zetterstedt)	Churchill, MB	BUIC-CHU0177	HM388952
Thricops innocuus (Zetterstedt)	Churchill, MB	BUIC-CHU0178	HM388953

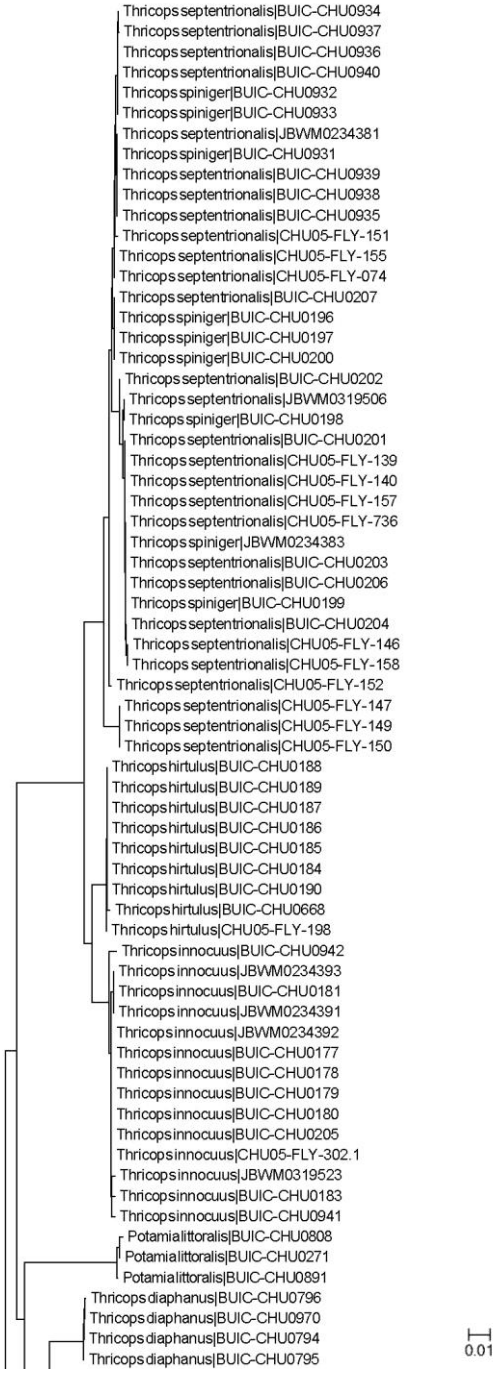
Appendix E. (continued)

Species	Collecting locality	Sample ID	Accession number
Thricops innocuus (Zetterstedt)	Churchill, MB	BUIC-CHU0179	HM388954
Thricops innocuus (Zetterstedt)	Churchill, MB	BUIC-CHU0180	HM388955
Thricops innocuus (Zetterstedt)	Churchill, MB	BUIC-CHU0181	HM388956
Thricops innocuus (Zetterstedt)	Churchill, MB	BUIC-CHU0183	HM388958
Thricops innocuus (Zetterstedt)	Churchill, MB	BUIC-CHU0205	HM388978
Thricops innocuus (Zetterstedt)	Churchill, MB	CHU05-FLY-302.1	N/A
Thricops innocuus (Zetterstedt)*	Churchill, MB	BUIC-CHU0813	N/A
Thricops innocuus (Zetterstedt)*	Churchill, MB	BUIC-CHU0176	N/A
Thricops innocuus (Zetterstedt)*	Churchill, MB	BUIC-CHU0182	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	JBWM0319506	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	JBWM0234381	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	JBWM0234383	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0931	HM891604
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0932	HM891605
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0933	HM891606
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0934	HM891607
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0935	HM891608
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0936	HM891609
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0937	HM891610
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0938	HM891611
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0939	HM891612
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Gaspésie, QC	BUIC-CHU0940	HM891613
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0196	HM388971
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0197	HM388972
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0198	HM388973
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0199	HM388974

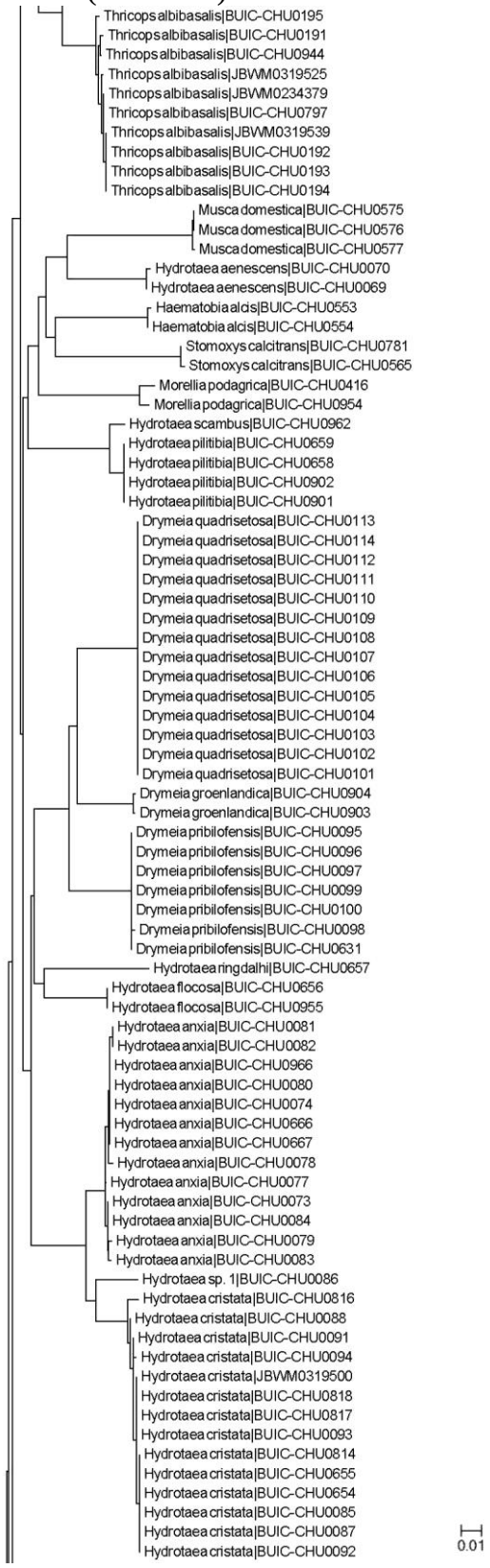
Appendix E. (concluded)

