Building a Collaborative DNA Barcode Library for the Diptera of Churchill, Canada: A Resource for Northern Research

DNA Barcode Library for Subarctic Diptera

Majoros, S.E.*[1], Wang, J.[1,2], Levesque-Beaudin, V.[3], Borkent, C.J.[4], Brodo, F.[5], Brooks, S.E.[6], Boucher, S.[7], Cumming, J.M.[6], Currie, D.C.[8,9], Ekrem, T.[10], Elliott, T.A.[1], Hebert, P.D.N.[3], Savage, J.[11], Schaefer, P.[8,9], Sinclair, B.J.[12], Skevington, J.H.[13], Solecki, A.M.[1,7], Stur, E.[10,] Wheeler, T.A.[7+], Woodcock, T.S.[1], Young, A. D.[1], Young, C.[14] & Adamowicz, S.J.[1]

*Author for correspondence: smajoros@uoguelph.ca

[1]University of Guelph, Guelph, Ontario, Canada

[2]China Agricultural University, Beijing, China

[3]Centre for Biodiversity Genomics, University of Guelph, Guelph, Ontario, Canada

[4]Biological Control Program and California State Collection of Arthropods, California Department of Food and Agriculture, Sacramento, California, USA

[5]Canadian Museum of Nature, Ottawa, Ontario, Canada

[6]Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

[7]McGill University, Ste-Anne-de-Bellevue, Quebec, Canada

[8]Royal Ontario Museum, Toronto, Ontario, Canada

[9]University of Toronto, Ontario, Canada

[10]Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology, Trondheim, Norway

[11]Bishop University, Sherbrooke, Quebec, Canada

[12]Canadian Food Inspection Agency, Ottawa, Ontario, Canada

[13]Carleton University, Ottawa, Ontario, Canada

[14]Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA

†Deceased author

Abstract

Churchill, Manitoba, Canada is a diverse Subarctic region that has been the focus of DNA barcoding efforts and research for decades. Despite this effort, there are still taxa in this region that are underrepresented in public databases, particularly hyperdiverse insect groups. We present a collaborative large-scale DNA barcode reference library for Diptera based on molecular data and expert taxonomic identification. The reference library contains 16786 specimens sampled around Churchill from 2005 to 2011. Specimens were identified by morphological means, sequenced for the animal barcode marker, and sorted into 2235 Molecular Operational Taxonomic Units (using Barcode Index Numbers: BINs) representing 68 families and 1212 species. Eighty-six species shared a BIN with another species, 98 species were split across several BINs, and 1027 species were assigned to a unique BIN. The intraspecific and nearest neighbour distances varied across families, but most species can be easily distinguished from their nearest neighbours. By combining molecular data with taxonomic expertise, the barcode library provides species-level information for 12612 specimens, supplying future researchers with detailed taxonomic information and the opportunity to perform barcode-based specimen identifications for large-scale studies through sequence matching. Comparison of the Diptera composition of Churchill to other geographic regions reflected likely postglacial colonization northwards as well as a Beringian component. This study provides a publicly available, detailed reference library for use in future research, as well as new insight into the current diversity of Diptera present among the diverse habitats in this Subarctic region.

Key Words: Subarctic, Cytochrome c oxidase subunit I (COI), Boreal habitats, Biodiversity

Introduction

DNA barcoding, the process in which standardized gene regions are used to identify, discover, and delineate species, is a useful tool that can allow us to document further the diversity of different regions and ecosystems (Hebert et al., 2003). These barcodes also enable further analysis and research, allowing us to study biodiversity and temporal trends in community composition.

There has been a concentrated effort to barcode species in various areas globally, including Costa Rica (Janzen & Hallwachs, 2011, Janzen et al., 2009), Finland (Pohjoismäki et al., 2016, Roslin et al., 2021), Greenland (Wirta et al., 2016), and Nunavut (deWaard, 2023, Pentinsaari et al., 2020). Regional barcode studies and libraries provide a valuable resource and allow for the diversity of regions to be elucidated, as well as provide the opportunity for further research. Wirta et al. (2016), for example, provided a barcode library including over 403 terrestrial animal species and 160 vascular plant species for the Zackenberg Valley in Northeast Greenland. The valuable library created by Wirta et al. (2016) has been used for a variety of novel studies, including to determine the prey of spiders and investigate niche overlap (Wirta et al., 2015b), to elucidate Arctic food webs and interactions between predators and prey (Wirta et al., 2015a), and to investigate the role of flies in the pollination of Arctic plant species (Tiusanen et al., 2016). By continuing to barcode specimens and build these regional libraries, we can further expand our knowledge of the communities and ecological processes within these regions. Across geographic regions, DNA barcoding has been shown to be a helpful and successful approach for Diptera, and it is a useful tool for identifying and delineating species within this group (Adeniran et al., 2019, Banerjee et al., 2015, Brodin et al., 2012, Changbunjong et al., 2018, Cywinska et al., 2010, de Souza Pinto, 2023, Machado et al., 2017, Posada- López et al., 2023, Rivera & Currie, 2009, Votýpka et al., 2019).

Since the 1800s, the Northern regions of Canada have been the focus of various biological surveys and sampling events. One notable sampling event that spanned several insect orders was the Canadian Northern Insect Survey, conducted from 1947 – 1962 (Danks, 1981, Freeman, 1958, Huckett et al., 1965a, McAlpine, 1965). In 2010-2011, the Northern Biodiversity Program resampled the sites of the Northern Insect Survey with the goal of comparing their more recent survey to the historical one (Northern Biodiversity Program, n.d.).

A taxon that has been of special research interest, both in historical and recent studies, is Diptera (true flies), particularly biting flies and those that act as vectors of disease. Diptera is a diverse and ecologically important group that contributes to ecosystem functioning, and it has a major impact on agriculture and public health (Adler & Courtney, 2019, Bedini et al., 2017, Bragard et al., 2020, Mesmin et al., 2019, Molinatto et al., 2020, Olafson et al., 2021, Shamshad, 2010, Wearing et al., 2013). Flies act as useful bioindicators (Czechowski et al., 2020, Hamerlík & Bitušík, 2009, Medeiros et al., 2020, Rossaro et al., 2022) and, in the Arctic particularly, act as an important pollinator group (Adler & Courtney, 2019, Cirtwill et al., 2022, Tiusanen et al., 2016). Flies are also vectors for several important, widespread diseases, including Malaria, Zika virus, Dengue, Chikungunya virus, and West Nile virus (Bonizzoni et al., 2013, World Health Organization, 2018, World Health Organization, 2020). There have been Zika virus outbreaks in over 30 countries, and *Aedes aegypti* (Linnaeus), the principal vector of this virus, as well as Dengue, Chikungunya, and yellow fever, is a highly adaptive and widespread species (World Health Organization, 2018). Similarly, *Aedes albopictus* (Skuse), a vector of Dengue and Chikungunya, is an extremely invasive species affecting an increasing proportion of the world (Bonizzoni et al., 2013). Developing a capacity for monitoring potential vectors of disease is important, as species may shift their geographic distributions in response to climate change. Non-biting fly species can also be a public health concern, transmitting microorganisms through their mouthparts, feces, and body

surface, and acting as vectors of parasites, disease, and food-borne pathogens (Förster et al., 2007, Graczyk et al., 2001, Pava-Ripoll et al., 2012).

Observations on Diptera diversity have been recorded in northern North America since the 1800s. Huckett et al. (1965a) provide a study of Muscidae (Diptera) specimens from Arctic and Subarctic regions of North America, Greenland, and Europe, and described the historical collection events and expeditions that have occurred in these regions. Various historical collection events, occurring from 1835 – 1954, recorded Muscidae flies in Northern regions such as the Hudson Bay Territory, Alaska, the Bering Sea Region, Northwest Territories, Ellesmere Island, Yukon territory and the Labrador coast (Aldrich, 1918, Coquillett, 1900, Huckett et al., 1965a, James and Huckett, 1952, Johnson, 1929, Malloch 1919, 1920a, 1921, 1923, Stein, 1898, 1920, Walker, 1849, Weber, 1950, 1954).

Another region that has been the focus of past sampling is Churchill, Manitoba, Canada. Churchill is a diverse Subarctic region containing a wide range of wildlife and plant species. Churchill is in the Coastal Hudson Bay Lowland ecoregion (Smith et al., 1998) (Fig 1a), an area characterized by short, cool summers; long, very cold winters; and widespread permafrost (Smith et al., 1998). This region contains a wide range of habitat types, including peat bogs, fens, tundra, wetlands, and boreal forests, and is bordered by Hudson Bay where sandy beaches and rocky outcrops can be found (Smith et al., 1998). The Churchill River is also an important water feature, in addition to several small, unconnected lakes throughout the region (Smith et al., 1998). The diversity of habitats within this region makes it a place of interest for investigating the diversity of Subarctic species and for research into Northern ecology and climate change. The establishment of the Churchill Northern Studies Centre in 1976 facilitated the usage of the Churchill area as a hub for Northern research (Churchill Northern Studies Centre, 2022). There have been several historical studies and surveys that included Churchill fly species, such as the Biting Fly Survey and Experimental Control Program and the Canadian Northern Insect

Survey (Hocking et al., 1950, Miller, 1951, Twinn et al., 1948, Danks, 1981, Freeman, 1958, Huckett et al., 1965a).

Despite this past sampling, work is still needed to fully understand the diversity of species present in Churchill and other Northern regions. By continuing sampling efforts, we can develop a solid baseline which can be used by future researchers. Historical sampling events and present-day efforts provide information on Diptera diversity across Northern regions and the opportunity to see how diversity changes over time. Renaud et al. (2012a) compared the historical surveys conducted during the Canadian Northern Insect Survey (1947 – 1962) to a more recent Diptera survey conducted in 2007. The sampling methods differed between these time periods, as historical samples were primarily collected using a sweep net, but the 2007 survey was done using Malaise traps and pan traps, in addition to sweep nets (Renaud et al., 2012a). This survey compared 10294 specimens representing 155 species of Muscidae from the 2007 survey to 2881 historical specimens representing 105 species (Renaud et al., 2012a). Eighty-seven percent of the species found in the historical records were rediscovered in the 2007 survey, and *Spilogona* Schnabl remained the most diverse genus in both time periods, with 38 species as of 1965 and 51 species as of 2007 (Renaud et al., 2012a). With consistent sampling over time, we can document how diversity changes and continue to add to our knowledge and number of known species.

Renaud et al. (2012b) added to our knowledge of Diptera diversity in Churchill by conducting a large-scale barcoding study of Muscidae that included 1114 individuals and 160 species. In addition, Churchill has been the focus of many barcoding studies for arthropod taxa such as Coleoptera (Woodcock et al. 2013), Ephemeroptera, Plecoptera, Trichoptera (Zhou et al. 2009, 2010), Hymenoptera (Fernandez-Triana et al., 2011, Stahlhut et al. 2013), Collembola (Porco et al. 2013), Araneae (Blagoev et al. 2013), Ostracoda (Laforest et al. 2013), Crustacea (Jeffery et al. 2011), and Acari (Young et al. 2012).

To add to this barcoding effort, we conducted a large-scale barcoding study of Diptera species in Churchill. Despite previous surveys, there are still gaps in our understanding of Diptera diversity, and there are many families which have not yet been the focus of dedicated barcoding efforts. Srivathsan et al. (2023) found that 20 insect families, 10 of which were from Diptera, made up 50% of the local diversity of flying insects at their sites worldwide. They also found that some of the most abundant taxa were still very poorly known (Srivathsan et al., 2023). In this paper, we present a large-scale barcoding study of Diptera including 16786 specimens and 2235 Barcode Index Numbers (BINs; Ratnasingham and Hebert, 2013) representing 68 families and 1212 species. Through a collaborative approach, we have conducted extensive field collections and created a taxonomically linked reference library that includes molecular data and expert taxonomic identifications. This approach is necessary due to the complex patterns of DNA sequence divergence within Diptera. For example, Lin et al. (2015) determined that a divergence threshold of 4–5% was suitable to delineate species within Chironomidae, reflecting the high genetic diversity within species of the family. However, Renaud et al. (2012b) found that a divergence threshold of 1.2% was more suitable for Muscidae, to optimize between cases of taxonomic lumping vs. over-splitting. Due to these complex and differing patterns of divergence and genetic diversity within Diptera, it is valuable to construct an extensive DNA barcode library so that new sequences can be linked with named species and their associated information. In this study we provide a taxonomically linked reference library, outline the diversity of Diptera currently present in Churchill across diverse habitat types, and contribute barcodes for thousands of individuals to the Barcode of Life Data (BOLD) Systems (https://boldsystems.org/; Ratnasingham & Hebert, 2007).

Materials and Methods

Study Site

Samples were collected from sites around Churchill, Manitoba (Latitude: 58.7, Longitude: -94) (Fig.1b) from 2005 to 2011. Sampling intensity varied across years, with the most sampling occurring in 2010 and the least occurring in 2011 (supplementary file 1). Sampling was conducted across several areas within the Churchill region, and areas closer to the town of Churchill and the Churchill Northern Studies Centre were sampled more frequently. Two hundred and sixty-seven sites were sampled, based on unique pairs of latitude and longitude coordinates. Sites were chosen to represent a wide variety of habitat types in the Churchill area, and accessibility of the sites was also considered.

Sampling Methods

Specimens were collected using various methods, including baited traps, Berlese traps, dip nets, fan traps, hand collection, intercept traps, light traps, Malaise traps, pan traps, pitfall traps, sweep nets and UV light traps. The sampling method used can result in a bias as to what types of species are collected. By using different trap types and sampling methods, a wider variety of species present in the community should be detected. Most specimens were caught using Malaise traps (Fig. 2). There were two main approaches used for sampling. The first is general collection, using a wide variety of methods and conducted by a variety of investigators, towards the reference library. The collector and other metadata are available through BOLD. The second is sampling conducted by Malaise traps by a single investigator (J.J. Wang) to obtain detailed data from five of the sites. These sites were repeatedly sampled throughout a single summer (2010). This subset of the data could be queried for a temporal analysis over a summer season by future researchers.

Barcoding Methods

DNA barcoding was conducted at the Canadian Centre for DNA Barcoding (https://ccdb.ca/) located at the University of Guelph. Three individuals per morphospecies per collecting event were generally selected for molecular analysis. DNA was extracted from each adult specimen using a single leg, and from each larvae using a section of the specimen. For some small-bodied specimens, the protocol from Porco et al. (2010) was used to extract DNA from the whole specimen. The protocol from Ivanova et al. (2006) was used for DNA extraction, and the final elution volume was 50 µl. The primers used for the amplification of the DNA barcode region of COI are included in supplementary file 2. The PCR reaction volume was 12.5 µl, and included 1.25 µl of 10x reaction buffer, 1.25 pmol of primer, 0.3U of Platinum Taq DNA polymerase, 5% trehalose, 2.5 mM of $MgCl_2$, 50 um of dNTP, and 2 µl of genomic DNA. The E-gel 96 System (Invitrogen, Carlsbad, Ca, USA) was used to visualize the PCR products on 2% agarose gels. The PCR products were bidirectionally sequenced using the protocols from Hajibabaei et al. (2005) and BigDye v3.1 on an ABI 3730xl DNA Analyzer. Using CodonCode Aligner v.3.0.2 (CodonCode Corporation), the forward and reverse sequences were used to assemble consensus sequences for each individual.

Specimen Identification

Specimens were identified using morphological characteristics to the family level (McAlpine, 1981). After sequencing, 1-5 specimens from each BIN were identified to species level for 19 of the families based on the specimen's morphology. BINs are clusters of barcode sequences similar to species and are the operational taxonomic units (OTUs) used by BOLD (Ratnasingham and Hebert, 2013). The goal was to identify a minimum of three specimens per BIN and per species, but some BINs were singletons or doubletons. Of the 16786 records, the taxonomy of 9159 records were assigned using morphological identification. The remaining 7303 records were identified using barcode matching to

BINs on the BOLD database. The reference materials used to identify the specimens are included in table 1.

Specimen and Data Storage

The specimens are stored in 95% ethanol, pinned or slide mounted material in various institutions including the Centre for Biodiversity Genomics (13222 specimens), the Wallis Roughley Museum of Entomology at the University of Manitoba (92 specimens), the Carnegie Museum of Natural History (251 specimens), the Insect Collection at Bishop's University (868 specimens), the Canadian National Collection of Insects, Arachnids and Nematodes (1312 specimens), and the Department of Natural History at NTNU University Museum (1041 specimens).

The specimens were each assigned a unique specimen ID and photographed before being uploaded to BOLD. The data are available in the dataset DS-CDIPTERA (dx.doi.org/10.5883/DS-CDIPTERA) (Majoros et al., 2023) and are also available on GenBank. A portion of the data has been previously published in association with Renaud et al. (2012b). A list of process IDs and GenBank accessions included in the dataset is available in supplementary file 3.

Quality Checking, Analyses and Summary Statistics

A phenogram was created using the neighbour-joining method (Saitou & Nei, 1987) for species in the dataset using the Taxon ID Tree tool available on BOLD version 4.0 (Ratnasingham & Hebert, 2007). For the phenogram, Kimura 2 parameter was used as the model for calculating distances between DNA sequences, and pairwise deletion was used for ambiguous base/gap handling. The sequences were aligned using the BOLD aligner (amino acid based hidden Markov model (HMM) (Eddy, 1998)). Known contaminants, records with stop codons, and flagged sequences were excluded, and the phenogram was coloured by family. This phenogram was used to look for any misplaced sequences at the family level, and any long branches not matching the typical patterns of genetic diversity in the

families. It is not equivalent or intended as a phylogeny. The mean and maximum intraspecific distances for each species and nearest neighbour distances (the shortest distance between a sequence in one species to a sequence in another species) were calculated using the Barcode Gap Analysis tool on BOLD. For these distance measures per species, pairwise distance (p-distance) was used as the sequence distance measure for comparative purposes, as this method is used by the BIN algorithm (Ratnasingham & Hebert, 2013). Discordant BINs (BINs where more than one taxonomic name was provided for the included records, at any taxonomic rank) were found using the BIN Discordance tool on BOLD, using records with a sequence length over 100 base pairs and excluding known contaminants, records with stop codons, and flagged records.

Other analyses and all summary statistics were completed in the R programming language version 4.3.0 (R Core Team, 2023). Various plots were created using the package ggplot2 version 3.4.2 (Wickham, 2016), including bar plots and scatterplots, to summarize and investigate different aspects of the dataset. Using the categories outlined in Ratnasingham and Hebert (2013), we determined the number of matches (species assigned to a unique BIN), splits (a single species split across several BINs), and merges (species that share a BIN with another species) in the dataset. A linear regression was performed to determine if there is a relationship between the number of specimens sequenced of each species and the number of BINs associated with that species. Only species with two or more specimens were included in the regression. Species and BIN accumulation curves were created using the iNEXT function, and Chao1 estimators (Chao, 1984) were calculated using the ChaoRichness function from the iNEXT package version 3.0.0 (Chao et al., 2014). As only 1-5 specimens per morphospecies were selected during sampling, there is a bias in analyses that rely on abundance data. To account for this, both abundance and incidence-based richness were calculated. For the iNEXT function, datatype was set to “abundance” and nboot was set to 1000. For the ChaoRichness function, datatype was set to

“abundance” to determine abundance-based richness, and to “incidence_freq” to determine incidence-based richness.