Species	Collecting locality	Sample ID	Accession number
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0200	HM388975
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0201	HM388976
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0202	HQ991819
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0203	HM388977
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0204	HQ991820
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0206	HM388979
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	BUIC-CHU0207	HM388980
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-074	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-139	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-140	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-146	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-147	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-149	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-150	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-151	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-152	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-155	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-157	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-158	N/A
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	Churchill, MB	CHU05-FLY-736	N/A

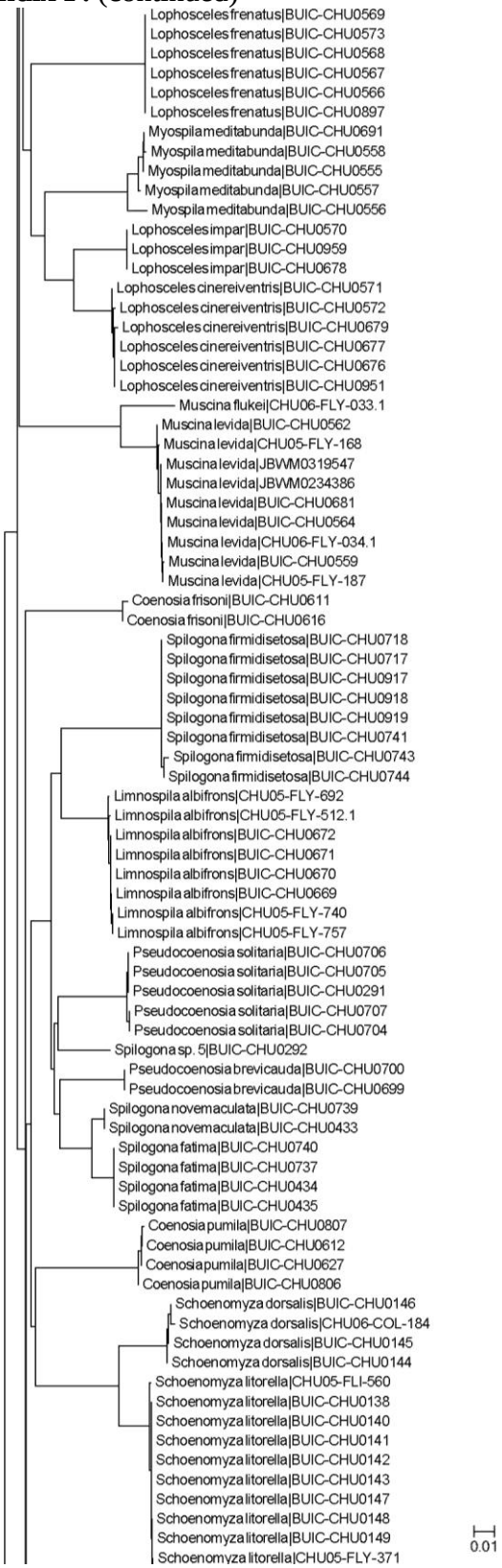
Appendix F. Kimura 2-parameter Neighbour-joining tree of 976 COI sequences of Muscidae from Churchill (Manitoba), Gaspésie (Québec), and Sweden. Numbers at the end of each taxon name refer to sample ID numbers (www.boldsystems.org) (Appendix E).



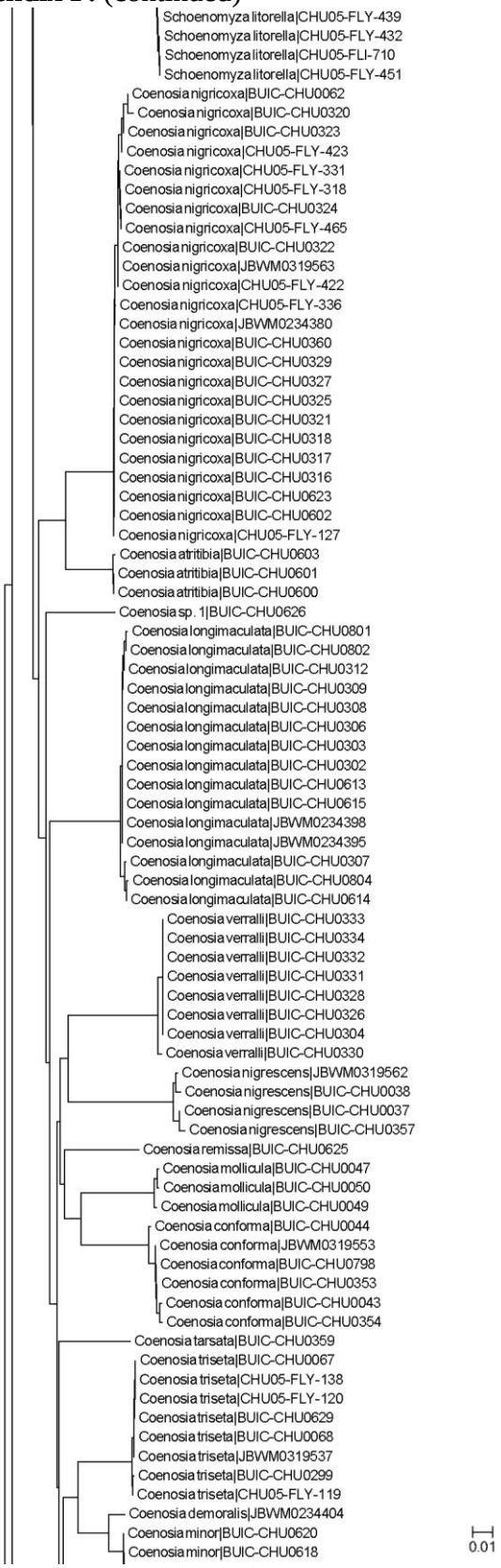
Appendix F. (continued)



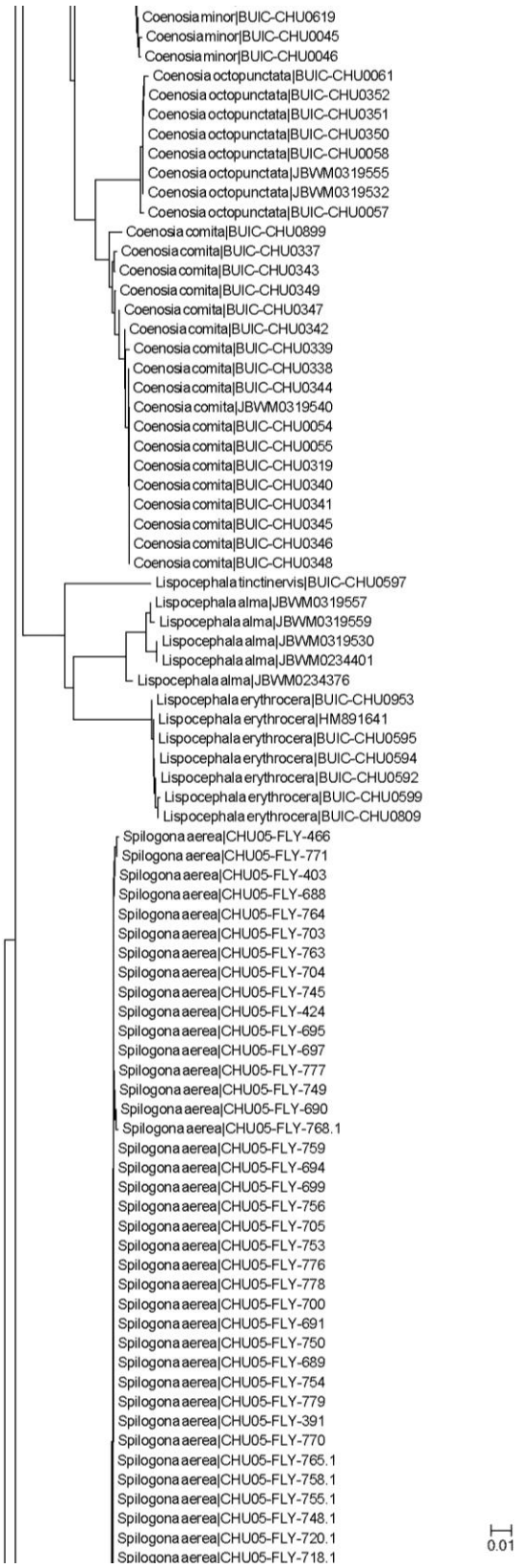
Appendix F. (continued)



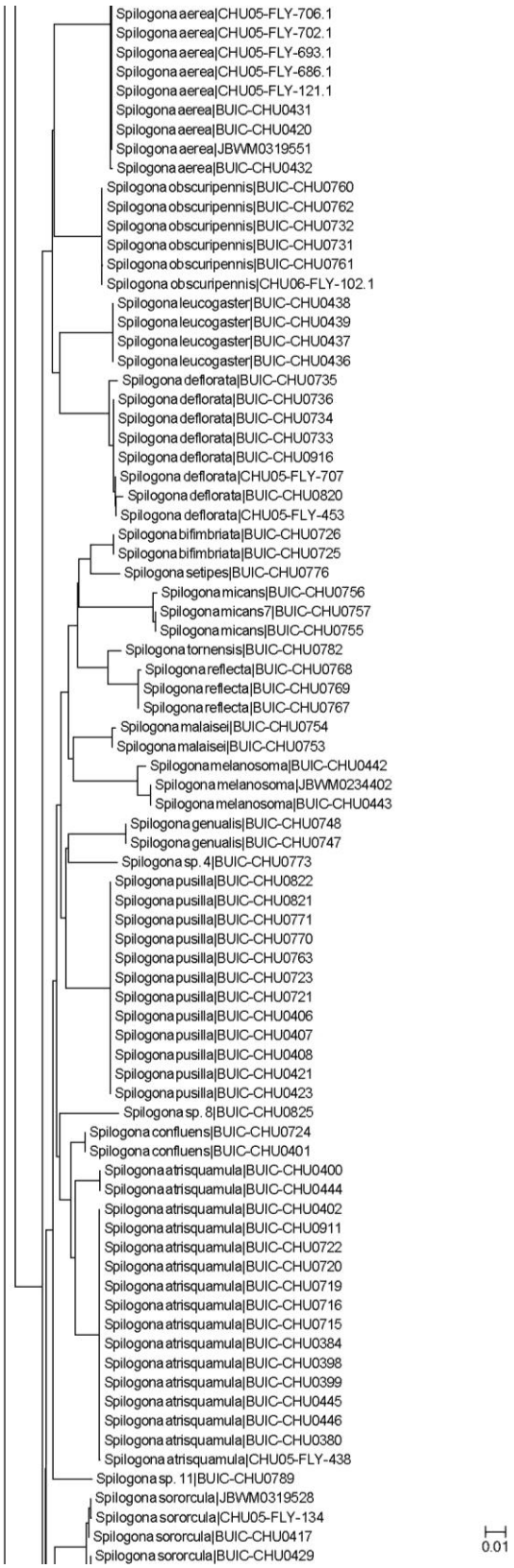
Appendix F. (continued)