To compare the intraspecific distances across families and species, a Maximum Likelihood (ML) tree was created using the 50 species with the most records. The ML tree used in this study is not intended to be equivalent to a phylogeny but to provide a visualization tool showing the intraspecific distances across families. The sequences were filtered to remove those with indels and stop codons using the R package Coil version 1.2.3 (Nugent et al., 2020). To ensure a consistent length among the sequences, only those with 669 base pairs were included as this was the most common sequence length in the dataset. A centroid sequence (the sequence with the minimum average distance to others in the species) was chosen for each species. These sequences were aligned using DECIPHER version 2.28.0, and followed its recommended steps (Wright, 2016). The AlignSeqs function uses the profile-to-profile method to align the sequences following a guide tree. The profile-to-profile method merges profiles along a guide tree until all the sequences in the set are aligned. As no guide tree was provided and the default parameters were used, the sequences were aligned first using a single-linkage guide tree based on a distance matrix of shared k-mers. The initial alignment was then re-aligned using an UPGMA guide tree. Finally, the alignment subsets were re-aligned to the rest of the alignment. Two alignments were created by this method, and the best was selected by the function using the sum-of-pairs score. The maximum likelihood tree was created using phangorn version 2.11.1 (Schliep, 2011). Models were tested using the ModelTest function from phangorn and the best model of molecular evolution for the data was determined to be TPM2u+G(4)+I, as this model had the lowest score for both the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC). The intraspecific distances were mapped to the ML tree using the species names and the function full_join from the package dplyr version 1.1.2 (Wickham et al. 2023). Upon constructing the tree and observing long branch lengths, several steps were taken to further investigate the sequences. The quality of the alignment created

using DECIPHER was checked by viewing it in MEGA-X: Molecular Evolutionary Genetics Analysis version 10.2.0 (Tamura et al., 2021). The identities of the sequences were confirmed using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). We compared these sequences to others of the same species available on BOLD and constructed trees using other sequences to check for long branches lengths. We also constructed trees based on the first, second and third codon positions, as well as an amino acid tree, in MEGA-X.

The dataset was compared to data from other Northern and Southern regions to determine how many BINs were shared. Data from Canadian provinces and territories, as well as Alaska, Greenland, Finland, and Norway, were pulled directly into the R environment using BOLD's application programming interface (API). Data was downloaded on Oct 2nd and 3rd, 2023. The number of shared BINs between each geographic region and Churchill was determined. The alpha diversity was calculated for each region using the Shannon Index and the Simpson Index, and the beta diversity between the regions and Churchill was calculated using the Bray-Curtis Index. Alpha diversity was obtained using the function diversity from the R package vegan version 2.6.4. (Oksanen et al., 2022) and beta diversity was obtained using the function vegdist from the same package. A list of BINs shared between Churchill and each region is available in supplementary file 4.

Land-use classification, visualization, and taxonomic breakdown by land-use class

We determined the family-level taxonomic composition across the varied landscape of Churchill based on the most common sequences encountered in different land-use and land-type categories, such as wetlands and coniferous forest. Land-use classification for the area surrounding Churchill was determined by extracting the Earth Observation for Sustainable Development (EOSD) categories for each unique set of GPS coordinates from the LUC_EOSD_Northeast dataset from the provincial government of Manitoba Conservation and Water Stewardship website (Manitoba Land Initiative,

accessed June 16th 2023). Briefly, the "GCS_North_American_1983" Geographic Coordinate System was selected to ensure accurate spatial reference. Next, the unique GPS coordinates were inputted into ArcGIS, and the "Longitude" and "Latitude" attributes were defined (Environmental Systems Research Institute, 2015). The "Spatial Analyst" toolbar was then accessed, and the "Extract Values to Points" tool was selected from the "Extraction" toolset. The shapefile served as the input point layer, while the raster layer was chosen as the input raster. To store the extracted land-classification values, specific output fields were specified and extracted. Classification data were then joined to the rest of the metadata for the unique sites using tidyverse v. 1.3.2 (Wickham et al., 2019). Definitions for various land-use categories can be found in supplementary file 5. The map itself (see Figure 1) was generated in mapview v. 2.11 in R (Appelhans et al., 2023). For the visualization, specimens lacking GPS coordinates were filtered out, along with seven sites that had <3 specimens and were geographically distant from the primary cluster of sampling sites closest to Churchill itself.

The specimen count by family and for each unique sampling site was determined by grouping data in R using the tidyverse v. 1.3.2 (Wickham et al., 2019) and janitor v. 2.2 (Firke, 2023) suite of data manipulation and data cleaning packages. A table containing the specimen count/site along with their respective EOSD classification is included in supplementary file 6. Pie charts for visualization were also created in R using ggplot2 v. 3.4.1 (Wickham, 2016) and viridis v. 0.6.3 (Garnier et al., 2023). To illustrate the phylogenetic relationships between different families, taxa within the same infraorder or superfamily (as determined by the phylogeny in Wiegmann et al (2011)), were assigned different shades of a similar colour using hex codes in R.

Results

Dataset Characteristics

We created a reference library containing 16786 specimens and 2235 BINs representing 68 families and 1212 species. Not all BINs were identified to the species level, and some species were split across multiple BINs. A phenogram showing the relationship between the taxa collected in this study shows that COI sequences within families generally group together for the Churchill area (supplementary file 7). However, the families of Tipuloidea were separated on the phenogram, as well as species within the family Limoniidae, despite being closely related. As this is not a phylogeny and Churchill is a small Northern region with lower species richness, these results are not unusual. Muscidae was the most abundant family in regard to specimen number, with 2478 specimens collected, while Chironomidae had the highest species richness, with 208 species recorded (Fig. 3). A total of 12612 specimens have been identified to species level. Species such as *Aedes nigripes* (Zetterstedt), *Aedes communis* (De Geer), *Aedes excrucians* (Walker), *Aedes campestris* Dyar & Knab, *Aedes flavescens* (Muller), *Aedes cinereus* (Meigen), *Chrysops nigripes* Zetterstedt, *Chrysops mitis* Osten Sacken, *Hybomitra affinis* (Kirby), *Hybomitra zonalis* (Kirby), and *Hybomitra hearlei* (Philip) have been previously sampled in historical studies (Hocking et al. 1950, Miller 1951, Twinn et al. 1948) and have been sampled again in our 2005-2011 survey.

Using the categories outlined in Ratnasingham and Hebert (2013), 1027 matches (species assigned to a unique BIN), 98 splits (a single species split across several BINs), and 86 merges (species that share a BIN with another species) were recorded. Out of the matches, 736 matches were for species represented by more than one sequence. Most species were assigned to a unique BIN. There was a significant relationship between the number of specimens sequenced of each species and the number of BINs associated with that species (p-value = 3.59×10^{-6} $R^2 = 0.02398$), and species with more

specimens appear to be more likely to be split across BINs (Fig. 4). This relationship persists even when excluding the smaller sample of species with a higher specimen count. A total of 948 BINs did not have a species-level identification. Of these, 491 have a genus level identification, and all have a family level identification. The majority of BINs are not unique to this study, but contain sequences from BINs already on BOLD that were found in previous works and other geographic regions (Fig. 5). Our study contains 142 unique BINs representing 33 species across 25 families. The families Cecidomyiidae and Chironomidae had the most unique BINs, with 38 unique BINs and 29 unique BINs, respectively. Forty discordant BINs were found, most of which appear to contain two species within the same genus.

Completeness of Sampling

A species accumulation curve and a BIN accumulation curve were created, and Chao1 estimators were calculated (Fig.6). Both curves appear to be approaching an asymptote. For species, 79.63% of the expected value was observed, and for BINs, 67.02% of the expected value was observed. These results were consistent for both abundance-based and incidence-based richness. As mentioned in the methods, only 1 -5 specimens per morphospecies were selected, so the species accumulation curve is overly conservative due to the sampling bias. These results indicate that the region is reasonably well sampled, though further work is still required to capture the full species richness.

Patterns of Sequence Divergence

The distance to nearest neighbour, and maximum and mean intraspecific distances were investigated for each family. The species within Coelopidae had the largest nearest neighbour distances, while Calliphoridae had the lowest (Fig. 7). For the mean intraspecific distances, the value for Canacidae was the highest, while Ulidiidae, Lonchopteridae, Chamaemyiidae, and Bolitophilidae all had mean intraspecific distances of zero. There is little overlap between the nearest neighbour distances and mean intraspecific distances, with most mean intraspecific distances being below 5%, and most nearest

neighbour distances being above 5%. The max intraspecific distances ranged from 0–16.06% and the nearest neighbour distances ranged from 0–16.23%. Most species appear to be easily distinguished from their nearest neighbours, with 1149 out of 1212 species having a greater interspecific distance than intraspecific (Fig. 8). In figure 9, we present maximum likelihood trees showing the maximum and mean intraspecific distances for the 50 best-sampled species. Genetic distances vary across the tree. The species with higher maximum intraspecific distance (highlighted green on the figure) are candidates for examination for potential cryptic species. The full results of the barcode gap analysis are available in supplementary file 8.

Comparing BIN Composition Across Geographic Regions

The BINs included in this study were also shared across several other northern and southern regions (Fig. 10). When looking at total counts, most BINs were shared with Alberta and the Northwest Territories, with 1258 BINs and 1046 BINs being shared, respectively. For Alpha diversity, Norway was the most diverse when using the Shannon Index and Simpson Index. For beta diversity, Churchill was most similar to the Northwest Territories, followed by Saskatchewan.

Taxonomic Composition by Habitat

The densest sampling was in wetlands, with a total of 6785 specimens and 62 families represented with at least one specimen. This was followed by water sites (n=5165), exposed/barren areas (n=3917) which were composed primarily of human adjacent/impacted areas and rock pool bluffs overlooking Hudson Bay, and coniferous (n=714) sites. Muscidae specimens were in the top 10 most sampled families by specimen number of all land-use areas, and the most abundant in two of the areas (wetlands, water), with Chironomidae being the second most abundant in water, wetland, and the most abundant in exposed/barren and bryoid sites (Figure 11). This trend continued with Dolichopodidae,

Syrphidae, Chloropidae, and Anthomyiidae found in four of the five areas. Bryoids were less densely sampled (n=198), with Chironomidae and Muscidae dominating as well.

Discussion

New Reference Library for Subarctic Diptera

In this study we presented a large barcode reference library containing 16786 barcode sequences for 1212 species of Diptera. Sixty-eight families are included in the study, with Muscidae being the most abundant. The sequences included in this study are publicly available on BOLD and have been sorted into 2235 BINs. Through this study we show the current diversity of Diptera within Churchill and provide opportunities for farther research into this community using the barcode information provided.

One hundred and forty-two BINs were unique to this project. Most unique BINs showed low abundance in this dataset, containing fewer than 10 records, except for one BIN identified only to the family level as Ephydridae, which contained 32 records, and the BIN identified as *Stictochironomus unguiculatus*, which contained 25 records. The unique BINs represented 25 families, and within these, species of the families Cecidomyiidae and Chironomidae were most abundant, with 38 and 29 unique BINs recorded. Almost all of the Cecidomyiidae BINs were only identified to family and reflected the taxonomic challenge that this family represents and the limited expertise available. Cecidomyiidae and Chironomidae differ in habitat, with Cecidomyiidae having mostly terrestrial larvae, and Chironomidae mostly aquatic (Gagné, 1981, Oliver, 1981). In regard to diet, Cecidomyiidae larvae are often phytophagous or mycophagous, and frequently form galls on plants, while Chironomidae larvae are mostly microphagous, feeding on small particles of detritus, plants, and microorganisms (Gagné, 1981,

Oliver, 1981). Chironomidae was the best-sampled family in our study, and it is possible that with increased sampling and research, more unique BINs could be discovered for other families.

Geographic Affiliation of Churchill Diptera

Most of the BINs were not unique to this study but contained members from other studies and geographic regions. Many of the BINs found in this study are shared with other regions, both Northern and Southern. Most BINs were shared with Alberta and the Northwest Territories when looking at total counts; and when calculating beta diversity, Churchill BIN composition was most similar to the Northwest Territories and Saskatchewan. Many BINs were also shared with the Northern Palearctic region, such as Finland and Norway, reflecting broader polar distributions. Churchill shared more BINs with Southern regions, but a high Northern affinity was observed when calculating beta diversity. The beta diversity results show that Churchill is most similar to other Northern regions in Canada, as well as nearby Southern regions, and is more similar to distant Northern regions such as Finland and Norway, than some Canadian Southern regions such as Ontario. The similarity to other Northern regions, such as the Northwest Territories, may reflect post glacial dispersal from the Beringia glacial refuge, while similarity to nearby Southern regions may reflect Northern and Southern dispersal. The similarity in BIN composition across Northern regions has been documented in other studies, such as Ekrem et al. (2018), who compared the species and BIN composition of Greenland Chironomidae to other Nearctic and Palearctic regions. They found that most taxa in Greenland were shared with at least one other geographic region, and that, when looking at taxon counts, Greenland was more similar to North America (Ekrem et al., 2018). While Greenland was one of the least similar regions compared to Churchill in our study, 91 BINs were still shared between them, and the overall sharing of dipteran BINs across Arctic regions here augments the findings for chironomids from Ekrem et al. (2018).

Genetic Divergence Patterns across Studies and Taxa

Based on the intraspecific distances, most species can be easily distinguished from their nearest neighbours using COI. For the dataset, across all Diptera families, the maximum intraspecific distances ranged from 0-16.06% and the nearest neighbour distances ranged from 0–16%. The high distance values, particularly those over 5% on the basis of other studies (e.g. Lin et al., 2015), are candidates for examination for cryptic species, particularly in understudied taxa. These distance values could also be high due to the biology of the species. The distances within individual families varied. For example, the mean nearest neighbour distance in Tachinidae was 6.97%. This value is comparable to previous research by Pohjioismaki et al. (2016), who determined that the mean nearest neighbour distance within the family Tachinidae was 5.52%. The maximum intraspecific distance for Drosophilidae ranged from 0–2.47%, similar to Machado et al. (2017) who found that the intraspecific distances ranged from 0–5.3%. The maximum intraspecific distances for Muscidae ranged from 0–4.25% here, similar to the results of Renaud et al. (2012b), who found distances of 0–3.01% for Churchill Muscidae, though there is some dataset overlap between our studies.

Some of the differences between our study and literature could be due to the geographic scope of the studies. The intraspecific divergence range can differ based on the area included in the study. Lin et al. (2015) found an average intraspecific divergence of 2.14% in a global scale study of Chironomidae, with maximum divergences reaching 21.1% due to cryptic species complexes. This is similar to our study, which found a maximum intraspecific divergence of 16.06%, but a much lower average of 1.56%. When focusing on the Baltic Sea region, Brodin et al. (2012) found that Chironomidae had intraspecific distances ranging from 0– 3.15%. For Psychodidae, the distances ranged from 0– 4.9% within the family in Brazil, and increased to 0– 8.32% when including multiple countries in the Neotropical region (de Souza Pinto et al., 2022, Posada-Lopez et al., 2023). When focusing only on Mexico, Adeniran et al. (2019) found intraspecific distances of 0.12–1.22%. This range increased to 0–9.48% when including

other sequences from North and South America (Adeniran et al., 2019). The maximum intraspecific distance was 1.07% for Psychodidae in our study, which is similar to other studies that focused on small geographic regions. Tabanidae has also been studied across several different countries with varying intraspecific distances, such as Thailand (0–4.4%), Africa (0–6.5%), India (0–6.34%), and Canada (0–3.3%) (Banerjee et al., 2015, Changbunjong et al., 2018, Cywinska et al., 2010, Votýpka et al., 2019). Our results were similar to previous Tabanidae results from Canada, with maximum intraspecific distances that ranged from 0–2.5%.

A family where the results differed from the literature was Mycetophilidae, which had intraspecific distances of 0–13.46%. Jürgenstein et al. (2015) conducted a study of *Mycetophila ruficollis* Meigen using 116 specimens collected throughout Europe from 1984 to 2014. Their study revealed intraspecific distances with a range of 0.08–0.8% (Jürgenstein et al., 2015), which is much lower than that of our study. The average intraspecific distance from our study, 0.7%, aligned more with Jürgenstein et al. (2015). Similarly, Kjærandsen (2022) found a mean intraspecific distance of 0.81% for Sciaroidea. This suggests that while the average genetic diversity in this family is low, there are some potential cryptic species to consider.

Nearest neighbour distances and intraspecific distances vary across insect orders. Lepidoptera has been shown to have lower levels of intraspecific variation than Diptera, with average intraspecific distances of 1.08% being found in species from Finland and Austria (Huemer et al., 2014), divergences ranging from 0-5.92% found in species from Nigeria (Nneji et al., 2020), and a threshold of <2% being found appropriate to differentiate most Lepidoptera species in Canada, though 7.7% of species had deeper divergences (Zahiri et al., 2014). The mean divergence between species of Lepidoptera was reported as 5.73% (Pentinsaari, 2016) and between Coleoptera was 11.99% (Pentinsaari et al., 2014). The mean intraspecific divergence and mean nearest neighbour distance reported in this study was 0.91% and 7.22% respectively. Though Lepidoptera has been reported to be lower than Diptera in the

literature, the mean intraspecific distance in our study is comparable to that seen in Lepidoptera. The mean nearest neighbour distance, however, appears to be lower in Lepidoptera. Both nearest neighbour and intraspecific distances appear to be higher in Coleoptera than Diptera, which aligns with what has been previously seen in the literature (Pentinsaari et al., 2014).

Taxonomic Composition by Habitat

We found a strong presence of families associated with aquatic habitats in the top 10 families for each land type classification, from aquatic larvae (e.g. Chironomidae, Culicidae, Simuliidae, Tipulidae) to predatory adults feeding near water (e.g. Dolichopodidae, Empididae) (Cook, 1981, Oliver, 1981, Stone, 1981, Peterson, 1981, Alexander & Byers, 1981, Robinson & Vockeroth, 1981, Steyskal & Knutson, 1981). This is not surprising considering that Churchill is located on the Hudson's Bay coast, and surrounded by diverse water body types like the Churchill River and a plethora of lakes. Despite the high occurrence of water in the area, shore flies (Ephydridae), which can be found around coastal areas, maritime marshes and even salt lakes, only made the top 10 in the wetlands. Furthermore, fungus gnats (Mycetophilidae, Sciaridae) were only abundant in the coniferous sites. As their larvae are typically fungivores, we can suspect a moist forest with abundant bracket fungi among others. Overall, the top 10 families are similar across the different habitats (with the exception of bryoids), which can suggest habitat fragmentation and edge effect uniformizing the family composition of each. A closer look at the species composition across habitats could provide more insight.

Findings for Further Investigation

Several of the findings outlined above could be elaborated upon in future studies, such as the comparison between Churchill and other geographic regions or the differences in Diptera community composition across different habitat types in the Churchill region. Significant differences in composition across land use cannot be evaluated, as samples were collected over many seasons by a variety of

people using different sampling methods and biases for what was sampled and later sequenced. We therefore used a graphical approach to explore the biodiversity across Churchill habitats. Future research could focus on comparing these different Churchill habitats with more consistent methods and timespan to determine if there are any significant differences in community composition.

A wide variety of collection methods were used to target a wider diversity of taxa and to obtain the specimens for this reference library. This resulted in a stronger reference library, and the benefits of using different trap types have been discussed in various studies (Ernst et al., 2015, Pentinsaari et al., 2020). However, we acknowledge there could still be biases and groups potentially missed or underrepresented. Different families are caught most often using different methods. For example, hand netting usually results in more specimens of Limoniidae being collected, while larger crane flies, like Tipulidae, are able to be caught in Malaise traps more often. As dipteran are strong flyers, most specimens were caught using Malaise traps. Hence, they are commonly used to collect these taxa, but there can be some biases depending on the type of Malaise trap used, the material used to make the trap, and the type of attractant used (Betts, 2010, Martinez et al., 2020, Skvarla et al., 2020, Uhler et al., 2022). Despite the potential limitations of each, we believe that combining a variety of trap types and sampling methods allowed us to construct a robust reference library for the region. Further research could be done to determine the effectiveness of trap type and their effect on species composition.

While many specimens were collected, there are still groups missing from the barcode data. About 20% of the known Churchill species of Empidoidea were not accounted for in the barcode data. It is possible that the habitats associated with these groups, such as running water habitats, were not well sampled, likely resulting in some species being missed. Future research could focus on the continued sampling of Churchill and other Northern regions to further expand the DNA barcode data available to other researchers.