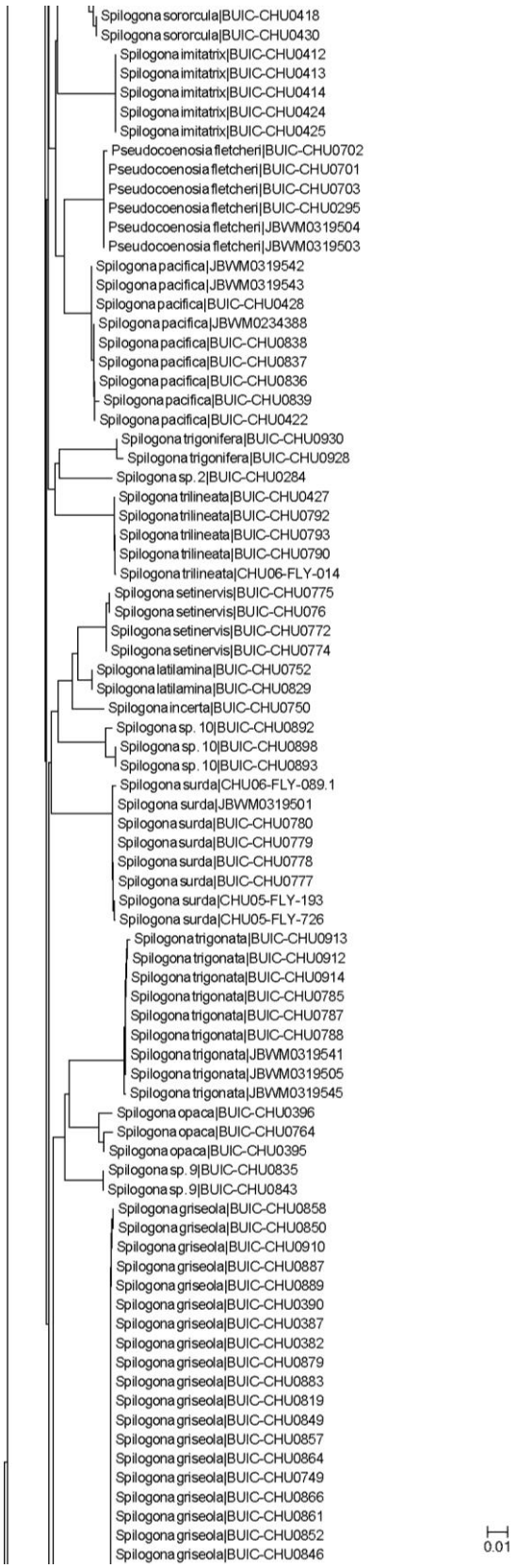
Appendix F. (continued)



Appendix F. (continued)

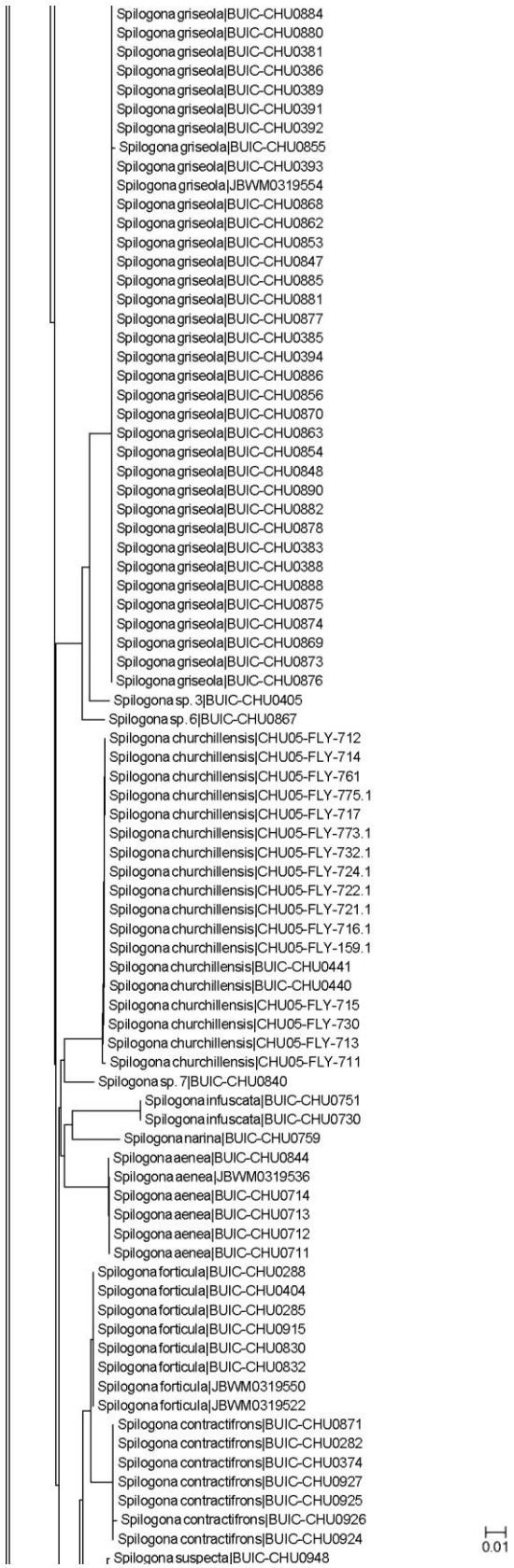


Appendix F. (continued)[2]

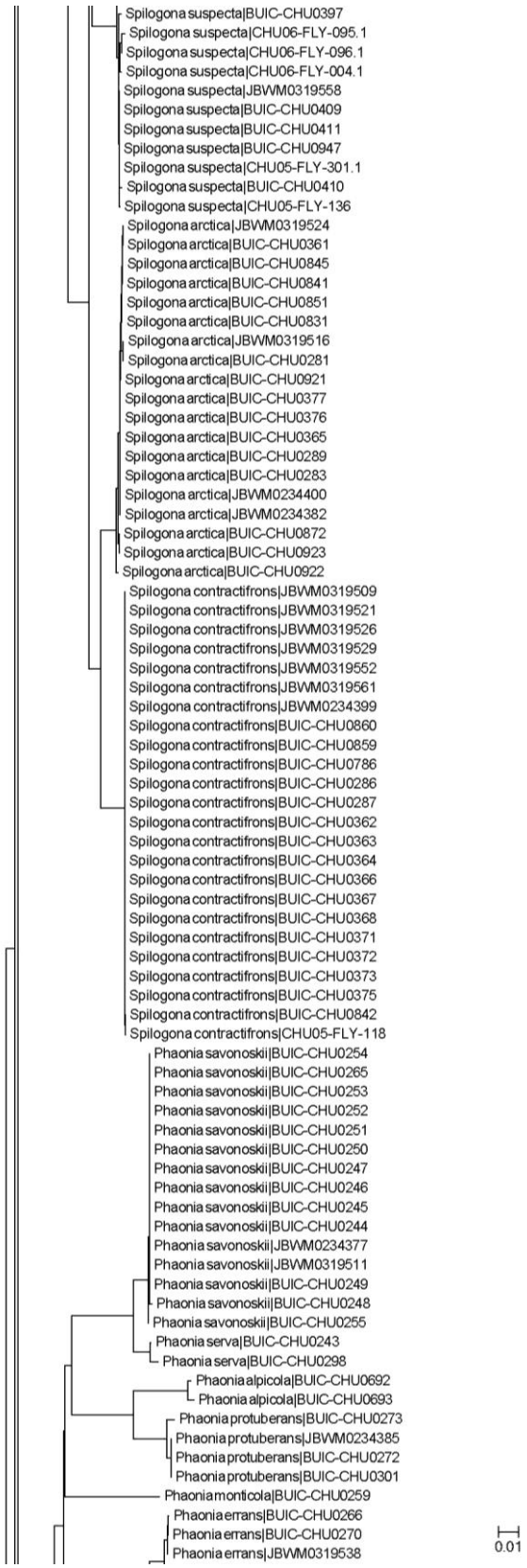


[2] *Spilogona latilamina* (Collin) is a junior synonym of *Spilogona zaitzevi* (Schnabl) (Michelsen 2006).

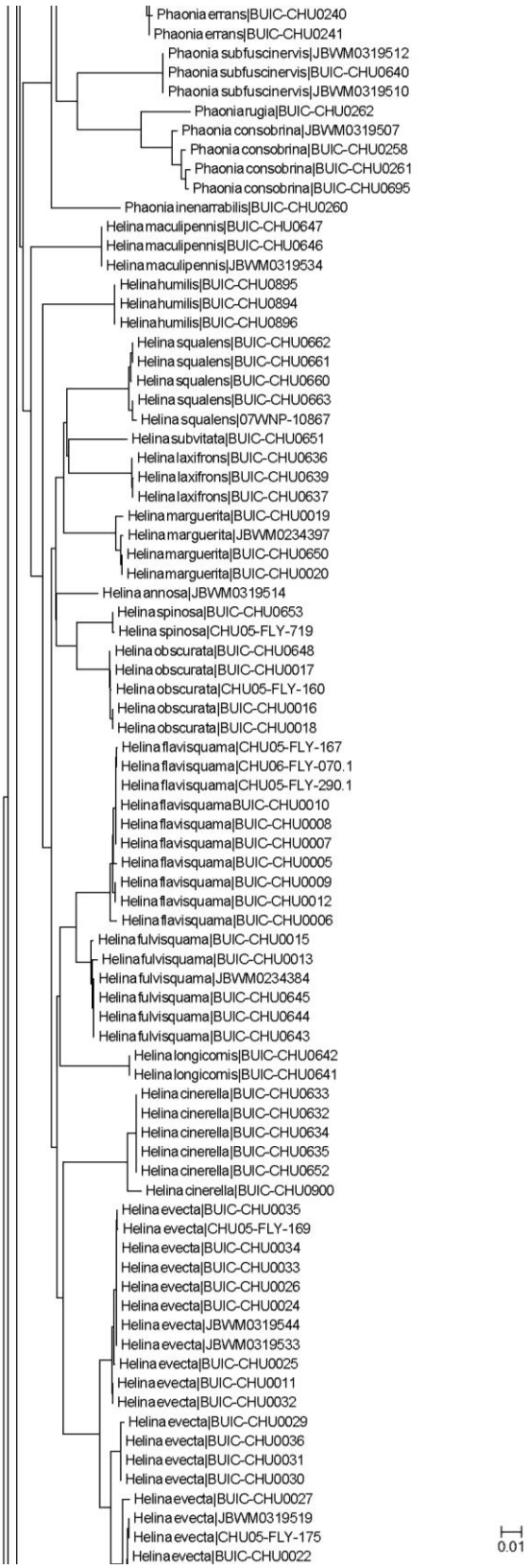
Appendix F. (continued)



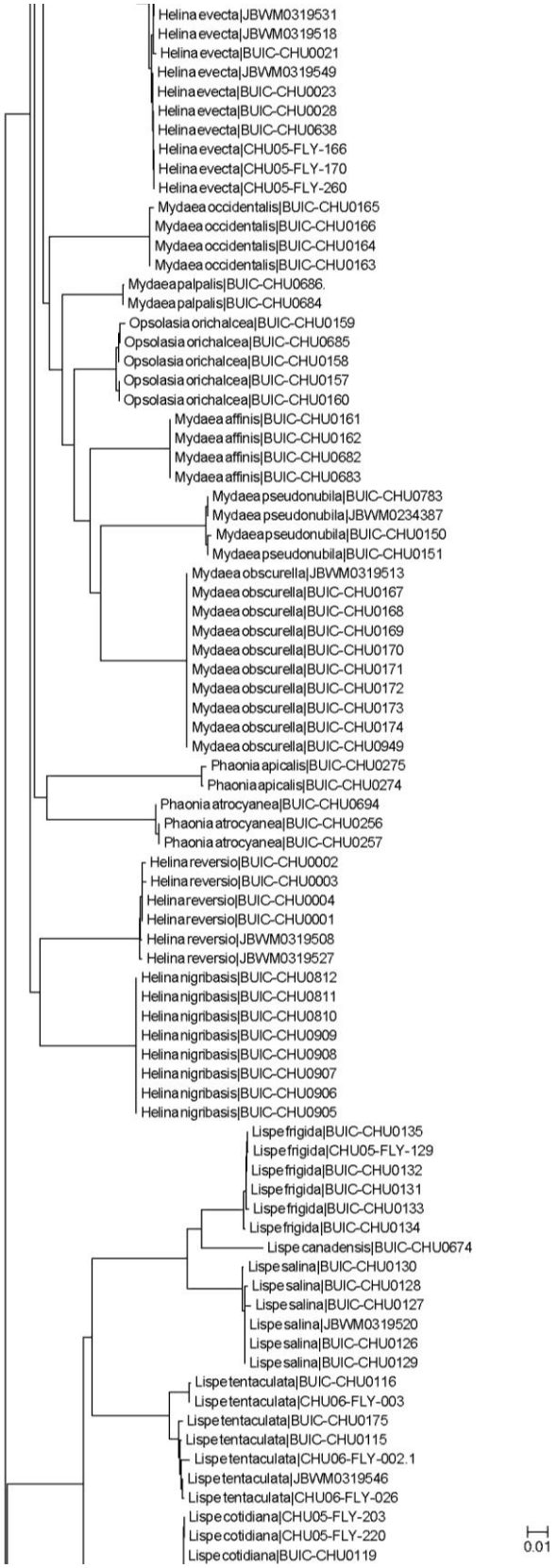
Appendix F. (continued)