Further research could also be done regarding the long branch lengths observed in the ML tree. After following the steps outlined in the methods, we determined no issues with the quality or identification of the sequences. These sequences, when BLASTed, returned the expected results and were similar to other sequences within the species. For *Tetanocera fuscinervis* (Zetterstedt), all codon positions showed the long branch length, but for *Bradysiopsis vittigera* (Zetterstedt), the long branch length only appeared when using the third codon position and could suggest a more rapid rate of evolution. Overall, the branch lengths do not appear to be caused by an issue with the sequence or alignment, but instead are something to do with the species itself. *Tetanocera fuscinervis* is a member of the family Sciomyzidae and is a predator of snails on shorelines (Foote, 1996). There is little information available regarding *Bradysiopsis vittigera*, but this species is a member of the family Sciaridae, where many larvae are saprophagous (Scudder & Cannings, 2006, Steffan, 1981). These species are the only members of their families on the tree. There is possibly something about these species and families that results in the long branch lengths and genetic differences. Further research could look into the reasons for these branch lengths and the relationships between these species and other Diptera. Further work could also be done to sequence the genomes of Diptera species. To look more closely at the phylogenetic relationships between the taxa recorded in Churchill, a range of nuclear and mitochondrial markers would need to be combined with morphological characters in order to get a more accurate picture, such as the phylogeny presented in Wiegmann et al. (2011), which uses nuclear loci, mitochondrial genomes, and morphological characters.

Opportunities for Using New Library

As outlined earlier in the discussion, there are differences across and within insect orders regarding the intraspecific divergence. The families within Diptera differed in their intraspecific distances, and these differences may be considered when clustering specimens into species. A single clustering threshold is not optimal for all of Diptera when performing *de novo* clustering of sequences

into taxonomic units as close to species as possible, but rather this threshold should differ based on the genetic diversity present in the family. If a threshold is used that is too large, this could result in closely related species being grouped together, or if one is too small, this could result in species being split across operational taxonomic units. These differences can impact estimates of species richness. However, through this collaboration between molecular analysts and taxonomic experts, researchers are not solely reliant on specific divergence thresholds. By using well-populated reference libraries, future researchers can perform identifications despite variation in genetic diversity within and among species. When sorting sequenced specimens into species, we recommend accessing reference libraries when possible.

References libraries are valuable resources that can help researchers more accurately identify and delineate species and provide opportunities for future research. The barcode library presented in this study reflects the current diversity of Diptera in Churchill, and the barcode sequences are publicly available on BOLD and accompanied by their voucher, taxonomic, and collection information. This library is an important resource for any researcher in need of species-level information. Taxonomic experts identified the specimens, and 12612 specimens were assigned species-level identification. While molecular information and BINs are an important resource, BINs do not always correspond to species. By combining molecular data with taxonomic identification, we can reduce the number of specimens that are misidentified and provide more accurate and detailed information to future researchers. Trait information is usually associated with species names, so having this taxonomic information available allows future researchers to make use of the molecular data to a greater extent. Future research could include comparing our reference library to past Churchill studies and future studies to see how these communities are changing. This region can also be compared to other Northern regions, such as those mentioned above. Overall, this barcode library can be applied to a wide range of studies investigating this Northern region and the ecology and evolution of Diptera. However, further work is needed, and

future studies could focus on a larger geographic scale, and include other Northern regions, as well as temperate and tropical regions, so we can further our knowledge of Diptera diversity and barcode variability at a global scale.

Conclusion

In conclusion, we present a large-scale barcoding study of Diptera from Churchill and contribute to the growing amount of barcode data available for research. We provide a detailed reference library, making use of molecular data and taxonomic knowledge, and make this library publicly available for future research. Our work demonstrates the utility of broad, multi-year collecting programs as well as collaborations to build well-populated DNA barcode reference libraries that will enable identification at the species level even for hyper-diverse arthropod groups. We outline the diversity of Diptera and contribute to the knowledge of this ecologically important group, including taxa that interact closely with humans and those that can be used for freshwater ecosystem health monitoring. By studying Northern Diptera communities and understanding current diversity, we can study the change over time, especially as Northern regions are impacted by the changing climate. By collecting and releasing barcode libraries we open the door for future research and allow other researchers to build off our work and further investigate taxa and regions of interest.

Acknowledgements

We dedicate this paper to Dr. Terry Wheeler, who was an Associate Professor and Director of the Lyman Entomological Museum, McGill University, and to Dr. Rob Roughley, who was a beetle specialist from the University of Manitoba. Terry was an excellent scientist, educator, mentor, and friend to many who contributed significantly to this research before he passed away. Dr. Rob Roughley was a major partner in the overall insect biodiversity survey of Churchill, and he contributed field work and

important specimens to the present paper. The authors are also grateful to the staff of the Churchill Northern Studies Centre, with special thanks to Dr. LeeAnn Fishback for supporting this project. Colleagues at the Centre for Biodiversity Genomics, University of Guelph, provided critical assistance in field collecting and in conducting the DNA sequencing work. We are grateful to Anais Renaud, Jostein Kjaerandsen, Terry L. Whitworth, Kai Heller, Norm E. Woodley, Kevin Barber, Brian V. Brown, Art Borkent, Ludovic Jolicoeur, Mikko Pentinsaari, Ayman K. Elsayed, Jeremy deWaard, Jukka E. Salmela, Michelle Locke, Ole A. Saether, Alyssa M. Anderson, Michel Rapinski, Xiaolong Lin, Susan E. Gresens, Lucas E. Roscoe and Jin-hua Ran for conducting additional specimen identifications. Thank you to Angela Telfer, Kate Perez, Allison Brown, Renee Miskie, and staff at the Centre for Biodiversity and Genomics for adding barcode-based IDs to portions of the data. Thank you to Jiaojiao Diao and Jessica Castellanos Labarcena for assisting with assigning land-use classification to sites and generating figures. Funding for this work was provided through a Natural Sciences and Engineering Research Council of Canada (NSERC) International Polar Year (IPY) grant to PDNH, NSERC Discovery Grants to SJA, grants in genomics and Bioinformatics and Computational Biology from Genome Canada through the Ontario Genomics Institute and from the Government of Ontario to SJA and PDNH, by the Government of Canada through the Canada First Research Excellence Fund to SJA and PDNH, and through a Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic Project Grant (Ecological Structure of Northern Arthropods: Adaptation to a Changing Environment) awarded to C.M. Buddle, TAW and DCC, plus their supporting partners and collaborators.

References

Adeniran, A.A., Fernández-Santos, N.A., Rodríguez-Rojas, J.J., Treviño-Garza, N., Huerta-Jiménez, H., Mis-Ávila, P.C., Pérez-Pech, W.A., Hernández-Triana, L.M. & Rodríguez-Pérez, M.A. (2019). Identification of phlebotomine sand flies (Diptera: Psychodidae) from leishmaniasis endemic

areas in southeastern Mexico using DNA barcoding. *Ecology and Evolution, 9*(23), 13543 – 13554. doi: 10.1002/ece3.5811

Adler, P.H. & Courtney, G.W. (2019). Ecological and societal services of aquatic Diptera. *Insects, 10*(3), 70. doi: 10.3390/insects10030070

Albrecht, A. (1990). Revision, phylogeny and classification of the genus *Dorylomorpha* (Diptera, Pipunculidae). *Acta Zoologica Fennica, 188,* 1-240.

Aldrich, J.M. (1918). The kelp flies of North America. *Proceedings of the California Academy of Sciences, 8,* 157-179.

Alexander, C.P. (1943). Family Tipulidae. Pp. 196–486 In Crampton, G.C. et al. (Eds.) Guide to the insects of Connecticut. Part VI. The Diptera or true flies of Connecticut. First Fascicle. *Bulletin Connecticut State Geological and Natural History Survey*, 64.

Alexander, C.P. & Byers, G.W. (1981). Tipulidae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), Manual of Nearctic Diptera. Volume 1. (pp. 247 – 256). Research Branch Agriculture Canada.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403-410. doi: 10.1016/S0022-2836(05)80360-2

Anderson, A. M., Stur, E., & Ekrem, T. (2013). Molecular and morphological methods reveal cryptic diversity and three new species of Nearctic *Micropsectra* (Diptera: Chironomidae). *Freshwater Science, 32*(3), 892-921. doi: 10.1899/12-026.1

Appelhans, T., Detsch, F., Reudenbach, C. & Woellauer, S. (2023). mapview: Interactive Viewing of Spatial Data in R. R package version 2.11. https://github.com/r-spatial/mapview.

Arntfield, P.W. (1975). A revision of *Graphomya* Robineau-Desvoidy (Diptera: Muscidae) from North America. *The Canadian Entomologist*, *107*(3), 257–302. doi: 10.4039/Ent107257-3

Banerjee, D., Kumar, V., Maity, A., Ghosh, B., Tyagi, K., Singha, D., Kundu, S., Laskar, B.A., Naskar, A. & Rath, S. (2015). Identification through DNA barcoding of Tabanidae (Diptera) vectors of surra disease in India. *Acta Tropica, 150*, 52-58. doi: 10.1016/j.actatropica.2015.06.023

Barták, M. (2002). Nearctic species of *Rhamphomyia* subgenus *Megacyttarus* (Diptera: Empididae). *Acta Universitatis Carolinae, Biologica*, *46,* 3–215.

Bedini, S., Flamini, G., Cosci, F., Ascrizzi, R., Echeverria, M.C., Guidi, L., Landi, M., Lucchi, A. & Conti, B. (2017). *Artemisia* spp. essential oils against the disease-carrying blowfly *Calliphora vomitoria. Parasites & Vectors, 10*(1), 80. doi: 10.1186/s13071-017-2006-y

Betts, D.J. (2010). *Investigation of potential trapping bias in Malaise traps due to mesh gauge, in two Habitats* [Master's Thesis, Brigham Young University]. https://scholarsarchive.byu.edu/etd/2561

Blagoev, G.A., Nikolova, N.I., Sobel, C.N., Hebert, P.D.N. & Adamowicz, S.J. (2013). Spiders (Araneae) of Churchill, Manitoba: DNA barcodes and morphology reveal high species diversity and new Canadian records. *BMC Ecology, 13*(1), 44. doi: 10.1186/1472-6785-13-44

Boesel, M. W. (1985). A brief review if the genus *Polypedilum* in Ohio, with keys to known stages of species occurring in northeastern United States (Diptera, Chironomidae). *Ohio Journal of Science, 85*(5), 245-262.

Bonizzoni, M., Gasperi, G., Chen, X. & James, A.A. (2013). The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. *Trends in Parasitology, 29*(9), 460-468. doi: 10.1016/j.pt.2013.07.003

Boucher, S. (2002). Revision of Nearctic species of *Cerodontha (Cerodontha)* (Diptera: Agromyzidae). *The Canadian Entomologist 134*, 577-603

Boucher, S. (2008). A new species and taxonomic notes on northern Nearctic *Cerodontha (Icteromyza)* (Diptera: Agromyzidae). *The Canadian Entomologist 140*, 557-562

Boucher, S. (2012a). Revision of the Nearctic species of *Cerodontha (Icteromyza)* (Diptera: Agromyzidae). *The Canadian Entomologist 144,* 122-157

Boucher, S. (2012b). Revision of the Canadian species of *Amauromyza* Hendel (Diptera: Agromyzidae). *The Canadian Entomologist 144*, 733-757

Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M., Jaques Miret, J..A., Justesen, A.F., Magnusson, C.S., Milonas, P., Navas-Cortes, J.A., Parnell, S., Potting, R., Reignault, P.L., Thulke, H., Van der Werf, W., Vicent Civera, A., Yuen, J., Zappalà, L., Czwienczek, E., Streissl, F. & MacLeod, A. (2020). Pest categorisation of *Liriomyza bryoniae. EFSA Journal, 18* (3), p.e06038-n/a. doi: 10.2903/j.efsa.2020.6038

Brodin, Y., Ejdung, G., Strandberg, J., Lyrholm, T. (2012). Improving environmental and biodiversity monitoring in the Baltic Sea using DNA barcoding of Chironomidae (Diptera). *Molecular Ecology Resources, 13*(6), 996-1004. doi: 10.1111/1755-0998.12053

Brodo, F. (1967). A review of the subfamily Cylindrotominae in North America (Diptera, Tipulidae). *The University of Kansas Science Bulletin*, *47*, 71–115.

Brodo, F. (1987). A revision of the genus Prionocera (Diptera: Tipulidae). *Evolutionary Monograph, 8*, 1–93.

Brodo, F. (2017). Taxonomic review of Angarotipula Savchenko, 1961 (Diptera: Tipulidae) in North America. *Canadian Entomologist, 150*, 12-34.

Brundin, L. (1947) Zur Kenntnis der schwedischen Chironomiden. *Arkiv för zoologi, 39 A(3)*, 1-95.

Brundin, L. (1949) Chironomiden und andere Bodentiere der südschwedischen Urgebirgsseen. Ein Beitrag zur Kenntnis der bodenfaunistischen Charakterzüge schwedischer oligotropher Seen. *Report/Institute of Freshwater Research, Drottningholm, 30*, 915.

Brundin, L. (1989) The adult males of Podonominae (Diptera: Chironomidae) of the Holarctic region - Keys and diagnoses. In: Wiederholm, T. (ed.): Chironomidae of the Holarctic region. Keys and diagnoses. Part 3 - Adult males. *Entomologica Scandinavica, Suppl., 34*, 23-36.

Byers, G.W. (1961). The crane fly genus Dolichopeza in North America. *The University of Kansas Science Bulletin, 42*, 665–924.

Changbunjong, T., Bhusri, B., Sedwisai, P., Weluwanarak, T., Nitiyamatawat, E., Chareonviriyaphap, T. & Ruangsittichai, J. (2018). Species identification of horse flies (Diptera: Tabanidae) in Thailand using DNA barcoding. *Veterinary Parasitology, 259*, 35-43. doi: 10.1016/j.vetpar.2018.07.002

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics, 11*(4), 265-270.

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K. & Ellison, A.M. (2014). Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs, 84*(1), 45-67. doi: 10.1890/13-0133.1

Chillcott, J.G. (1962). A revision of the *Platypalpus juvenis* complex in North America (Diptera: Empididae). *The Canadian Entomologist*, *94*(2), 113–143. doi: 0.4039/Ent94113-2

Churchill Northern Studies Centre. (2022). *The Centre.* Churchill Northern Studies Centre. https://churchillscience.ca/about/the-centre/

Cirtwill, A.R., Kaartinen, R., Rasmussen, C., Redr, D., Wirta, H., Olesen, J.M., Tiusanen, M., Ballantyne, G., Cunnold, H., Stone, G.N., Schmidt, N.M. & Roslin, T. (2022). Stable pollination service in a generalist high Arctic community despite the warming climate. *Ecological Monographs, 93*(1). doi: 10.1002/ecm.1551

Collin, J.E. (1930). A revision of the Greenland species of the anthomyid 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*(2), 255–281. doi: 10.1111/j.1365-2311.1930.tb00387.x

Cook, E.F. (1981). Chaoboridae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), Manual of Nearctic Diptera. Volume 1. (pp. 257 – 292). Research Branch Agriculture Canada.

Coquillett, D.W. (1900). Papers from the Harriman Alaska Expedition. IX. Entomological Results (3): Diptera. *Proceedings of the Washington Academy of Sciences, 2*, 389-464.

Cranston, P. S., Oliver, D. R. (1988) Additions and corrections to the Nearctic Orthocladiinae (Diptera: Chironomidae). *Canadian entomologist, 120*(5), 425-462. doi: 10.4039/Ent120425-5

Cranston, P. S., Dillon, M. E., Pinder, L. C. V., Reiss, F. (1989a). The adult males of Chironominae (Diptera: Chironomidae) of the Holarctic region - Keys and diagnoses. In: Wiederholm, T. (ed.): Chironomidae of the Holarctic region. Keys and diagnoses. Part 3 - Adult males. *Entomologica Scandinavica, Suppl., 34*, 353-502.

Cranston, P. S., Oliver, D. R., Sæther, O. A. (1989b) The adult males of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region - Keys and diagnoses. - In: Wiederholm, T. (ed.): Chironomidae of the Holarctic region. Keys and diagnoses. Part 3 - Adult males. *Entomologica Scandinavica, Suppl., 34*, 165-352.

Cywinska, A., Hannan, M.A., Kevan, P.G., Roughley, R.E., Iranpour, M. & Hunter, F.F. (2010). Evaluation of DNA barcoding and identification of new haplomorphs in Canadian deerflies and horseflies. *Medical and Veterinary Entomology, 24*(4), 382-410. doi: 10.1111/j.1365-2915.2010.00896.x

Czechowski, P., Stevens, M.I., Madden, C. & Weinstein, P. (2020). Steps towards a more efficient use of chironomids as bioindicators for freshwater bioassessment: Exploiting eDNA and other genetic tools. *Ecological Indicators, 110*. doi: 10.1016/j.ecolind.2019.105868

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

De Meyer, M. (1989). Systematics of the Nearctic species of the genus *Cephalops* Fallén (Diptera, Pipunculidae). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie, 59,* 99-130.

de Souza Pinto, I., Rodrigues, B.L., de Araujo-Pereira, T., Shimabukuro, P.H.F., de Pita-Pereira, D., Britto, C. & Brazil, R.P. (2023). DNA barcoding of sand flies (Diptera, Psychodidae, Phlebotominae) from the western Brazilian Amazon. *PLOS ONE, 18*(2). doi: 10.1371/journal.pone.0281289

deWaard, J. (2023). *Welcome to Arctic BIOSCAN (ARCBIO).* Arctic BIOSCAN. https://arcticbioscan.ca/

Eddy, S.R. (1998). Profile hidden Markov models. *Bioinformatics, 14*(9), 755-763. doi: 10.1093/bioinformatics/14.9.755

Ekrem, T. (2007). A taxonomic revision of the genus *Stempellinella* (Diptera: Chironomidae). *Journal of Natural History, 41*(21-24), 1367-1465. doi: 10.1080/00222930701437360

Ekrem, T., Sublette, M. F., & Sublette, J. E. (2003). North American *Tanytarsus* I. Descriptions and keys to species in the *eminulus, gregarius, lugens* and *mendax* species groups (Diptera: Chironomidae). *Annals of the Entomological Society of America, 96*(3), 265-328. doi: 10.1603/0013-8746(2003)096[0265:NATIDA]2.0.CO;2

Ekrem, T., Stur, E., Orton, M.G. & Adamowicz, S.J. (2018). DNA barcode data reveal biogeographic trends in Arctic non-biting midges. *Genome, 61*(11), 787-796. doi: 10.1139/gen-2018-0100

Environmental Systems Research Institute. (2015). ArcGIS Desktop: Release 10.3 [Software]. Redlands, CA: Environmental Systems Research Institute.

Epler, J. H. (1987). Revision of the Nearctic *Dicrotendipes* Kieffer, 1913 (Diptera: Chironomidae). *Evolutionary Monographs, 9*, 1-102

Ernst, C.M., Loboda, S. & Buddle, C.M. (2015). Capturing northern biodiversity: diversity of arctic, Subarctic and north boreal beetles and spiders are affected by trap type and habitat. *Insect Conservation and Diversity, 9*(1), 63-73. doi: 10.1111/icad.12143

Fedoseeva, L.I. 1971. A revision of the Nearctic species of grass flies of the genus Meromyza Mg. (Diptera: Chloropidae). Entomological Review, 50(4): 520—529.

Fedoseeva, L.I. 2003 Chloropid flies of the genus Meromyza Meigen, 1830 (Diptera: Chloropidae) of the fauna of North America. Russian Entomological Journal, 12(4): 425—430.

Fernandez-Triana, J., Smith, M.A., Boudreault, C., Goulet, H., Hebert, P.D.N., Smith, A.C. & Roughley, R. (2011). A poorly known high-latitude parasitoid wasp community: unexpected diversity and dramatic changes through time. *PLOS ONE, 6*(8). doi: 10.1371/journal.pone.0023719

Ferrington, L. C. Jr & Sæther, O. A. (2011) A revision of the genera *Pseudosmittia* Edwards, 1932, *Allocladius* Kieffer, 1913, and *Hydrosmittia* gen. n. (Diptera: Chironomidae, Orthocladiinae). *Zootaxa, 2849*(1), 1-314. doi: 10.11646/zootaxa.2849.1.1

Firke, S. (2023). janitor: Simple Tools for Examining and Cleaning Dirty Data. R package version 2.2. https://github.com/sfirke/janitor

Fittkau, E. J. (1962) Die Tanypodinae (Diptera, Chironomidae). Die Tribus Anatopyniini, Macropeloplini und Pentaneurini. *Abh. Larvalsyst. Insekten, 6,* 1-453.