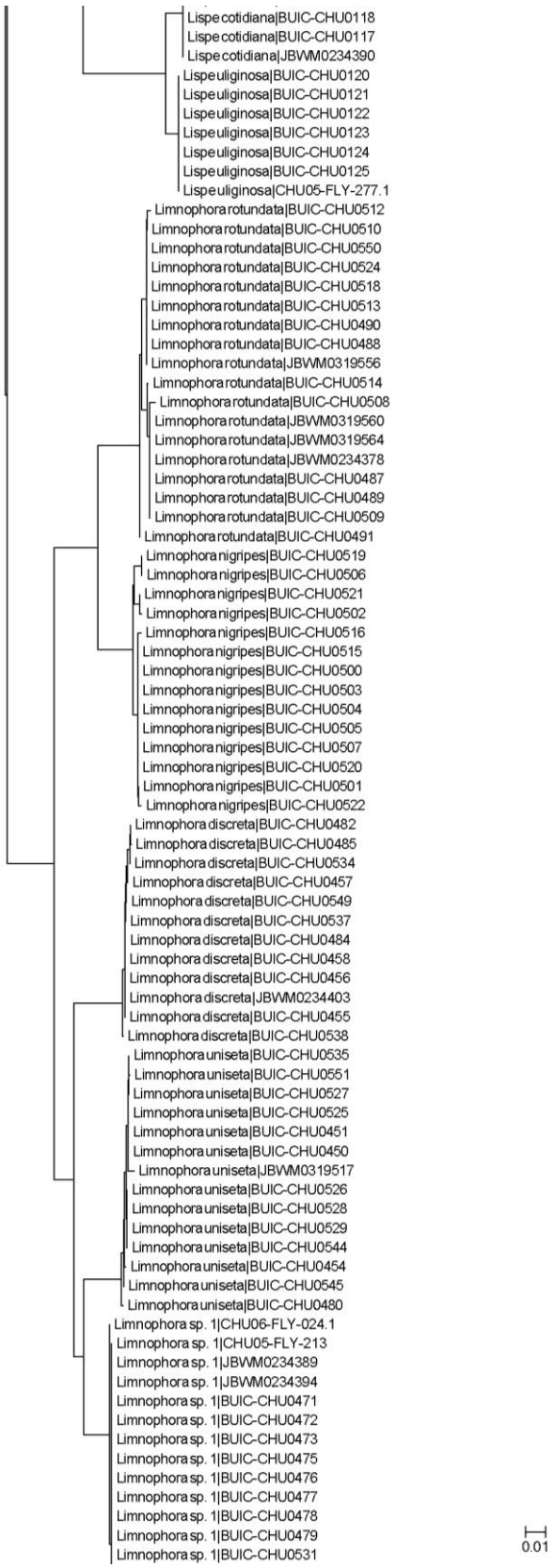
Appendix F. (continued)



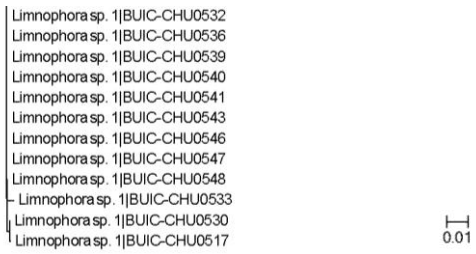
Appendix F. (continued)



Appendix F. (continued)



Appendix F. (concluded)



Appendix G. Average and maximum intraspecific distance of COI and distance to nearest neighbour (NN) for 148 taxa (976 specimens) of Muscidae from Churchill (Manitoba), Gaspésie (Québec), and Sweden following the taxonomic reassessment.