Fittkau, E. J. & Lehmann, J. (1970) Revision der Gattung *Microcricotopus* Thien. u. Harn. (Dipt., Chironomidae). *Internationale Revue der gesamten Hydrobiologie, 55*(3), 391-402.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mitochondrial DNA. Part B. Resources, 5*(2), 1622-1624. doi: 10.1080/23802359.2020.1745100

Foote, B.A. (1996). Biology and immature stages of snail-killing flies belonging to the genus *Tetanocera* (Insecta: Diptera: Sciomyzidae). 1. Introduction and life histories of predators of shoreline snails. *Annals of the Carnegie Museum, 65*, 1-12. doi: 10.5962/p.226629

Förster, M., Klimpel, S., Mehlhorn, H., Sievert, K., Messler, S. & Pfeffer, K. (2007). Pilot study on synanthropic flies (e.g. *Musca, Sarcophaga, Calliphora, Fannia, Lucilia, Stomoxys*) as vectors of pathogenic microorganisms. *Parasitology Research, 101*(1), 243-246. doi: 10.1007/s00436-007-0522-y

Freeman, T.N. (1958). A historical account of insect collecting in northern Canada. *Proceedings of the 10th International Congress of Entomology.*

Fu, Y. & Sæther, O. A. (2012) *Corynoneura* Winnertz and *Thienemanniella* Kieffer from the Nearctic Region (Diptera: Chironomidae: Orthocladiinae). *Zootaxa, 3536*(1), 1-61. doi: 10.11646/zootaxa.3536.1.1

Gagné, R.J. (1981). Cecidomyiidae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), *Manual of Nearctic Diptera. Volume 1.* (pp. 257 – 292). Research Branch Agriculture Canada.

Garnier, S., Ross, N., Rudis, R., Camargo, P.A., Sciaini, M. & Scherer, C. (2023). Viridis: Colorblind-Friendly Color Maps for R. R package version 0.6.3. https://sjmgarnier.github.io/viridis/

Giłka, W. (2001). A review of Polish *Cladotanytarsus* Kieffer (Diptera: Chironomidae) with description of three new species. *Polish Journal of Entomology, 70*, 307-328.

Gowin, F. & Thienemann, A. (1942) Zwei neue Orthocladiinen-Arten aus Lunz (Niederdonau). Chironomiden aus dem Lunzer Seengebiet VII. *Zoologischer Anzeiger, 140*(7/8), 101-109.

Graczyk, T.K., Knight, R., Gilman, R.H. & Cranfield, M.R. (2001). The role of non-biting flies in the epidemiology of human infectious diseases. *Microbes and Infection, 3*(3), 231-235. doi: 10.1016/S1286-4579(01)01371-5

Gregor, F., Rozkošný, R., Barták, M. & Vaňhara, J. (2002). The Muscidae (Diptera) of Central Europe. *Folia Facultatis Scientiarium Naturalium Universitatis Masarykianae Brunensis. Biol*. *107*, 1–280.

Griffiths, G.C.D. (1991a). Anthomyiidae [part]. In Griffiths, G.C.D. (Eds), *Flies of the Nearctic Region*, (pp. 1049–1240). E. Schweizerbart, Stuttgart, Germany.

Griffiths, G.C.D. (1991b). Anthomyiidae [part]. In Griffiths, G.C.D. (Eds), *Flies of the Nearctic Region*, (pp. 1633–1872). E. Schweizerbart, Stuttgart, Germany.

Griffiths, G.C.D. (1991c). Anthomyiidae [part]. In Griffiths, G.C.D. (Eds), *Flies of the Nearctic Region*, (pp. 2289–2484). E. Schweizerbart, Stuttgart, Germany.

Griffiths, G.C.D. (1993a). Anthomyiidae [part]. In Griffiths, G.C.D. (Eds), *Flies of the Nearctic Region*, (pp. 1417–1632). E. Schweizerbart, Stuttgart, Germany.

Griffiths, G.C.D. (1993b). Anthomyiidae [part]. In Griffiths, G.C.D. (Eds), *Flies of the Nearctic Region*, (pp. 1873–2120). E. Schweizerbart, Stuttgart, Germany.

Griffiths, G.C.D. (1964). The Agromyzid fauna of Iceland and the Faroes, with Appendices on the *Phytomyza millii* and *robustella* Groups. *Entomologiske Meddelelser 32*, 393- 450

Grodhaus, G. (1987). *Endochironomus* Kieffer, *Tribelos* Townes, *Synendotendipes*, n. gen., and *Endotribelos*, n. gen. (Diptera: Chironomidae) of the Nearctic Region. *Journal of the Kansas Entomological Society, 60*(2), 167-247.

Hajibabaei, M., DeWaard, J.R., Ivanova, N.V., Ratnasingham, S., Dooh, R.T., Kirk, S.L., Mackie, P.M. & 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*(1462), 1959-1967. doi: 10.1098/rstb.2005.1727

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. (2006). DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences, 103*(4), 968-971. doi: 10.1073/pnas.0510466103

Hamerlík, L. & Bitušík, P. (2009). The distribution of littoral chironomids along an altitudinal gradient in High Tatra Mountain lakes: Could they be used as indicators of climate change? *Annales de Limnologie, 45*(3), 145-156. doi: 10.1051/limn/2009021

Hardy, D.E. (1943). A revision of Nearctic Dorilaidae (Pipunculidae). *University of Kansas Science Bulletin, 29,* 1-231.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & DeWaard, J.R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1512), 313– 21. doi: 10.1098/rspb.2002.2218

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S. & Francis, C.M. (2004). Identification of birds through DNA barcodes. *PLOS Biology, 2*(10). doi: 10.1371/journal.pbio.0020312

Hennig, W. (1955–1964). Muscidae. In E. Lindner (Eds.), *Die Fliegen der palaearktischen Region 63b 7(2*). (pp. 1–1110). Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Hestenes, T. C. & Sæther, O. A. (2000) Three new Nearctic *Thienemanniella* Kieffer species with a review of the Nearctic species. In: Hoffrichter, O. (ed.): Late 20th Century Research on Chironomidae: an Anthology from the 13th International Symposium on Chironomidae, pp. 103-127. Shaker Verl., Aachen.

Hirvenoja, M. (1962) *Cladotanytarsus*- Arten (Dipt., Chironomidae) aus Finnisch-Lappland. *Annales Entomologici Fennici*, *28*(4), 173-181.

Hirvenoja, M. (1973) Revision der Gattung *Cricotopus* van der Wulp und ihrer Verwandten (Diptera, Chironomidae). *Annales Zoologici Fennici, 10*(1), 1-363.

Hirvenoja, M. & Hirvenoja, E. (1988) *Corynoneura brundini* spec. nov. Ein Beitrag zur Systematik der Gattung *Corynoneura* (Diptera, Chironomidae). In: Fittkau, E. J. (ed.): Festschrift zu Ehren von Lars Brundin. *Spixiana Suppl., 14*, 213-238.

Hocking, B., Richards, W.R. & Twinn, C.R. (1950). Observations on the bionomics of some Northern mosquito species (Culicidae: Diptera). *Canadian Journal of Research, 28*(2), 58-80. doi: 10.1139/cjr50d-006

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*(3), 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*. T*ransactions of the American Entomological Society, 60*(1), 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*(2), 133–198.

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*(3), 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*(2), 316–342. doi: 10.1093/aesa/47.2.316

Huckett, H.C. (1965a). The Muscidae of Northern Canada, Alaska, and Greenland (Diptera). *Memoirs of the Entomological Society of Canada, 97,* 5-369. doi: 10.4039/entm9742fv

Huckett, H.C. (1965b). Family Muscidae. In A. Stone, C.W. Sabrosky, W.W. Wirth, R.H. Foote, and J.T.P. Coulson (Eds.), *A catalog of the Diptera of America north of Mexico. Handbook no. 276.* (pp. 869–915). U.S. Department of Agriculture, Washington, DC.

Huckett, H.C. & Vockeroth, J.R. (1987). Muscidae. In J.F. McAlpine, B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth, and D.M. Wood (Eds), *Manual of Nearctic Diptera. Vol. 2.* (pp. 1115–1131). Agriculture Canada Monograph No. 28.

Huemer, P., Mutanen, M., Sefc, K.M. & Hebert, P.D.N. (2014). Testing DNA barcode performance in 1000 species of European Lepidoptera: large geographic distances have small genetic impacts. *PLOS ONE, 9*(12). doi: 10.1371/journal.pone.0115774

Ivanova, N.V., DeWaard, J.R. & Hebert, P.D.N. (2006). An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes, 6*(4), 998-1002. doi: 10.1111/j.1471-8286.2006.01428.x

Jackson, G. A. (1977) Nearctic and Palaearctic *Paracladopelma* Harnisch and *Saetheria* n. gen. (Diptera: Chironomidae). *Journal of the Fisheries Research Board of Canada, 34*(9), 1321-1359.

James, M.T. & Huckett, H.C. (1952). The Diptera collected by I.O. Buss in southwestern Yukon Territory during the summer of 1950. *The Canadian Entomologist, 84*(9), 265-269.

Janzen, D.H. & Hallwachs, W. (2011). Joining inventory by parataxonomists with DNA barcoding of a large complex tropical conserved wildland in Northwestern Costa Rica. *PLOS ONE, 6*(8). doi: 10.1371/journal.pone.0018123

Janzen, D.H., Hallwachs, W., Blandin, P., Burns, J.M., Cadiou, J-M., Chacon, I., Dapkey, 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., Woodley, N.E. & Wilson, J.J. (2009) Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Molecular Ecology Resources, 9*(1), 1-16. doi: 10.1111/j.1755-0998.2009.02628.x

Jeffery, N.W., Elías-Gutiérrez, M. & Adamowicz, S.J. (2011). Species diversity and phylogeographical affinities of the Branchiopoda (Crustacea) of Churchill, Manitoba, Canada. *PLOS ONE, 6*(5). doi: 10.1371/journal.pone.0018364

Johnson, C.W. (1929). Diptera of Labrador. *Psyche, 36*(2), 129-146. doi: 10.1155/1929/84643

Jürgenstein, S., Kurina, O. & Põldmaa, K. (2015). The *Mycetophila ruficolli*s Meigen (Diptera, Mycetophilidae) group in Europe: elucidating species delimitation with COI and ITS2 sequence data. *ZooKeys, 508*(508), 15-51. doi: 10.3897/zookeys.508.9814

Kjærandsen, J. (2022). Current state of DNA barcoding of Sciaroidea (Diptera)—Highlighting the need to build the reference library. *Insects, 13*(2), 147. doi: 10.3390/insects13020147

Kyerematen, R. A. K., Sæther, O. A., & Andersen, T. (2000). A review of the *Rheotanytarsus pellucidus* group (Diptera: Chironomidae). In O. Hoffrichter (Ed.), Late 20th century research on Chironomidae: an anthology from the 13th international symposium on Chironomidae (pp. 147-170). Shaker Verlag.

Laforest, B.J., Winegardner, A.K., Zaheer, O.A., Jeffery, N.W., Boyle, E.E. & Adamowicz, S.J. (2013). Insights into biodiversity sampling strategies for freshwater microinvertebrate faunas through bioblitz campaigns and DNA barcoding. *BMC Ecology, 13*(1), 13. doi: 10.1186/1472-6785-13-13

Langton, P. H. (1985) Review of type specimens of the *limbatellus* group, with a provisional key to known females of *Psectrocladius* Kieffer (Diptera: Chironomidae). *Entomologica Scandinavica, 15,* 477-485.

Langton, P. H. & Pinder, L. C. V. (2007) Keys to the adult male Chironomidae of Britain and Ireland; 2 vols. *Freshwater Biological Association*, *64*, 239 + 168.

Lehmann, J. (1970). Revision der Europäischen Arten (Imagines) der Gattung *Parachironomus* Lenz (Diptera, Chironomidae). *Hydrobiologia, 36*,(1), 129-158. doi: 10.1007/bf00751287

Lehmann, J. (1970). Revision der europäischen Arten (Imaginees und Puppen) der Gattung *Rheotanytarsus* Bause (Diptera, Chironomidae). *Zoologischer Anzeiger, 185*(5/6), 343-378.

Lehmann, J. (1972). Revision der europäischen Arten (Puppen ♂♂ und Imagines ♂♂) der Gattung *Eukiefferiella* Thienemann (Diptera: Chironomidae). *Beiträge zur Entomologie, 22*(7/8), 347-405.

Lin, X., Stur, E. & Ekrem, T. (2015). Exploring genetic divergence in a species-rich insect genus using 2790 DNA barcodes. *PLoS One, 10*(9), p.e0138993. doi: 10.1371/journal.pone.0138993

Lin, X.-L., Stur, E., & Ekrem, T. (2018). DNA barcodes and morphology reveal unrecognized species in Chironomidae (Diptera). *Insect Systematics & Evolution, 49*(4), 329-398. doi: 10.1163/1876312X-00002172

Lindeberg, B. (1967). Sibling species delimitation in the *Tanytarsus lestagei* aggregate (Diptera, Chironomidae). *Annales Zoologici Fennici, 4*(1), 45-86.

Lindeberg, B., & Wiederholm, T. (1979). Notes on the taxonomy of European species of *Chironomus* (Diptera: Chironomidae). *Entomologica scandinavica Suppl., 10*, 99-116.

Liu, Y. & Wang, X. (2005) *Synorthocladius* Thienemann from China, with a review on the genus (Diptera: Chironomidae: Orthocladiinae). *Zootaxa, 1057*(1), 51-60. doi: 10.11646/zootaxa.1057.1.3

MacDonald, J.F. (1994). Review of the Nearctic species of the genus *Chelifera* Macquart (Diptera: Empididae; Hemerodromiinae) of America north of Mexico. *Proceedings of the Entomological Society of Washington*, *96*(2), 236–275.

Machado, S., dos Santos, J.P.J., Fonseca, P.M., Bolzan, A.R., David, J., da Silva Loreto, E.L., Gottschalk, M.S. & Robe, L.J. (2017). Neotropical mycophagous drosophilids (Diptera: Drosophilidae): DNA barcoding as a way of overcoming the taxonomic impediment. *Insect Conservation and Diversity, 10*(3), 271-281. doi: 10.1111/icad.12223

[dataset] Majoros, S.E., Wang, J., Levesque-Beaudin, V., Borkent, C.J., Brodo, F., Brooks, S.E., Boucher, S., Cumming, J.M., Currie, D.C., Ekrem, T., Elliott, T.A., Hebert, P.D.N., Roughley, R.E., Savage, J., Schaefer, P., Sinclair, B.J., Skevington, J.H., Solecki, A.M., Stur, E., Wheeler, T.A., Woodcock, T.S., Young, A.D., Young, C. & Adamowicz, S.J.; 2023; DS-CDIPTERA Collaborative DNA Barcode Library for Diptera of Churchill, Canada: A Resource for Northern Research; Barcode of Life Data Systems; dx.doi.org/10.5883/DS-CDIPTERA

Malloch, J. R. (1915) The Chironomidae, or midges, of Illinois, with particular reference to the species occuring in the Illinois River. *Bulletin of the Illinois State Laboratory of Natural History, 10*, 273-591.

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, 3. *Pt. C, Dipt., pp.* 60c-90c. Ottawa.

Malloch, J.R. (1920a). Scientific results of the Katmai Expedition of the National Geographic Society. XII. Descriptions of Diptera of the families Anthomyidae and Scatophagidae. *The Ohio Journal of Science*, *20*, 267-288.

Malloch, J.R. (1920b). Descriptions of new North American Anthomyiidae (Diptera). *Transactions of the American Entomological Society, 46*(2), 133–196.

Malloch, J.R. (1921). Dipterous insects of the family Anthomyiidae from the Pribilof Islands, Alaska. *Proceedings of the California Academy of Sciences, 11*, 178-182.

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*(3), 227-282.

Manitoba Land Initiative (n.d.) LUC_EOSD_Northeast [Dataset]. Manitoba Land Initiative. Accessed from https://mli.gov.mb.ca/landuse/index.html

Martinez, A., Reeves, N., Perry, R. & Horton, M. (2020). Do attractants bias the results of Malaise trap research? *Journal of Emerging Investigators, 3*.

McAlpine, J.F. (1965). Insects and related terrestrial invertebrates of Ellef Ringnes Island. *Arctic, 18*(2), 73-103. doi: 10.14430/arctic3455

McAlpine, J.F. (1981). Key to families-adults. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M (Eds), *Manual of Nearctic Diptera. Volume 1*. (pp 89 -124). Research Branch Agriculture Canada.

Medeiros, A.S., Milošević, Đ., Francis, D.R., Maddison, E., Woodroffe, S., Long, A., Walker, I.R., Hamerlík, L., Quinlan, R., Langdon, P., Brodersen, K.P. & Axford, Y. (2020). Arctic chironomids of the northwest North Atlantic reflect environmental and biogeographic gradients. *Journal of Biogeography, 48*(3), 511-525. doi: 10.1111/jbi.14015

Mesmin, X., Vincent, M., Tricault, Y., Estorgues, V., Daniel, L., Cortesero, A., Faloya, V. & Le Ralec, A. (2019). Assessing the relationship between pest density and plant damage: a case study with the belowground herbivore *Delia radicum* (Diptera: Anthomyiidae) on broccoli. *Applied Entomology and Zoology, 54*(2), 155-165. doi: 10.1007/s13355-019-00607-3

Messing, J. (1983). New M13 vectors for cloning. *Methods in Enzymology, 101,* 20-78. 10.1016/0076-6879(83)01005-8

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

Miller, L.A. (1951). Observations on the bionomics of some Northern species of Tabanidae (Diptera). *Canadian Journal of Zoology, 29*(3), 240-265. doi: 10.1139/z51-023

Molinatto, G., Demichelis, S., Bodino, N., Giorgini, M., Mori, N. & Bosco, D. (2020). Biology and prevalence in Northern Italy of *Verrallia aucta* (Diptera, Pipunculidae), a parasitoid of *Philaenus spumarius* (Hemiptera, Aphrophoridae), the main vector of *Xylella fastidiosa* in Europe. *Insects, 11*(9), 607. doi: 10.3390/insects11090607

Murray, D. A. (1987) *Conchapelopia aagaardi* n. sp., a new species of Tanypodinae (Diptera: Chironomidae) from Norway. In: Sæther, O. A. (ed.): A conspectus of contemporary studies in Chironomidae (Diptera). Contributions from the IXth International Symposium on Chironomidae, Bergen, Norway, 1985. *Entomologica Scandinavica, Suppl., 29*, 161-166.

Murray, D. A., Fittkau, E. J. (1989) The adult males of Tanypodinae (Diptera: Chironomidae) of the Holarctic region - Keys and diagnoses. In: Wiederholm, T. (ed.): Chironomidae of the Holarctic region. Keys and diagnoses. Part 3. Adult males. *Entomologica Scandinavica, Suppl.,* 34, 37-123.

Nneji, L.M., Adeola, A.C., Ayoola, A.O., Oladipo, S.O., Wang, Y-Y., Malann, Y.D., Anyaele, O., Nneji, I.C., Rahman, M.M. & Olory, C.S. (2020). DNA barcoding and species delimitation of butterflies (Lepidoptera) from Nigeria. *Molecular Biology Reports, 47*(12), 9441-9457. doi: 10.1007/s11033-020-05984-5

Northern Biodiversity Program. (n.d.) *About the NMP.* Northern Biodiversity Program. Retrieved from https://northernbiodiversity.wordpress.com/about-the-nbp/

Nugent, C.M., Elliott, T.A., Ratnasingham, S., Adamowicz, S.J. (2020). Coil: an R package for cytochrome c oxidase I (CO1) DNA barcode data cleaning, translation, and error evaluation. Genome, 63(6), 291-305. doi: 10.1139/gen-2019-0206

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C. & Weedon, J. (2022). vegan: Community Ecology Package. R package version 2.6-4. https://CRAN.R-project.org/package=vegan.

Olafson, PU., Aksoy, S., Attardo, G.M., Buckmeier, G., Chen, X., Coates, C.J., Davis, M., Dykema, J., Emrich, S.J., Friedrich, M., Holmes, C.J., Ioannidis, P., Jansen, E.N., Jennings, E.C., Lawson, D., Martinson, E.O., Maslen, G.L., Meisel, R.P., Murphy, T.D., Nayduch, D., Nelson, D.R., Oyen, K.J., Raszick, T.J., Ribeiro, J.M.C., Robertson, H.M., Rosendale, A.J., Sackton, T.B., Saelao, P., Swiger, S.L., Sze, S., Tarone, A.M., Taylor, D.B., Warren, W.C., Waterhouse, R.M., Weirauch, M.T., Werren, J.H., Wilson, R.K., Zdobnov, E.M. & Benoit, J.B. (2021). The genome of the stable fly, *Stomoxys calcitrans*, reveals potential mechanisms underlying reproduction, host interactions, and novel targets for pest control. *BMC Biology, 19*(1), 41. doi: 10.1186/s12915-021-00975-9

Oliver, D.R. (1981). Chironomidae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), *Manual of Nearctic Diptera. Volume 1.* (pp. 423 – 458). Research Branch Agriculture Canada

Oliver, D. R. (1989). The adult males of Diamesinae (Diptera: Chironomidae) of the Holarctic region - Keys and diagnoses. In: Wiederholm, T. (ed.): Chironomidae of the Holarctic region. Keys and diagnoses. Part 3 - Adult males. *Entomologica Scandinavica, Suppl., 34*, 129-154.

Oliver, D. R., & Dillon, M. E. (1994). Systematics of some species of *Micropsectra* (Diptera: Chironomidae) living in low-order streams in southern Ontario, Canada. *The Canadian Entomologist, 126*(2), 199-217. doi: 10.4039/Ent126199-2

Oliver, D. R. & Roussel, M. E. (1983) Redescription of *Brillia* Kieffer (Diptera: Chironomidae) with descriptions of Nearctic species. *Canadian entomologist, 115*, 257-279. doi: 10.4039/Ent115257-3

Oosterbroek, P. (1984). A revision of the crane-fly genus Nephrotoma Meigen, 1803, in North America (Diptera, Tipulidae). Part II. The non-dorsalis species-groups. *Beaufortia, 34,* 117–180.

Pava-Ripoll, M., Pearson, R.E.G., Miller, A.K. & Ziobro, G.C. (2012). Prevalence and relative risk of *Cronobacter* spp., *Salmonella* spp., and *Listeria monocytogenes* associated with the body surfaces and guts of individual filth flies. *Applied and Environmental Microbiology, 78*(22), 7891-7902. doi: 10.1128/AEM.02195-12

Pentinsaari, M. (2016). *Utility of DNA barcodes in identification and delimitation of beetle species, with insights into COI protein structure across the animal kingdom.* [Doctoral Dissertation, University of Oulu]. http://jultika.oulu.fi/files/isbn9789526212104.pdf

Pentinsaari, M., Blagoev, G.A., Hogg, I.D., Levesque-Beaudin, V., Perez, K., Sobel, C.N., Vandenbrink, B. & Borisenko, A. (2020). A DNA barcoding survey of an Arctic arthropod community: Implications for future monitoring. *Insects, 11*(1), 46. doi: 10.3390/insects11010046

Pentinsaari, M., Hebert, P.D.N. & Mutanen, M. (2014). Barcoding beetles: a regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. *PLOS ONE, 9*(9). doi: 10.1371/journal.pone.0108651

Peterson, B.V. (1981). Simuliidae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), Manual of Nearctic Diptera. Volume 1. (pp. 247 – 256). Research Branch Agriculture Canada.

Pohjoismäki, J.L.O., Kahanpää, J. & Mutanen, M. (2016). DNA barcodes for the Northern European Tachinid flies (Diptera: Tachinidae). *PLOS ONE, 11*(11). doi: 10.1371/journal.pone.0164933

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 Soós Á, Papp L. (Eds.), *Catalogue of Palaearctic Diptera, volume 11:Scathophagidae-Hypodermatidae.* (pp. 55-215). Budapest: Akadémiai Kiadó.

Pont, A.C. (2011). The Muscidae described by J.W. Zetterstedt (Insecta: Diptera). *Zootaxa, 2852*(1), 1-83. doi: 10.11646/zootaxa.2852.1.1

Porco, D., Rougerie, R., Deharveng, L. & Hebert, P.D.N. (2010). Coupling non-destructive DNA extraction and voucher retrieval for small soft-bodied Arthropods in a high-throughput context: the

example of Collembola. *Molecular Ecology Resources, 10*(6), 942-945. doi: 10.1111/j.1755-0998.2010.2839.x

Porco, D., Skarżyński, D., Decaëns, T., Hebert, P.D.N. & Deharveng, L. (2013). Barcoding the Collembola of Churchill: a molecular taxonomic reassessment of species diversity in a Sub-Arctic area. *Molecular Ecology Resources, 14*(2), 249-261. doi: 10.1111/1755-0998.12172

Posada-López, L., Rodrigues, B.L., Velez, I.D. & Uribe, S. (2023). Improving the COI DNA barcoding library for Neotropical phlebotomine sand flies (Diptera: Psychodidae). *Parasites & Vectors, 16*(1), 198. doi: 10.1186/s13071-023-05807-z

R Core Team. (2023). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Ratnasingham, S. & Hebert, P.D.N. (2007). Bold: the barcode of life data system. *Molecular Ecology Notes, 7*(3), 355-364. doi:10.1111/j.1471-8286.2007.01678.

Ratnasingham, S. & Hebert, P.D.N. (2013). A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. *PLoS ONE, 8*(7), e66213.

Reiss, F., & Fittkau, E. J. (1971). Taxonomie und Ökologie europäisch verbreiteter *Tanytarsus*-Arten (Chironomidae, Diptera). *Archiv für Hydrobiologie Suppl., 40*, 75-200.

Reiss, F., & Säwedal, L. (1981). Keys to males and pupae of the Palaearctic (excl. Japan) *Paratanytarsus* Thienemann & Bause, 1913, n. comb., with descriptions of three new species (Diptera: Chironomidae). *Entomologica Scandinavica, Suppl., 15*, 73-104.

Renaud, A.K., Savage, J. & Roughley, R.E. (2012a). Muscidae (Diptera) diversity in Churchill, Canada, between two time periods: evidence for limited changes since the Canadian Northern Insect Survey. *The Canadian Entomologist, 144*(1), 29-51. doi: 10.4039/tce.2012.6

Renaud, A.K., Savage, J. & Adamowicz, S.J. (2012b). DNA barcoding of Northern Nearctic Muscidae (Diptera) reveals high correspondence between morphological and molecular species limits. *BMC Ecology, 12*(1), 24. doi: 10.1186/1472-6785-12-24

Rivera, J. & Currie, D.C. (2009). Identification of Nearctic black flies using DNA barcodes (Diptera: Simuliidae). *Molecular Ecology Resources, 9*(s1), 224-236. doi: 10.1111/j.1755-0998.2009.02648.x

Roback, S. S. (1971) The adults of the subfamily Tanypodinae (= Pelopiinae) in North America (Diptera: Chironomidae). *Monographs, Academy of Natural Sciences of Philadelphia, 17,* 1-410.

Robinson, H. & Vockeroth, J.R. (1981). Dolichopodidae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), Manual of Nearctic Diptera. Volume 1. (pp. 247 – 256). Research Branch Agriculture Canada.

Roslin, T., Somervuo, P., Pentinsaari, M., Hebert, P.D.N., Agda, J., Ahlroth, P., Anttonen, P., Aspi, J., Blagoev, G., Blanco, S., Chan, D., Clayhills, T., deWaard, J., deWaard, S., Elliot, T., Elo, R., Haapala, S., Helve, E., Ilmonen, J., Hirvonen, P., Ho, C., Itämies, J., Ivanov, V., Jakovlev, J., Juslén, A., Jussila, R., Kahanpää, J., Kaila, L., Jari-PekkaKaitila, Kakko, A., Kakko, I., Karhu, A., Karjalainen, S., Kjaerandsen, J., Koskinen, J., Laasonen, E.M., Laasonen, L., Laine, E., Lampila, P., Levesque-Beaudin, V., Lu, L., Lähteenaro, M., Majuri, P., Malmberg, S., Manjunath, R., Martikainen, P.,

Mattila, J., McKeown, J., Metsälä, P., Miklasevskaja, M., Miller, M., Miskie, R., Muinonen, A., Veli-MattiMukkala, Naik, S., Nikolova, N., Nupponen, K., Ovaskainen, O., Österblad, I., Paasivirta, L., Pajunen, T., Parkko, P., Paukkunen, J., Penttinen, R., Perez, K., Pohjoismäki, J., Prosser, S., Raekunnas, M., Rahulan, M., Rannisto, M., Ratnasingham, S., Raukko, P., Rinne, A., Rintala, T., Romo, S.M., Salmela, J., Salokannel, J., Savolainen, R., Schulman, L., Sihvonen, P., Soliman, D., Sones, J., Steinke, C., Ståhls, G., Tabell, J., Tiusanen, M., Várkonyi, G., Vesterinen, E.J., Viitanen, E., Vikberg, V., Viitasaari, M., Vilen, J., Warne, C., Wei, C., Winqvist, K., Zakharov, E. & Mutanen, M. (2021). A molecular-based identification resource for the arthropods of Finland. *Molecular Ecology Resources, 22*(2), 803-822. doi: 10.1111/1755-0998.13510

Rossaro, B., Marziali, L. & Boggero, A. (2022). Response of Chironomids to key environmental factors: perspective for biomonitoring. *Insects, 13*(10), 911. doi: 10.3390/insects13100911

Sabrosky, C.W. 1936. A synopsis of the Nearctic species of Oscinella and Madiza, based on a study of the types (Diptera, Chloropidae). Annals Entomological Society of America, 29: 707—728.

Sabrosky, C.W. 1938. Taxonomic notes on the dipterous family Chloropidae. Journal of the New York Entomological Society. 46(4): 417—434.

Sabrosky, C.W. 1940. Twelve New North American species of Oscinella (Diptera: Chloropidae). The Canadian Entomologist. 72(11): 214—230.

Sabrosky, C.W. 1943 A revised synopsis of Nearctic Thaumatomyia (=Chloropisca) (Diptera: Chloropidae). The Canadian Entomologist, 75(6): 109-117.

Sabrosky, C.W. 1948. A synopsis of the Nearctic species of Elachiptera and related genera (Diptera: Chloropidae). Journal of the Washington Academy of Sciences, 38(11): 365—382.

Sabrosky, C.W. 1950 A new species of Eribolus from California (Diptera, Chloropidae. The Pan-Pacific Entomologist, 26: 91--92.

Sabrosky, C.W. 1951 A revision of nearctic species of Gaurax (Diptera: Chloropidae) The American Midland Naturalist, 45(2): 407—431.

Sabrosky, C.W. and Valley, K.R. 1987. A new Elachiptera from salt marshes, with a redescription of E. penita and partially revised key to nearctic Elachiptera (Diptera: Chloropidae). Proceedings of the Entomological Society of Washington. 89: 581—586.

Sæther, O. A. (1969). Some nearctic Podonominae, Diamesinae, and Orthocladiinae (Diptera: Chironomidae). *Bulletin/Fisheries Research Board of Canada, 170*, 1-154.

Sæther, O. A. (1975a). Nearctic and Palaearctic *Heterotrissocladius* (Diptera: Chironomidae). *Bulletin/Fisheries Research Board of Canada,* 193, 67.

Sæther, O. A. (1975b). Twelve new species of *Limnophyes* Eaton, with keys to Nearctic males of the genus (Diptera: Chironomidae). *Canadian entomologist,* 107(10), 1029-1056. doi: 10.4039/Ent1071029-10

Sæther, O. A. (1977). Taxonomic studies on Chironomidae: *Nanocladius*, *Pseudochironomus*, and the *Harnischia* complex. *Bulletin/Fisheries Research Board of Canada, 196*, 1-143.

Sæther, O. A. (1986). A review of the genus *Rheocricotopus* Thienemann & Harnisch, 1932, with the description of three new species (Diptera, Chironomidae) In: Fittkau, E. J. (ed.): Beiträge zur Systematik der Chironomidae, Diptera. *Spixiana Suppl, 11,* 59-108.

Sæther, O. A. (1989). *Metriocnemus* van der Wulp: a new species and a revision of species described by Meigen, Zetterstedt, Stæger, Holmgren, Lundström and Strenzke (Diptera: Chironomidae). *Entomologica Scandinavica, 19*(4), 393-430.

Sæther, O. A. (1990). A review of the genus *Limnophyes* Eaton from the Holarctic and Afrotropical regions (Diptera: Chironomidae, Orthocladiinae). *Entomologica Scandinavica, Suppl., 35*, 135.

Sæther, O. A. (1995). *Metriocnemus* van der Wulp: Seven new species, revision of species, and new records (Diptera: Chironomidae). *Annales de limnologie, 31*(1), 35-64. doi: 10.1051/limn/1995002

Sæther, O. A. (2004a). A review of *Orthocladius* subgenus *Symposiocladius* Cranston (Diptera: Chironomidae). *Aquatic Insects, 25*(4), 281-317. doi: 10.1076/aqin.25.4.281.26221

Sæther, O. A. (2004b). Three new species of *Orthocladius* subgenus *Eudactylocladius* (Diptera: Chironomidae) from Norway. *Zootaxa, 508*(1), 1-12. doi: 10.11646/zootaxa.508.1.1

Sæther, O. A. (2009). *Cryptochironomus* Kieffer from Lake Winnipeg, Canada, with a review if Nearctic species (Diptera: Chironomidae). *Zootaxa, 2208*(1), 1-24. doi: 10.11646/zootaxa.2208.1.1

Sæther, O. A. (2010).*Cryptotendipes* Lenz from Manitoba, Canada, with keys to known immatures of the genus (Diptera: Chironomidae). *Zootaxa, 2412*, 1-2.

Sæther, O. A. & Halvorsen, G. A. (1981). Diagnoses of *Tvetenia* Kieff. emend., *Dratnalia* n. gen., and *Eukiefferiella* Thien. emend., with a phylogeny of the *Cardiocladius* group (Diptera: Chironomidae). *Entomologica Scandinavica, Suppl., 15,* 269-285.

Sæther, O. A. & Oyewo, E. A. (2008). Keys, phylogenies and biogeography of *Polypedilum* subgenus *Uresipedilum* Oyewo and Sæther (Diptera, Chironomidae). *Zootaxa, 1806*, 1-34.

Sæther, O. A. & Sublette, J. E. (1983). A review of the genera *Doithrix* n. gen., *Georthocladius* Strenzke, *Parachaetocladius* Wülker and *Pseudorthocladius* Goetghebuer (Diptera: Chironomidae, Orthocladiinae). *Entomologica Scandinavica, Suppl*., *20,* 100.

Sæther, O. A. & Wang, X. (1995). Revision of the genus *Paraphaenocladius* Thienemann, 1924 of the world (Diptera: Chironomidae, Orthocladiinae). *Entomologica Scandinavica, Suppl*., *48,* 1-69.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*(4), 406-425. doi: 10.1093/oxfordjournals.molbev.a040454

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

Säwedal, L. (1981). Description of *Micropsectra tori* n. sp. from Greenland, with notes on the *recurvata*-group (Diptera: Chironomidae). *Entomologica Scandinavica, 12*, 27-30.

Schlee, D. (1968) Vergleichende Merkmalsanalyse zur Morphologie und Phylogenie der *Corynoneura*-Gruppe (Diptera, Chironomidae). Zugleich eine Allgemeine Morphologie der Chironomiden-Imago (?) *Stuttgarter Beiträge zur Naturkunde, 180*, 150.

Schliep K.P. (2011). phangorn: phylogenetic analysis in R. Bioinformatics, 27(4), 592-593. doi: 10.1093/bioinformatics/btq706

Scudder, G.G.E. & Cannings, R.A. (2006). *The Diptera Families of British Columbia.* University of British Columbia.

Shamshad, A. (2010). The development of integrated pest management for the control of mushroom sciarid flies, *Lycoriella ingenua* (Dufour) and *Bradysia ocellaris* (Comstock), in cultivated mushrooms. *Pest Management Science, 66*(10), 1063-1074. doi: 10.1002/ps.1987

Sinclair, B.J. (2008). The systematics of New World *Clinocera* Meigen (Diptera: Empididae: Clinocerinae). NRC Research Press, Ottawa. viii + 245 pp.

Sinclair, B.J., Brooks, S.E., Cumming, J.M., & Coovert, G.A. (2011). Revision of the Nearctic species of *Heleodromia* (Diptera: Empidoidea: Brachystomatidae). *The Canadian Entomologist*, *143*(6), 629–649. doi: 10.4039/n11-036

Skevington, J. & Marshall, S.A. (1998). Systematics of New World *Pipunculus* (Diptera: Pipunculidae). Thomas Say Publications in Entomology: Monographs. Lanham, Maryland: Entomological Society of America. 201 pp.

Skevington, J.H. (2005). Revision of Nearctic *Nephrocerus* Zetterstedt (Diptera: Pipunculidae). *Zootaxa, 977*, 1-36.

Skvarla, M.J., Larson, J.L., Fisher, J.R. & Dowling, A.P.G. (2020). A review of terrestrial and canopy Malaise traps. *Annals of the Entomological Society of America, 114*(1), 27-47. doi: 10.1093/aesa/saaa044

Smith, R.E., Veldhuis, H., Mills, G.F., Eilers, R.G., Fraser, W.R. & Lelyk, G.W. (1998). *Terrestrial Ecozones, Ecoregions, and Ecodistricts of Manitoba: An Ecological Stratification of Manitoba's Natural Landscapes.* Agriculture and Agri-Food Canada. Available at: https://sis.agr.gc.ca/cansis/publications/ecostrat/provDescriptions/mbteee/mbteee_report.pdf

Snow, W.A. (1891). The moose fly – a new *Haematobia*. *The Canadian Entomologist, 23*(4), 87–89. doi: 10.4039/Ent2387-4

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 Latreille (Diptera: Muscidae). *American Museum Novitates, 1675*, 1–40.

Soponis, A. R. (1977) A revision of the Nearctic species of *Orthocladius* (*Orthocladius*) van der Wulp (Diptera: Chironomidae). *Memoirs of the Entomological Society of Canada, 102*, 1-187.

Soponis, A. R. (1990) A revision of the Holarctic species of *Orthocladius* (*Euorthocladius*) (Diptera: Chironomidae). *Spixiana Suppl.,* 13, 1-56.

Spencer, K.A. (1969). The Agromyzidae of Canada and Alaska. *Memoirs of the Entomological Society of Canada 64*, 1-311

Spencer, K.A. (1976). The Agromyzidae of Fennoscandia and Denmark. Part II. *Fauna Entomologica Scandinavica, 5,* 305-606

Spencer, K.A. & Steyskal, G.C. (1986). Manual of the Agromyzidae (Diptera) of the United States. U. S. Department of Agriculture, *Agriculture Handbook 638*. 478 pp

Srivathsan, A., Ang, Y., Heraty, J.M., Hwang, W.S., Jusoh, W.F.A., Kutty, S.N., Puniamoorthy, J., Yeo, D., Roslin, T. & Meier, R. (2023). Convergence of dominance and neglect in flying insect diversity. *Nature Ecology & Evolution.*

Stahlhut, J.K., Fernández-Triana, J., Adamowicz, S.J., Buck, M., Goulet, H., Hebert, P.D.N., Huber, J.T., Merilo, M.T., Sheffield, C.S., Woodcock, T. & Smith, M.A. (2013). DNA barcoding reveals diversity of Hymenoptera and the dominance of parasitoids in a Sub-Arctic environment. *BMC Ecology, 13*(1), 2. doi: 10.1186/1472-6785-13-2

Starý, J. and Brodo, F. (2009). Arctic species the subgenus Symplecta sensu stricto (Diptera: Limoniidae). *Canadian Entomologist, 141,* 1–30.

Steffan, W.A. (1981). Sciaridae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), *Manual of Nearctic Diptera. Volume 1.* (pp. 247 – 256). Research Branch Agriculture Canada.