Species	Average intra	Max intra	NN	Distance to NN
Coenosia atritibia Ringdahl	0.1	0.15	*C. nigricoxa*	4.63
Coenosia comita (Huckett)	0.47	1.55	*C. octopunctata*	2.96
Coenosia conforma Huckett	0.23	0.49	*C. octopunctata*	6.69
Coenosia demoralis Huckett	N/A	N/A	*C. minor*	1.47
Coenosia frisoni Malloch	0	----	*C. minor*	7.39
Coenosia longimaculata Stein	0.13	0.48	*C. comita*	6.69
Coenosia minor Huckett	0.07	0.17	*C. demoralis*	1.47
Coenosia mollicula (Fallén)	0.31	0.46	*C. conforma*	7.35
Coenosia nigrescens Stein	0.61	0.92	*C. comita*	9.06
Coenosia nigricoxa Stein	0.13	0.86	*C. atritibia*	4.63
Coenosia octopunctata (Zetterstedt)	0.12	0.49	*C. comita*	2.96
Coenosia pumila (Fallén)	0.05	0.15	*C. comita*	7.54
Coenosia remissa Huckett	N/A	N/A	*C. octopunctata*	6.96
Coenosia sp. 1	N/A	N/A	*C. comita*	7.04
Coenosia tarsata Huckett	N/A	N/A	*C. comita*	5.71
Coenosia triseta Stein	0.07	0.16	*C. minor*	4.93
Coenosia verralli Collin	0.11	0.46	*C. comita*	7.56
Drymeia groenlandica (Lundbeck)	0.15	0.15	*D. pribilofensis*	5.89
Drymeia pribilofensis Malloch	0.04	0.15	*D. groenlandica*	5.89
Drymeia quadrisetosa Malloch	0	----	*D. groenlandica*	5.89
Haematobia alcis (Snow)	0.15	0.15	*P. littoralis*	8.71
Helina annosa (Zetterstedt)	N/A	N/A	*H. fulvisquama*	4.39
Helina cinerella (van der Wulp)	0.37	1.11	*H. fulvisquama*	5.56
Helina evecta (Harris)	1.17	2.36	*H. annosa*	4.56
Helina flavisquama (Zetterstedt)	0.26	0.62	*H. fulvisquama*	2.64
Helina fulvisquama (Zetterstedt)	0.15	0.46	*H. flavisquama*	2.64
Helina humilis (Stein)	0	----	*H. fulvisquama*	6.37
Helina laxifrons (Zetterstedt)	0	----	*H. annosa*	5.88
Helina longicornis (Zetterstedt)	0	----	*H. fulvisquama*	4.89
Helina maculipennis (Zetterstedt)	0	----	*H. fulvisquama*	6.02
Helina marguerita Snyder	0.41	0.77	*H. annosa*	5.38
Helina nigribasis Malloch	0	----	*H. annosa*	9.59
Helina obscurata (Meigen)	0.09	0.15	*H. spinosa*	3.45
Helina reversio (Harris)	0.2	0.31	*S. aenea*	9.7
Helina spinosa (Walker)	0	----	*H. obscurata*	3.45
Helina squalens (Zetterstedt)	0.31	0.61	*H. subvittata*	6.2
Helina subvittata (Seguy)	N/A	N/A	*H. fulvisquama*	5.38
Hydrotaea aenescens (Wiedemann)	0.16	0.16	*T. albibasalis*	9.41
Hydrotaea anxia (Zetterstedt)	0.29	0.77	*H. cristata*	2.96
Hydrotaea cristata Malloch	0.23	0.8	*H. anxia*	2.96
Hydrotaea floccosa (Fallén)	0	----	*H. anxia*	7.35
Hydrotaea pilitibia Stein	0	----	*H. scambus*	1.47

Appendix G. (continued)

Species	Average intra	Max intra	NN	Distance to NN
Hydrotaea ringdalhi Stein	N/A	N/A	*H. floccosa*	8.39
Hydrotaea scambus (Zetterstedt)	N/A	N/A	*H. pilitibia*	1.47
Hydrotaea sp. 1	N/A	N/A	*H. anxia*	3.76
Limnophora discreta Stein	0.03	0.16	*L.* sp. 1	4.07
Limnophora nigripes (R.-D.)	0.37	0.92	*L. rotundata*	4.2
Limnophora rotundata Collin	0.37	0.92	*L. nigripes*	4.2
Limnophora uniseta Stein	0.16	0.47	*L.* sp. 1	3.24
Limnophora sp. 1	0.04	0.46	*L. uniseta*	3.24
Limnospila albifrons Zetterstedt	0.07	0.16	*S. novemaculata*	5.48
Lispe canadensis Snyder	N/A	N/A	*L. frigida*	5.21
Lispe cotidiana Snyder	0	----	*L. uliginosa*	1.54
Lispe frigida Erichson	0.09	0.31	*L. canadensis*	5.21
Lispe salina Aldrich	0.2	0.46	*L. frigida*	5.23
Lispe tentaculata (DeGeer)	0.97	1.76	*L. uliginosa*	9.75
Lispe uliginosa Fallén	0	----	*L. cotidiana*	1.54
Lispocephala alma (Meigen)	1.05	1.86	*L. erythrocera*	6.74
Lispocephala erythrocera (R.-D.)	0	----	*L. alma*	6.74
Lispocephala tinctinervis Malloch	N/A	N/A	*L. alma*	7.59
Lophosceles cinereiventris Zetterstedt	0.1	0.31	*L. impar*	4.57
Lophosceles frenatus Holmgren	0	----	*L. cinereiventris*	9.06
Lophosceles impar (Zetterstedt)	0	----	*L. cinereiventris*	4.57
Morellia podagrica Loew	1.23	1.23	*H. floccosa*	10.09
Musca domestica Linnaeus	0.1	0.15	*H. aenescens*	10.26
Muscina flukei Snyder	N/A	N/A	*M. levida*	4.32
Muscina levida (Harris)	0.03	0.17	*M. flukei*	4.32
Mydaea affinis Meade	0	----	*O. orichalcea*	6.62
Mydaea obscurella Malloch	0	----	*O. orichalcea*	9.11
Mydaea occidentalis Malloch	0.08	0.15	*O. orichalcea*	8.91
Mydaea palpalis Stein	0	----	*O. orichalcea*	6.55
Mydaea pseudonubila Huckett	0.18	0.31	*O. orichalcea*	8.38
Myospila meditabunda Fabricius	0.76	1.7	*L. impar*	8.37
Opsolasia orichalcea (Zetterstedt)	0.25	0.48	*M. palpalis*	6.55
Phaonia alpicola (Zetterstedt)	0.46	0.46	*P. protuberans*	4.58
Phaonia apicalis Stein	0.32	0.32	*H. annosa*	11.35
Phaonia atrocyanea Ringdahl	0.1	0.15	*S. opaca*	10.34
Phaonia consobrina (Zetterstedt)	0.74	1.08	*P. rugia*	4.26
Phaonia errans Meigen	0.36	0.8	*P. luteva*	3.27
Phaonia inenarrabilis Huckett	N/A	N/A	*P. errans*	7.95
Phaonia luteva (Walker)	0.69	1.78	*P. errans*	3.27
Phaonia monticola Malloch	N/A	N/A	*P. inenarrabilis*	8.56
Phaonia protuberans Malloch	0.31	0.61	*P. alpicola*	4.58
Phaonia rugia (Walker)	N/A	N/A	*P. consobrina*	4.26
Phaonia savonoskii Malloch	0.02	0.16	*P. serva*	1.7
Phaonia serva (Meigen)	0.46	0.46	*P. savonoskii*	1.7
Phaonia subfuscinervis (Zetterstedt)	0	----	*P. savonoskii*	9.43