Stein, P. (1898). Nordamerikanische Anrhomyiden. Bcitrag zur Dipterenfauna der Vereinigten Staaten. *Berliner Entomolog. Zeitschr.* (1897). *42*, 161-288

Stein, P. (1920). Nordamerikanische Anthomyiden, 2. Beitrag. *Arch. Naturgesch.* (1918). *84A*(9), 1-106

Steyskal, G.C. & Knutson, L.V. (1981). Empididae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), Manual of Nearctic Diptera. Volume 1. (pp. 247 – 256). Research Branch Agriculture Canada.

Stone, A. (1981). Culicidae. In McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M. (Eds), Manual of Nearctic Diptera. Volume 1. (pp. 247 – 256). Research Branch Agriculture Canada.

Stur, E., Silva, F. L. da, Ekrem, T. (2019) Back from the past: DNA barcodes and morphology support *Ablabesmyia americana* Fittkau as a valid species (Diptera: Chironomidae). *Diversity [Basle]*, *11*(9, paper 173), 15.

Sublette, J. E. (1964). Chironomidae (Diptera) of Lousiana I. Systematics and immature stages of some lentic chironomids of West-Central Lousiana. Tulane Studies in Zoology, 11(4), 109-150. doi: 10.5962/bhl.part.7051

Sublette, J. E. (1967a). Type specimens of Chironomidae (Diptera) in the Canadian National Collections, Ottawa. *Journal of the Kansas Entomological Society, 40*(3), 290-331.

Sublette, J. E. (1967b). Type specimens of Chironomidae (Diptera) in the Cornell University collection. *Journal of the Kansas Entomological Society, 40*(4), 477-564.

Sublette, J. E. (1970). Type specimens of Chironomidae (Dipt.) in the Illinois Natural History Survey collection, Urbana. *Journal of the Kansas Entomological Society, 43*(1), 44-95.

Tamura, K., Stecher, G. & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022-3027. doi: 10.1093/molbev/msab120

Tangelder, I. (1983). A revision of the Crane Fly genus Nephrotoma Meigen, 1803, in North America (Diptera, Tipulidae), Part 1, the dorsalis species-group. *Beaufortia, 33,* 111–205.

Thompson, F.C. *A Conspectus of Nearctic Syrphidae.* Unpublished.

Thompson, F. C. (1991). A conspectus of the flower flies (Diptera: Syrphidae) of the Nearctic Region. Unpublished manuscript.

Tiusanen, M., Hebert, P.D.N., Schmidt, N.M. & Roslin, T. (2016). One fly to rule them all – muscid flies are the key pollinators in the Arctic. *Proceedings of the Royal Society: Biological Sciences, 283*(1839), 20161271. doi: 10.1098/rspb.2016.1271

Townes, H. K. Jr. (1945). The Nearctic species of Tendipedini [Diptera; Tendipedidae (= Chironomidae)]. The American Midland Naturalist Journal, 34(1), 1-206.

Tuiskunen, J. (1986). The Fennoscandian species of *Parakiefferiella* Thienemann (Diptera, Chironomidae, Orthocladiinae). *Annales Zoologici Fennici, 23*(2), 175-196.

Twinn, C.R., Hocking, B., McDuffie, Wm.C. & Cross, H.F. (1948). A preliminary account of the biting flies at Churchill, Manitoba. *Canadian Journal of Research, 26*(6), 334-357. doi: 10.1139/cjr48d-025

Uhler, J., Haase, P., Hoffmann, L., Hothorn, T., Schmidl, J., Stoll, S., Welti, E.A.R., Buse, J. & Müller, J. (2022). A comparison of different Malaise trap types. *Insect Conservation and Diversity, 15*(6), 666-672. doi: 10.1111/icad.12604

Vockeroth, J.R. (1992) The flower flies of the subfamily Syrphinae of Canada, Alaska and Greenland (Vol. 18). Ottawa: Canada Communications Group - Publishing. 456 pp.

Vockeroth, J.R. & Thompson, F.C. (1987). Manual of Nearctic Diptera.

Votýpka, J., Brzoňová, J., Ježek, J. & Modrý, D. (2019). Horse flies (Diptera: Tabanidae) of three West African countries: A faunistic update, barcoding analysis and trypanosome occurrence. *Acta Tropica, 197*. doi: 10.1016/j.actatropica.2019.105069

Walker, F. (1849). List of the specimens of dipterous insects in the collection of the British Museum. *Pt. 4. pp.* 923-972. London.

Wearing, C. H., Marshall, R. R., Attfield, B. & Colhoun, C. (2013). Phenology and distribution of the apple leafcurling midge (*Dasineura mali* (Kieffer)) (Diptera: Cecidomyiidae) and its natural enemies on apples under biological and integrated pest management in Central Otago, New Zealand. *New Zealand Entomologist, 36*(2), 87-106. doi: 10.1080/00779962.2012.712887

Weber, N.A. (1950). A survey of the insects and related arthropods of arctic Alaska. Part 1. *Transactions of the American Entomological Society, 76*(3), 147-206.

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

Wheeler, T.A. 1994. A revision of the genus Epichlorops Becker (Diptera: Chloropidae). Insect Systematics and Evolution, 25(4): 393—414.

Wickham, H. (2016). ggplot2: *Elegant graphics for data analysis.* Springer-Verlag New York. https://ggplot2.tidyverse.org

Wickham, H., François, R., Henry, L., Müller, K. & Vaughan, D. (2023). *dplyr: A Grammar of Data Manipulation*. https://dplyr.tidyverse.org, https://github.com/tidyverse/dplyr

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K. & Yutani, H. (2019). Welcome to the Tidyverse. *The Journal of Open Source Software, 4*(43), 1686. doi: 10.21105/joss.01686

Wiederholm, T. (ed.) (1989) Chironomidae of the Holarctic region. Keys and diagnoses. Part 3. Adult males. - *Entomologica Scandinavica, Suppl. 34,* 532.

Wiegmann, B.M., Trautwein, M.D., Winkler, I.S. & Yeates, D.K. (2011) Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences of the USA*, *108*(14), 5690-5695. doi: 10.1073/pnas.1012675108

Wirta, H.K., Vesterinen, E.J., Hambäck, P.A., Weingartner, E., Rasmussen, C., Reneerkens, J., Schmidt, N.M., Gilg, O. & Roslin, T. (2015a). Exposing the structure of an Arctic food web. *Ecology and Evolution, 5*(17), 3842-3856. doi: 10.1002/ece3.1647

Wirta, H.K., Weingartner, E., Hambäck, P.A. & Roslin, T. (2015b). Extensive niche overlap among the dominant arthropod predators of the High Arctic. *Basic and Applied Ecology, 16*(1), 86-92. doi: 10.1016/j.baae.2014.11.003

Wirta, H., Várkonyi,, G., Rasmussen, C., Kaartinen, R., Schmidt, N.M., Hebert, P.D.N., Barták, M., Blagoev, G., Disney, H., Ertl, S., Gjelstrup, P., Gwiazdowicz, D.J., Huldén, L., Ilmonen, J., Jakovlev, J., Jaschhof, M., Kahanpää, J., Kankaanpää, T., Krogh, P.H., Labbee, R., Lettner, C., Michelsen, V., Nielsen, S.A., Nielsen, T.R., Paasivirta, L., Pedersen, S., Pohjoismäki, J., Salmela, J., Vilkamaa, P., Väre, H., von Tschirnhaus, M., Roslin, T. (2016). Establishing a community-wide DNA barcode library as a new tool for arctic research. *Molecular Ecology Resources, 16*(3), 809-822. doi: 10.1111/1755-0998.12489

Woodcock, T.S., Boyle, E.E., Roughley, R.E., Kevan, P.G., Labbee, R.N., Smith, A.B.T., Goulet, H., Steinke, D. & Adamowicz, S.J. (2013). The diversity and biogeography of the Coleoptera of Churchill: insights from DNA barcoding. *BMC Ecology, 13*(1), 40-40. doi: 10.1186/1472-6785-13-40

World Health Organization. (2016). *Mosquito control: can it stop Zika at source?* https://reliefweb.int/report/world/mosquito-control-can-it-stop-zika-source

World Health Organization (2020). *World Malaria Report 2020: 20 Years of Global Progress and Challenges.* https://www.who.int/publications/i/item/9789240015791

Wright, E.S. (2016). Using DECIPHER v2.0 to analyze big biological sequence data in R. The R Journal, 8(1), 352-359. Doi: 10.32614/RJ-2016-025

Wulder, M.A., Nelson, T.A. EOSD Land Cover Classification Legend Report. Version 2. (2003) Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC. https://cfs.nrcan.gc.ca/publications?id=33752

Wülker, W. (1956) Zur Kenntnis der Gattung *Psectrocladius* Kieff. (Dipt., Chironom.). Individuelle Variabilität, Grenzen und Möglichkeiten der Artentrennung, Ökologie und Verbreitung. *Archiv für Hydrobiologie, Suppl., 24*(1), 1-66.

Wülker, W., Ryser, H. M., & Scholl, A. (1981). Revision der Gattung *Chironomus* Meigen (Dipt.). VI. *C. holomelas* Keyl, *C. saxatilis* n. sp., *C. melanescens* Keyl. *Revue Suisse De Zoologie, 88*(4), 903-924. doi: 10.5962/bhl.part.82418

Young, M.R., Behan-Pelletier, V.M. & Hebert, P.D.N. (2012). Revealing the hyperdiverse mite fauna of Subarctic Canada through DNA barcoding. *PLOS ONE, 7*(11). doi: 10.1371/journal.pone.0048755

Zahiri, R., Lafontaine, J.D., Schmidt, B.C., deWaard, J.R., Zakharov, E.V. & Hebert, P.D.N. (2014). A transcontinental challenge — a test of DNA barcode performance for 1,541 species of Canadian Noctuoidea (Lepidoptera). *PLOS ONE, 9*(3). doi: 10.1371/journal.pone.0092797

Zelentsov, N. I. (1985). Tablitsy dlya opredeleniya podrod i vidov roda *Psectrocladius* Kieff. (Diptera, Chironomidae). [Keys for the identification of subgenera and species in the genus Psectrocladius (Diptera, Chironomidae)]. In: Vodnye soobshchestva i biologiya gidrobiontov [Aquatic communities and biology of aquatic organisms]. *Akademija Nauk SSSR, Institut Biologii Vnutrennich Vod, Trudy, 52*, 119-137. [in Russian].

Zhou, X., Adamowicz, S.J., Jacobus, L.M., DeWalt R.E. & Hebert, P.D.N. (2009). Towards a comprehensive barcode library for arctic life - Ephemeroptera, Plecoptera, and Trichoptera of Churchill, Manitoba, Canada. *Frontiers in Zoology, 6*(1), 30. doi: 10.1186/1742-9994-6-30

Zhou, X., Jacobus, L.M., DeWalt, R.E., Adamowicz, S.J. & Hebert, P.D.N. (2010). Ephemeroptera, Plecoptera, and Trichoptera fauna of Churchill (Manitoba, Canada): insights into biodiversity patterns from DNA barcoding. *Freshwater Science, 29*(3), 814-837. doi: 10.1899/09-121.1

Zlobin, V.V. (1995). Review of mining flies of the genus *Metopomyza* Enderlein (Diptera: Agromyzidae). *International Journal of Dipterological Research 6*(2), 143-168

Zorina, O. V. (2004). Novye i maloizvestnye vidy komarov-zvontsov roda *Demicryptochironomus* Lenz (Diptera, Chironomidae) s ostrova Sakhalin. (New and little known species of the genus *Demicryptochironomus* Lenz (Diptera, Chironomidae) from Sakhalin Island). In: Bogatov, V. V., Barkalov, V. Yu., Lelej, A. S., Makarchenko, E. A., Storozhenko, S. Yu. (eds): Flora and fauna of Sakhalin Island (Materials of International Sakhalin Island Project), Pt 1: 224-237. Dal'nauka, Vladivostok.

Data and Accessibility and Benefit-Sharing Section

Data Accessibility Statement

The DNA barcode reference library is publicly available on the Barcode of Life Data Systems in the dataset DS-CDIPTERA (dx.doi.org/10.5883/DS-CDIPTERA) (Majoros et al., 2023). The sequences are also available on GenBank. A list of process IDs and GenBank accessions included in the dataset is available in supplementary file 3.

Benefit-Sharing Statement

Benefits from this research accrue from the public sharing of the DNA barcode reference library on the Barcode of Life Data Systems.

Author Contributions

All authors reviewed and edited the manuscript. SEM, TAE and SJA wrote the manuscript. SEM and TAE ran analyses and created figures. FB and CY identified crane flies from the families Cylindrotomidae, Limoniidae, Pediciidae and Tipulidae. JW conducted field work, identified specimens, processed the specimens for barcoding, did family-level determinations, and species-level determinations for Chloropidae. VLB did family-level determinations, co-ordination of sending representative vouchers to collaborators for determination, and contributed to manuscript writing. CJB identified the Mycetophilidae specimens. SEB, JMC and BJS identified the Empidoidea (Empididae and Dolichopodidae) specimens. SB identified the Agromyzidae specimens. DCC and PS did collecting and determinations for Simuliidae, Culicidae, and Tabanidae. TE and ES did collecting and determinations for Chironomidae. PDNH and RER contributed to the collecting. JS did Muscidae determinations for the 2010 clusters and the Anthomyiidae determinations. JHS did Syrphidae and Pipunculidae collecting and determinations. AMS conducted field work and specimen identifications. TAW did the Chloropidae, Piophilidae and Heleomyzidae identifications. TSW contributed to collecting and sorting the malaise traps to family. ADY identified Syrphidae specimens.

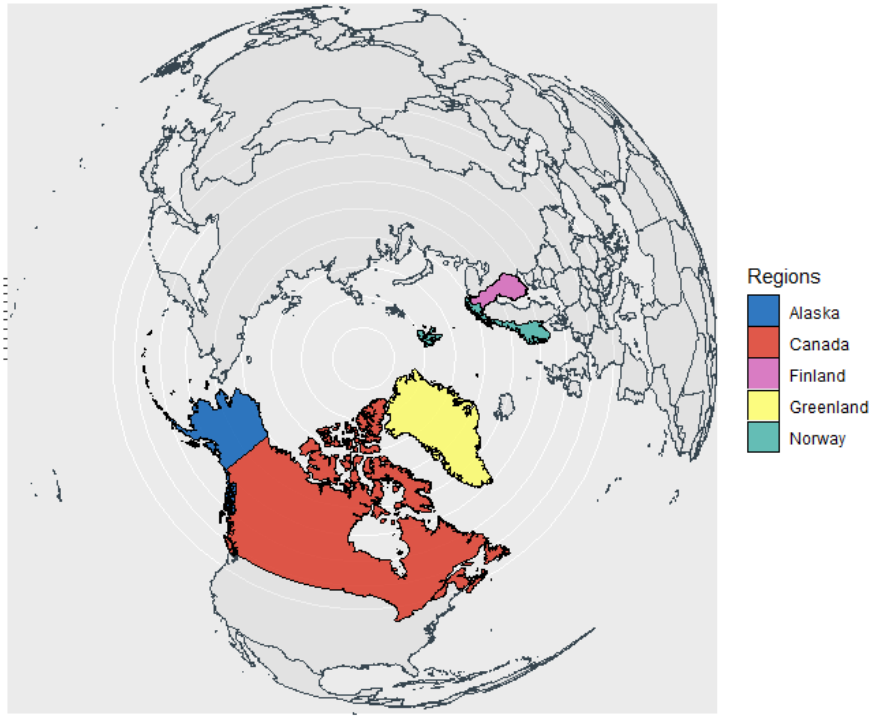
Tables and Figures

Taxa	Reference Material
Muscidae	Arntfield (1975), Collin (1930), Gregor et al. (2002), Hennig (1955-1964), Huckett (1932, 1934a, 1934b, 1936, 1954, 1965a, 1965b), Huckett and Vockeroth, (1987), Malloch (1918, 1919, 1920b, 1923), Michelsen (2006), Pont (1984, 1986, 2011), Savage (2003), Snow (1891), and Snyder (1949a, 1949b, 1954)
Empidoidea (Empididae and Hybotidae)	Comparisons were made with the Canadian National Collection of Insects (CNC) and the following publications: Barták (2002), Chillcott (1962), MacDonald (1994), Sinclair (2008), and Sinclair et al. (2011).
Tipuloidea (Cylindrotomidae, Limoniidae, Pediciidae and Tipulidae)	Alexander (1943), Brodo (1967, 1987, 2017), Byers (1961), Oosterbroek (1984), Starý and Brodo (2009), Tangelder (1983). Identifications were checked by comparing with specimens in the CNC and in the private collection of F. Brodo.
Syrphidae	Vockeroth (1992), Thompson (1991), Thompson (unpublished manuscript keys), and Vockeroth and Thompson (1987). Specimens were then compared to reference material in the University of Guelph Insect collection (DEBU) and to specimens in the CNC.
Pipunculidae	Albrecht (1990), De Meyer (1989), Hardy (1943), Skevington and Marshall (1998) and Skevington (2005) and compared to specimens in the CNC.
Agromyzidae	Boucher (2002, 2008, 2012a, 2012b), Griffiths (1964), Spencer (1969, 1976), Spencer & Steyskal (1986) and Zlobin (1995).
Chloropidae	Fedoseeva (1971, 2003), Sabrosky (1936, 1938, 1940, 1943, 1948, 1950, 1951, Sabrosky and Valley (1987), and Wheeler (1994 and unpublished keys). Specimens were then compared to reference material in the Lyman Entomological Museum (LEMQ) and specimens in the CNC and the Smithsonian Museum of Natural History (USNM).
Chironomidae	Chironomidae: Brundin (1949), Langton & Pinder (2007), Sæther (1977) and Wiederholm (1989). Tanypodinae: Fittkau (1962), Murray (1987), Murray & Fittkau (1989), Roback (1971) and Stur et al. (2019). Diamesinae & Podonominae: Brundin (1989) and Oliver (1989). Orthocladiinae: Brundin (1947), Cranston et al. (1989b), Cranston

	& Oliver (1988), Ferrington & Sæther (2011), Fittkau & Lehmann (1970), Fu & Sæther (2012), Gowin & Thienemann (1942), Hestenes & Sæther (2000), Hirvenoja (1973), Hirvenoja & Hirvenoja (1988), Langton (1985), Lehmann (1972), Liu & Wang (2005), Oliver & Roussel (1983), Sæther (1969, 1975a, 1975b, 1986, 1989, 1990, 1995, 2004a, 2004b), Sæther & Halvorsen (1981), Sæther & Sublette (1983), Sæther & Wang (1995), Schlee (1968), Soponis (1977, 1990), Sublette (1967a, 1967b, 1970), Tuiskunen (1986), Wülker (1956) and Zelentsov (1985). Chironominae: Anderson et al. (2013), Boesel (1985), Cranston et al. (1989a), Ekrem (2007), Ekrem et al. (2003), Epler (1987), Giłka (2001), Grodhaus (1987), Hirvenoja (1962), Jackson (1977), Kyerematen et al. (2000), Lehmann (1970a, 1970b), Lin et al. (2018), Lindeberg (1967), Lindeberg & Wiederholm (1979), Malloch (1915), Oliver & Dillon (1994), Reiss & Fittkau (1971), Reiss & Säwedal (1981), Sæther (1977, 2009, 2010), Sæther & Oyewo (2008), Säwedal (1981), Sublette (1964), Townes (1945), Wülker et al. (1981) and Zorina (2004). Specimens were identified morphologically, and slide mounts were made to look at structures with a compound microscope.
Anthomyiidae	Griffiths (1991a, 1991b, 1991c, 1993a, 1993b)

Table 1: The reference materials used to identify specimens for each Diptera family.

a)


Regions
Alaska
Canada
Finland
Greenland
Norway

b)

Yukon
Northwest Territories
Nunavut
British Columbia
Alberta
Saskatchewan
Manitoba
Ontario
Quebec
Churchill
Newfoundland and Labrador
Prince Edward Island
Nova Scotia
New Brunswick

c)

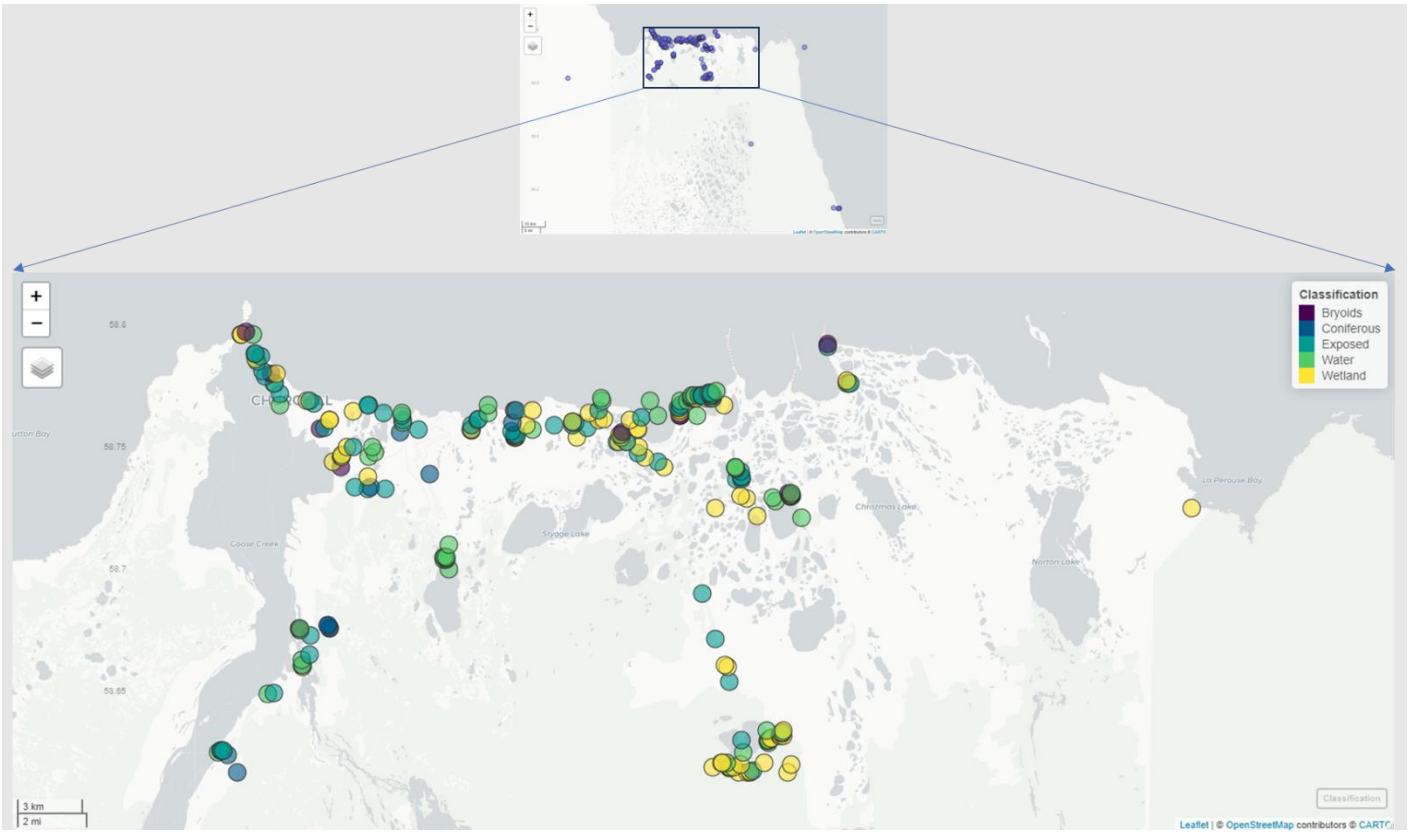


Figure 1: The location of Churchill, Manitoba, and the study sites within the region. a) shows the placement of Canada in relation to other Northern countries and regions. b) shows the location of Churchill within Canada. Churchill is located on the coast of Hudson Bay in Northern Manitoba. It is a Subarctic region located within the Coastal Hudson Bay Lowland ecoregion. c) shows the locations where samples were collected in the Churchill region and the land-use classification categories of sampling sites, with the most samples inferred using the EOSD classifications from satellite data. For details on the classification status for each site, please refer to Supplementary File 6.

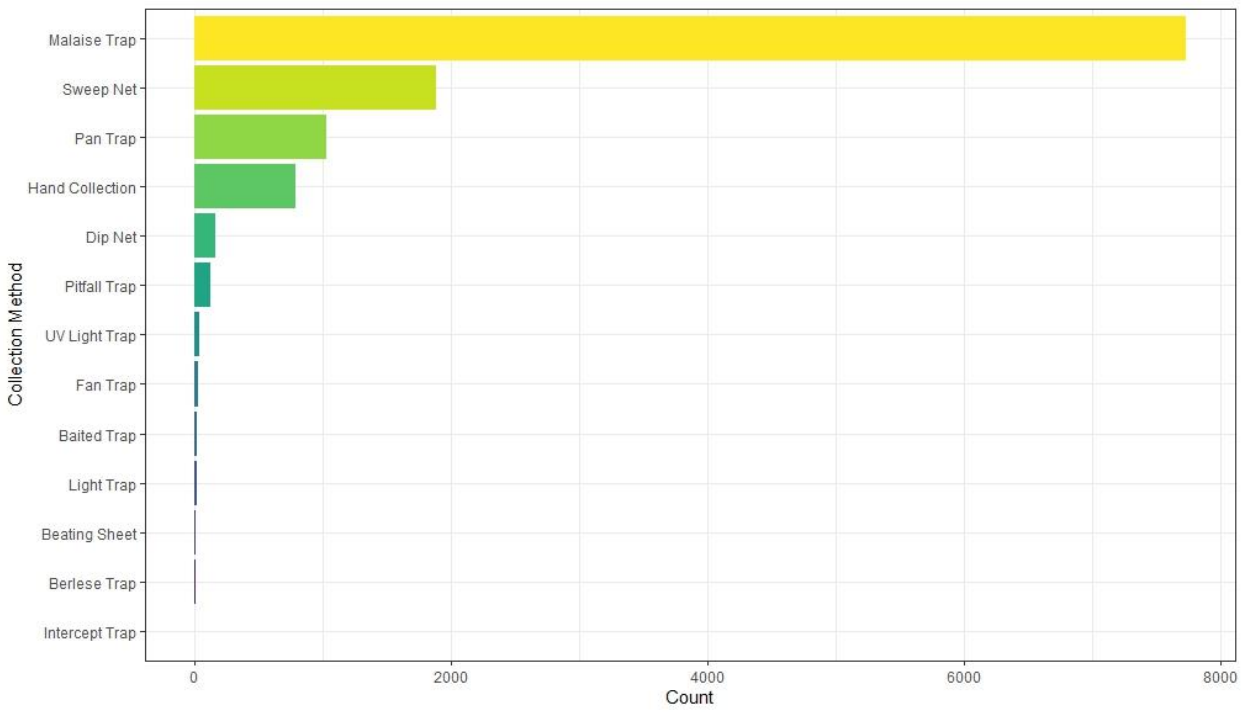
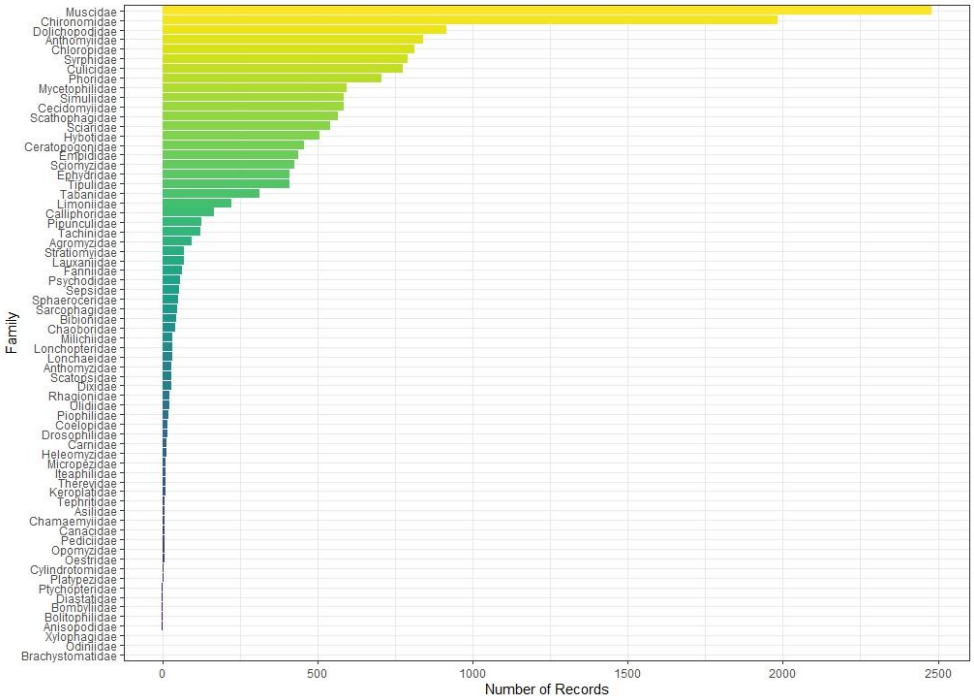


Figure 2: The number of Diptera specimens sampled with each collection method. The majority of specimens were caught using Malaise traps.

a)



b)

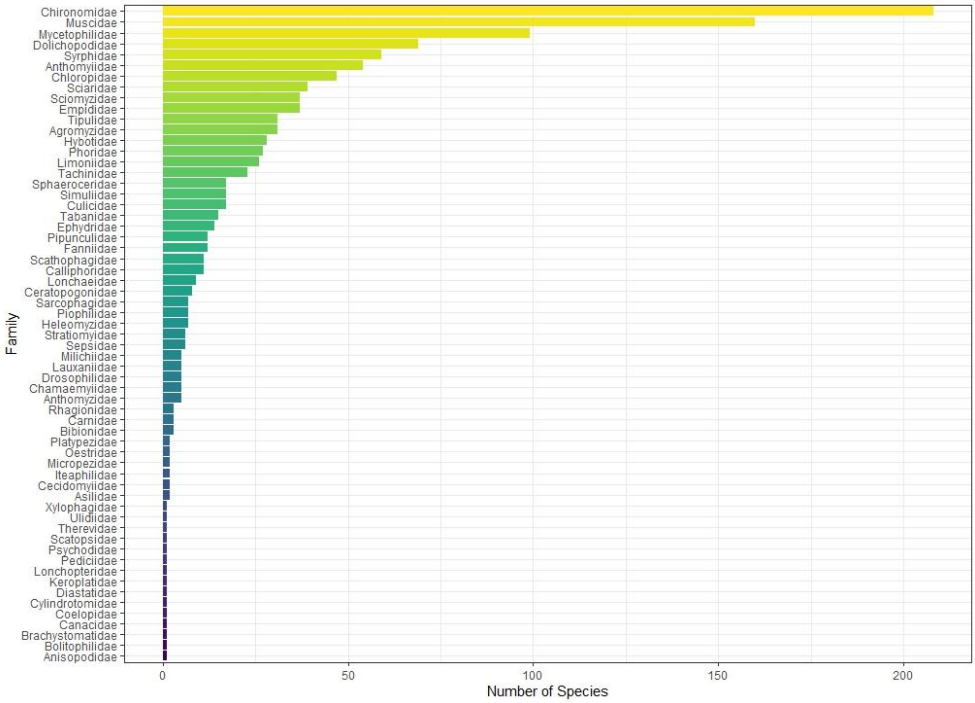


Figure 3: The number of a) records and b) species collected for each family. Muscidae was the most abundant family in regard to specimens, with 2478 specimens collected, while Chironomidae had the highest species richness, with 208 species recorded.

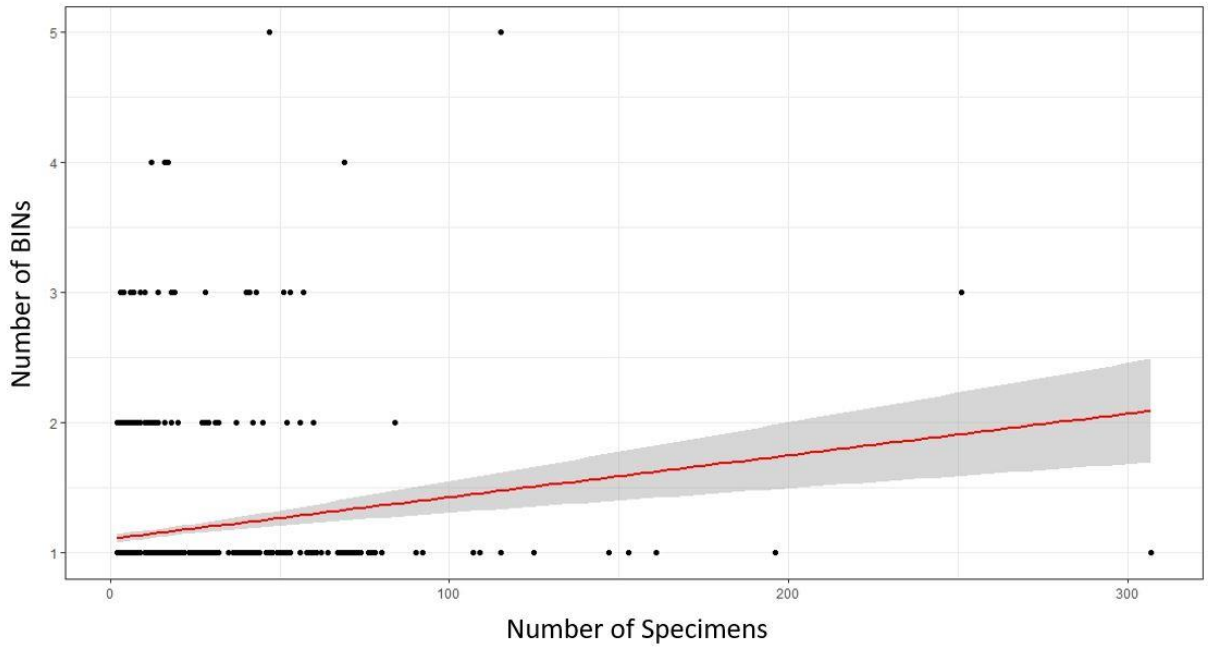


Figure 4: The relationship between the number of specimens of each species and the number of BINs associated with each species. Each dot represents a species. Considering only those species with 2 or more specimens, there was a significant relationship between these factors (p-value = 3.59×10^{-6}, R^2 = 0.02398), and species with more specimens are more likely to be split across BINs. This relationship persists even when excluding the smaller sample of species with a higher specimen count.

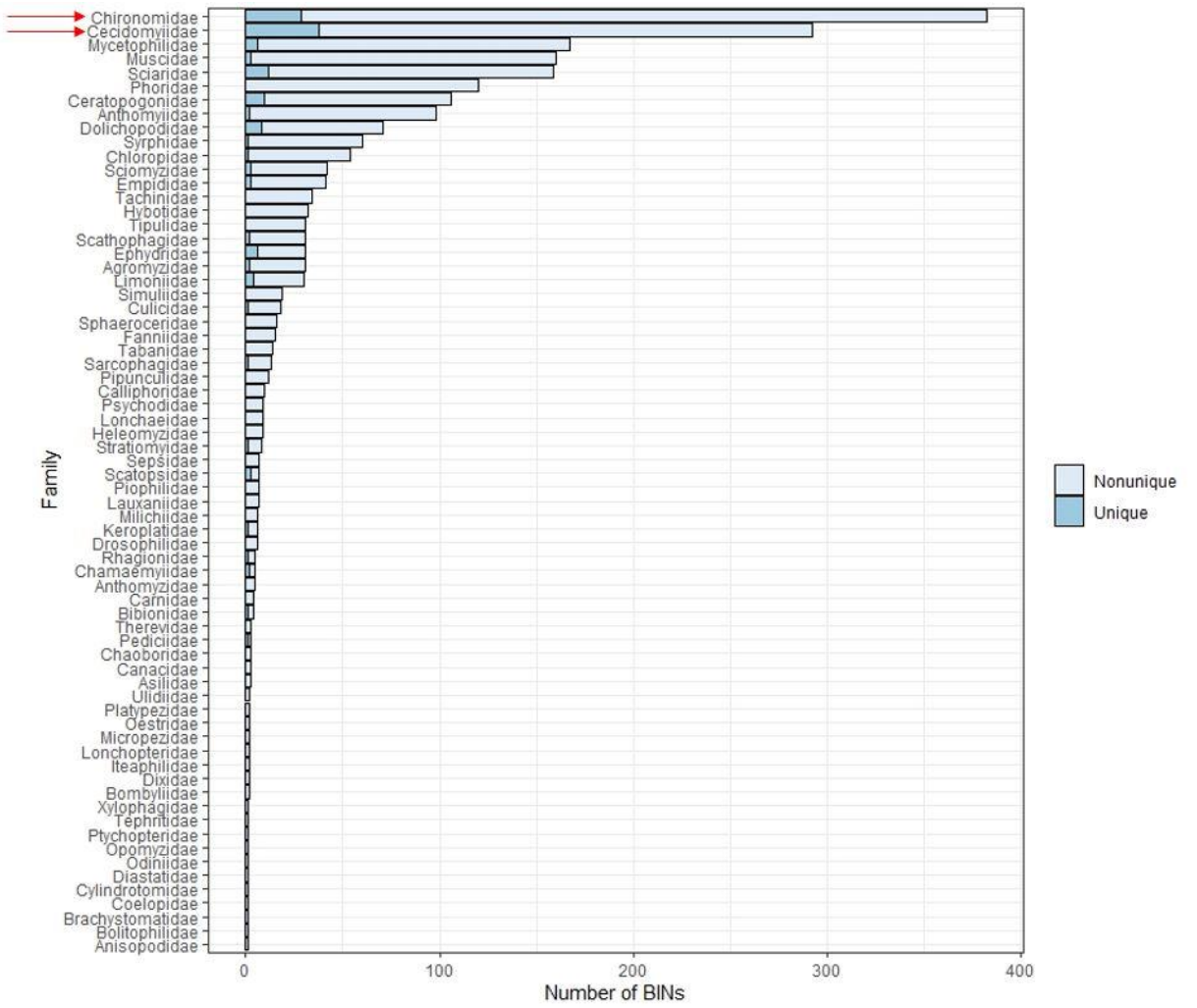


Figure 5: The number of BINs unique to the project on BOLD and the number of nonunique BINs for each family. The families Cecidomyiidae and Chironomidae (indicated with red arrows) had the most unique BINs, with 38 and 29 respectively.

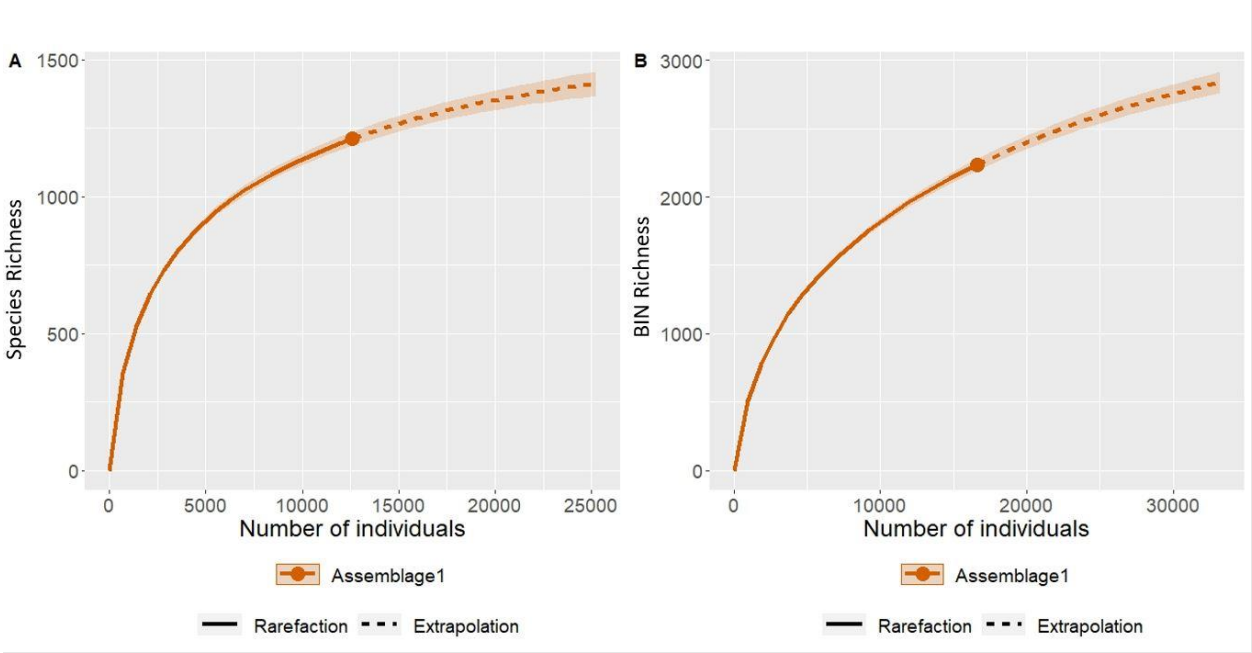


Figure 6: Accumulation curves for the sampled Churchill region. Both curves appear to be approaching an asymptote. A) shows the species richness. The observed value was 1212 and the estimated value was 1522. B) shows the BIN richness. The observed value was 2235 and the estimated value was 3335. These results indicate that the region is reasonably well sampled.