Appendix G. (continued)

Species	Average intra	Max intra	NN	Distance to NN
Potamia littoralis R.-D.	0.1	0.16	*T. diaphanus*	7.02
Pseudocoenosia brevicauda Huckett	0	----	*S. novemaculata*	5.23
Pseudocoenosia fletcheri (Malloch)	0.05	0.15	*S. pacifica*	3.23
Pseudocoenosia solitaria (Zetterstedt)	0.09	0.15	*S. fatima*	5.72
Schoenomyza dorsalis Loew	0.08	0.17	*S. litorella*	3.93
Schoenomyza litorella (Fallén)	0.02	0.16	*S. dorsalis*	3.93
Spilogona aenea Huckett	0	----	*S. forticula*	3.91
Spilogona aerea (Fallén)	0.02	0.46	*S. leucogaster*	5.25
Spilogona arctica (Zetterstedt)	0.1	0.31	*S. contractifrons*	1.92
Spilogona atrisquamula Hennig	0.58	2.5	*S. confluens*	2.01
Spilogona bifimbriata Huckett	0	----	*S. setipes*	2.64
Spilogona churchillensis Huckett	0.02	0.16	*S.* sp. 7	3.38
Spilogona confluens Huckett	0	----	*S. atrisquamula*	2.01
Spilogona contractifrons (Zetterstedt)	0	----	*S. arctica*	1.92
Spilogona deflorata (Holmgren)	0.27	0.66	*S. leucogaster*	5.38
Spilogona fatima Huckett	0	----	*S. novemaculata*	1.7
Spilogona firmidisetosa Huckett	0.07	0.16	*L. albifrons*	7.28
Spilogona forticula Huckett	0	----	*S.* sp. 12	1.23
Spilogona genualis Huckett	0	----	*S. atrisquamula*	4.73
Spilogona griseola Collin	0.02	0.31	*S.* sp. 3	2.08
Spilogona imitatrix (Malloch)	0	----	*S. sororcula*	4.41
Spilogona incerta Huckett	N/A	N/A	*S. zaitzevi*	2.65
Spilogona infuscata Huckett	0	----	*S. confluens*	5.73
Spilogona leucogaster (Zetterstedt)	0	----	*S. pacifica*	5.18
Spilogona malaisei (Ringdahl)	0.15	0.15	*S. confluens*	4.07
Spilogona melanosoma Huckett	0.72	1.08	*S. confluens*	5.71
Spilogona micans (Ringdahl)	0.2	0.31	*S. setipes*	5.71
Spilogona narina (Walker)	N/A	N/A	*S. churchillensis*	4.83
Spilogona novemaculata (Zetterstedt)	0	----	*S. fatima*	1.7
Spilogona obscuripennis (Stein)	0	----	*S. confluens*	4.55
Spilogona opaca Schnabl	0.82	1.39	*S.* sp. 9	3.29
Spilogona pacifica (Meigen)	0.11	0.33	*S. sororcula*	2.9
Spilogona pusilla Huckett	0	----	*S. atrisquamula*	3.92
Spilogona reflecta Huckett	0.1	0.15	*S. tornensis*	2.17
Spilogona setinervis Huckett	0.1	0.15	*S. zaitzevi*	2.17
Spilogona setipes Huckett	N/A	N/A	*S. bifimbriata*	2.64
Spilogona sororcula (Zetterstedt)	0.27	0.46	*S. pacifica*	2.9
Spilogona sp. 2	N/A	N/A	*S. churchillensis*	4.66
Spilogona sp. 3	N/A	N/A	*S.* sp. 6	2.02
Spilogona sp. 4	N/A	N/A	*S. atrisquamula*	4.91
Spilogona sp. 5	N/A	N/A	*S. novemaculata*	5.38
Spilogona sp. 6	N/A	N/A	*S.* sp. 3	2.02

Appendix G. (concluded)

Species	Average intra	Max intra	NN	Distance to NN
Spilogona sp. 7	N/A	N/A	*S. churchillensis*	3.38
Spilogona sp. 8	N/A	N/A	*S. confluens*	4.36
Spilogona sp. 9	0	----	*S. opaca*	3.29
Spilogona sp. 10	0.51	0.77	*S. confluens*	4.41
Spilogona sp. 11	N/A	N/A	*S. confluens*	3.75
Spilogona sp. 12	0.04	0.15	*S. forticula*	1.23
Spilogona surda (Zetterstedt)	0.04	0.16	*S. zaitzevi*	4.88
Spilogona suspecta (Malloch)	0.17	0.46	*S. forticula*	2.01
Spilogona tornensis (Ringdahl)	N/A	N/A	*S. reflecta*	2.17
Spilogona trigonata (Zetterstedt)	0.03	0.16	*S. opaca*	4.42
Spilogona trigonifera (Zetterstedt)	0.31	0.31	*S. pusilla*	5.07
Spilogona trilineata Huckett	0	----	*S. sororcula*	4.88
Spilogona zaitzevi (Schnabl)	0	----	*S. setinervis*	2.17
Stomoxys calcitrans Linnaeus	0.15	0.15	*H. alcis*	10.96
Thricops albibasalis (Zetterstedt)	0.28	0.77	*T. diaphanus*	3.25
Thricops diaphanus (Wiedemann)	0	----	*T. albibasalis*	3.25
Thricops hirtulus (Zetterstedt)	0.03	0.15	*T. innocuus*	1.7
Thricops innocuus (Zetterstedt)	0.19	0.61	*T. hirtulus*	1.7
Thricops septentrionalis (Stein)/*T. spiniger* (Stein)	0.53	1.88	*T. innocuus*	1.86

N/A: taxa represented by only one specimen where intraspecific distance could not be calculated.