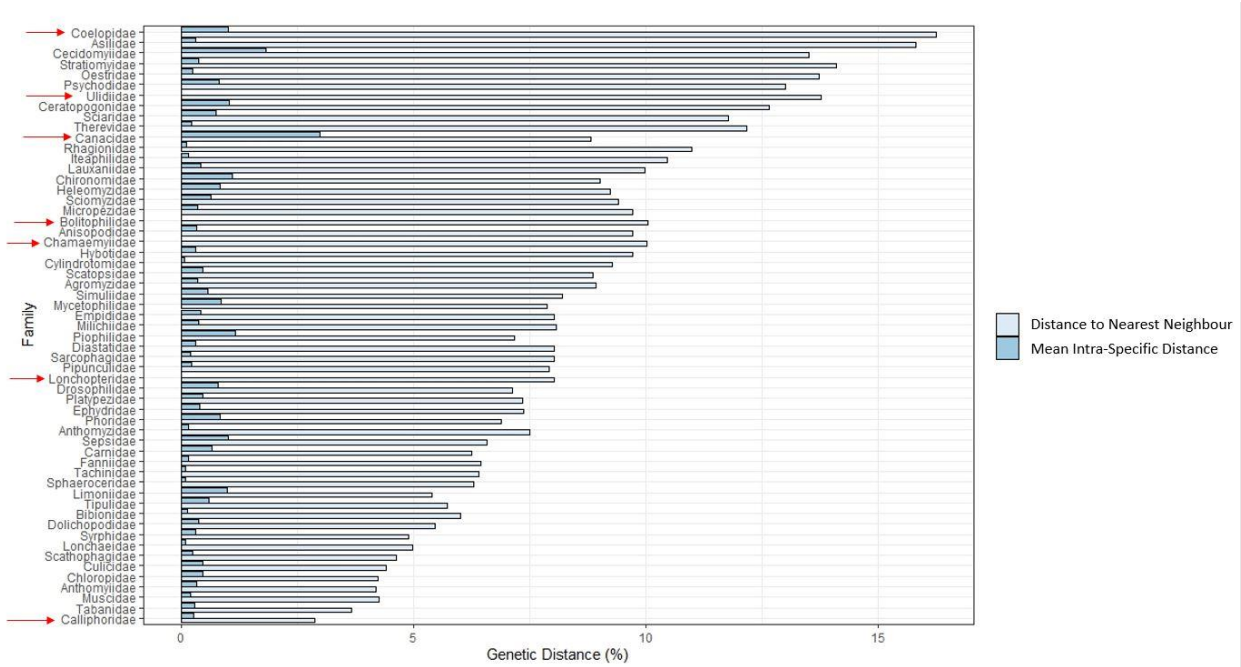


Figure 7: The mean distance to nearest neighbour and mean intraspecific distance for species within each family. The species within Coelopidae had the largest nearest neighbour distances, while Calliphoridae had the lowest. For the intraspecific distances, the value for Canacidae was the highest, while Ulidiidae, Lonchopteridae, Chamaemyiidae, and Bolitophilidae all had mean intraspecific distances of zero. The families mentioned are indicated with red arrows.

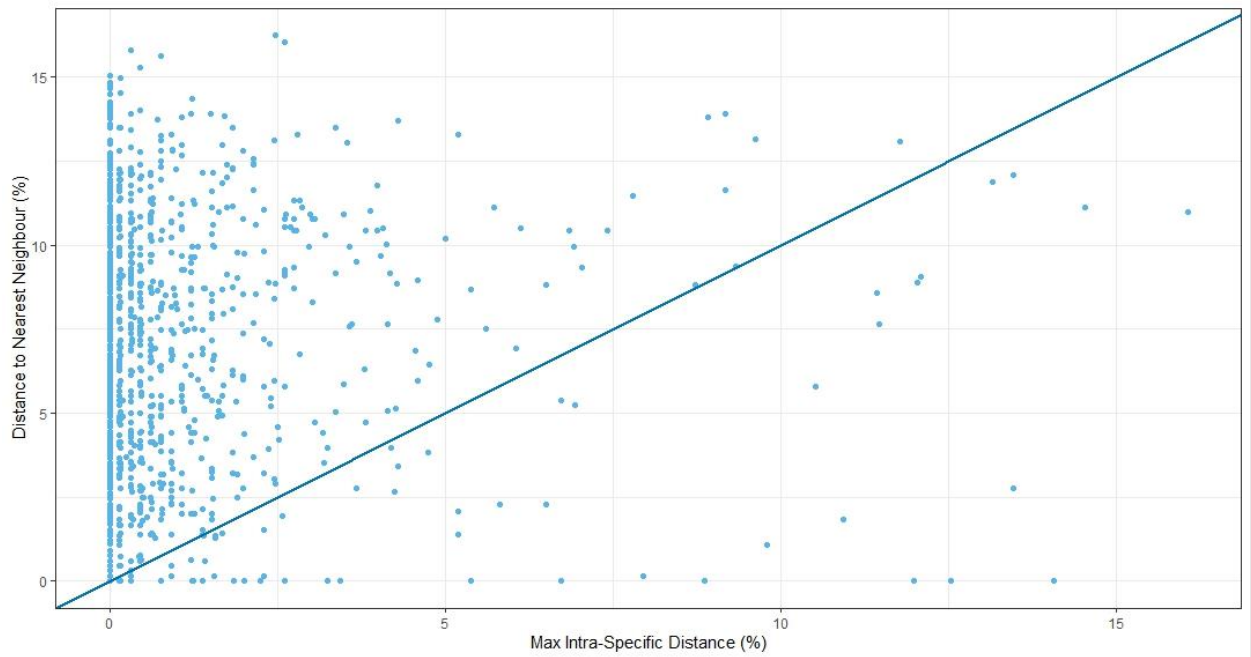
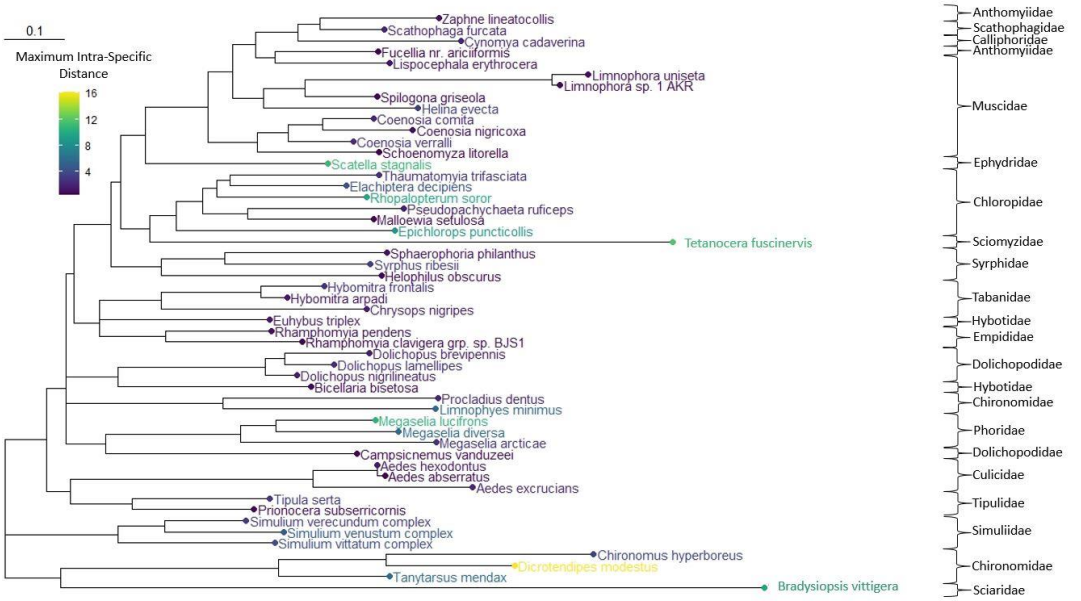


Figure 8: The overlap of the maximum intraspecific distances and the distances to nearest neighbours. Each dot represents a species. Out of the total sample size of 1212 species, 1149 lie above the line, and 63 lie below the line. This indicates that most species have a larger interspecific distance than intraspecific distance, and most species can be easily distinguished from their nearest neighbour.

a)



b)

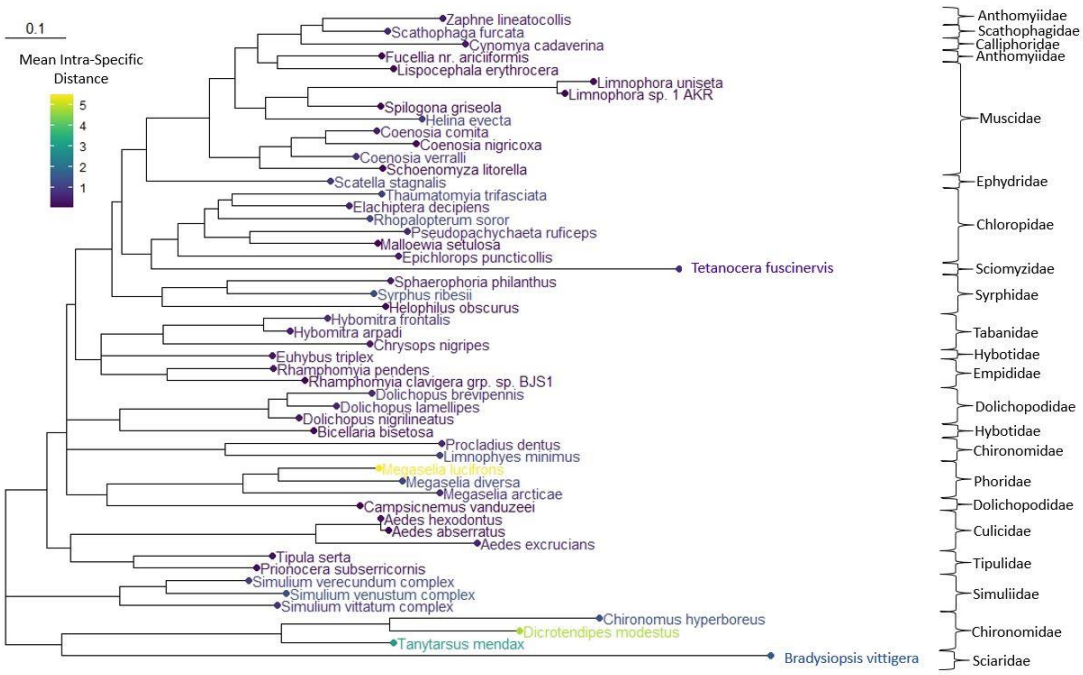
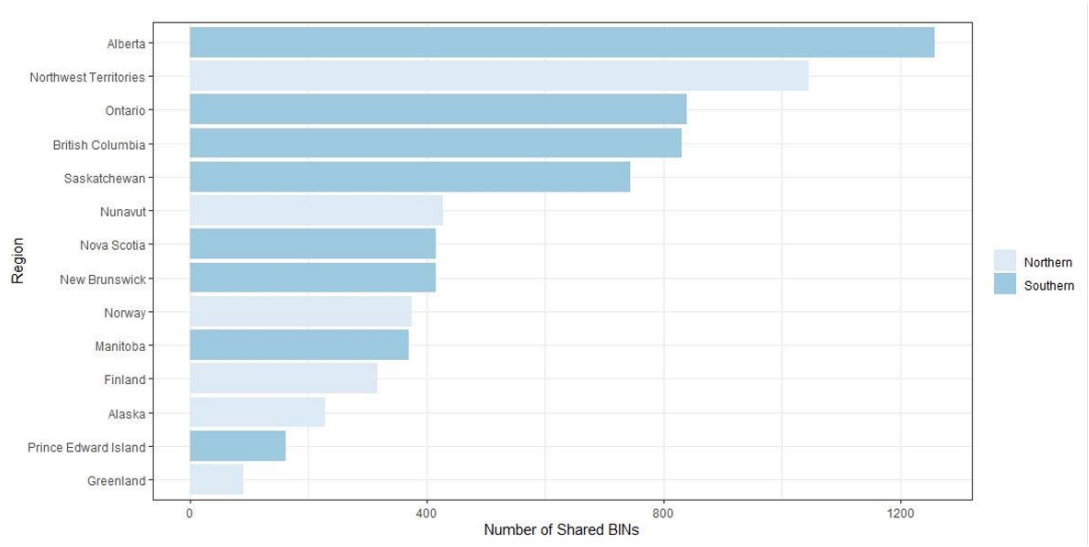


Figure 9: Maximum Likelihood tree of the 50 best-sampled species in the dataset. The a) maximum intraspecific distance (p-distance in %) and b) mean intraspecific distance (p-distance in %) for each species is shown on the tips using the colour scale. The sequences were aligned using DECIPHER version 2.28.0 (Wright, 2016) and the maximum likelihood tree was created using phangorn version 2.11.1 (Schliep, 2011). Higher divergence values were present in scattered locations on the tree.

a)



b)

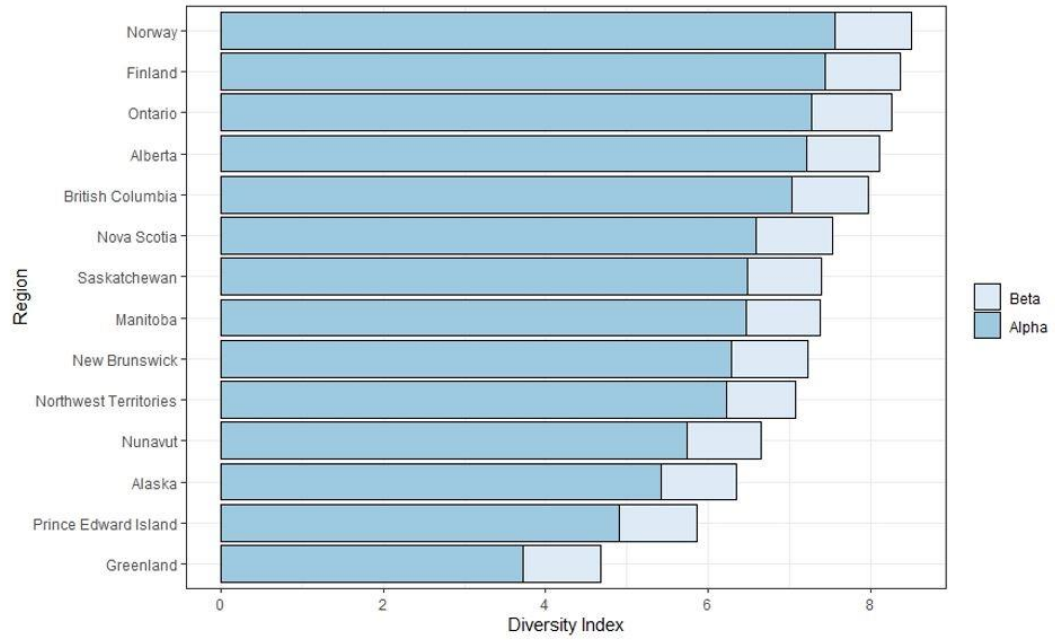


Figure 10: The number of BINs shared between our Churchill barcode library and other geographic regions. a) shows the total count of shared BINs between the regions, while b) shows the alpha diversity of the geographic regions and the beta diversity between the region and Churchill. For a), most BINs were shared with Alberta, with 1258 BINs being shared. b) indicates that Churchill is most similar to the Northwest Territories with a beta diversity of 0.855. Norway and Finland had the highest alpha diversity. Churchill shared BINs with both Northern and Southern regions. For total counts, Churchill shared more with Southern regions, but when calculating beta diversity, Churchill was more similar to other Northern regions.

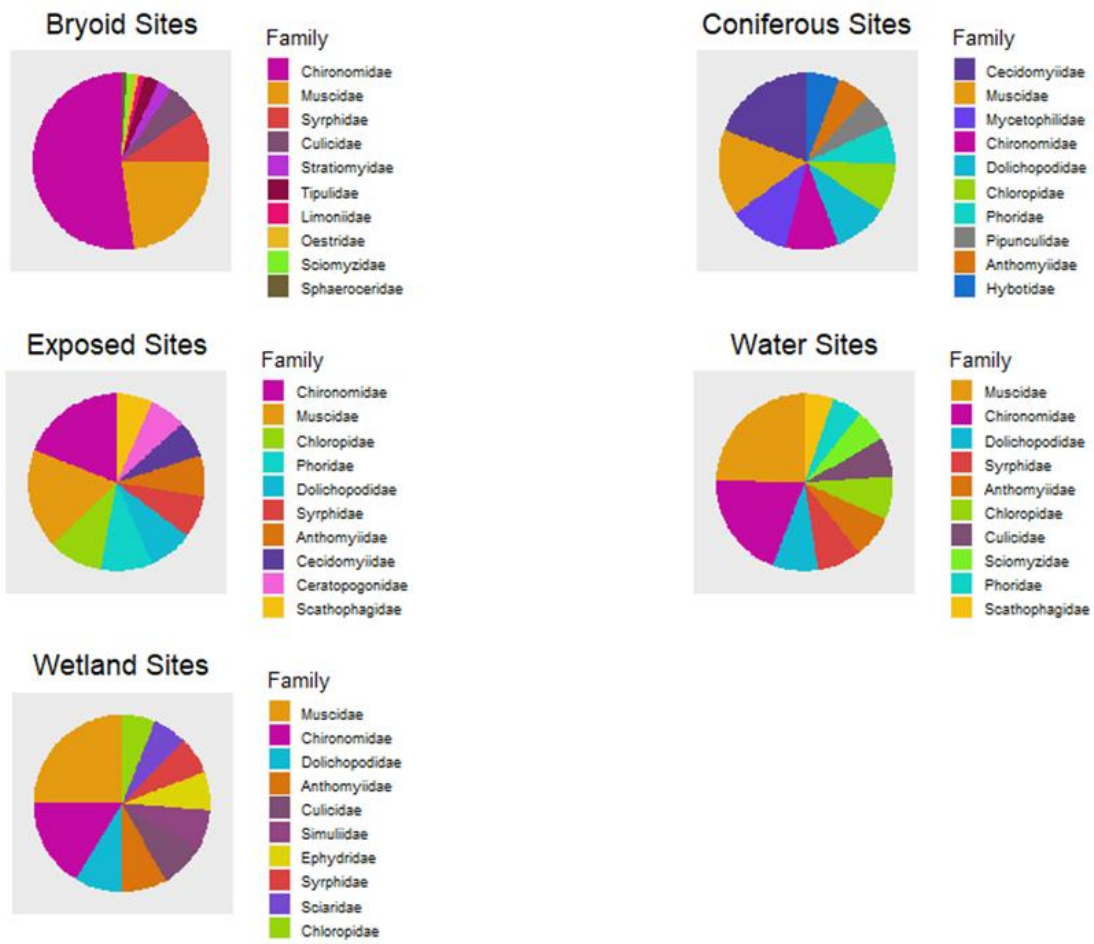


Figure 11. The top 10 families for each land type classification. Each chart depicts the number of specimens in the top 10 most abundant families in that site classification, and the order of the associated legend proceeds from the most to least abundant. Portions appear in each chart from most to least abundant in a counter-clockwise manner.