Diversity of wetland non-biting midges (Diptera: Chironomidae) and their responses to environmental factors in Alberta

by

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Abstract

Wetlands provide a wide range of services, including improving water quality, providing habitats for wildlife, and storing floodwaters. In Alberta, wetlands cover about 21% of the landscape of the province. In Alberta, as elsewhere, wetlands have suffered from human activities and many have declined in water quality and value as habitat. Non-biting midges (Diptera: Chironomidae) often dominate aquatic macroinvertebrate assemblages, both in number and diversity. They have been successfully used as indicators to assess water quality and human disturbance in streams. In contrast, the ecology of chironomids and value as indicators in wetlands are less explored. In this thesis, I use samples and environmental data from the Alberta Biodiversity Monitoring Institute (ABMI) to first explore what taxa of chironomids are present in Albertan wetlands. I created an atlas of all 40 chironomid genera identified, including a detailed glossary describing taxonomically important features of chironomids and a description of morphological and ecological features of each genus. This will provide baseline information and a good taxonomic tool for future chironomid studies in Alberta. Then I use multi- and univariate statistical approaches to assess the relationships between various aspects of chironomid community (i.e. chironomid assemblage structure, Shannon–Wiener index, total genus-richness, total abundance, and abundance of each genus) and both their associated habitats and measures of 'human footprint' (i.e., land with altered natural cover by human activities). Although several environmental factors and human footprint were identified as significantly correlated with chironomid variables, the overall relationships were weak (i.e. low variance explained). The weak correlation between chironomids and environmental variables could be due to the lack of important but unmeasured environmental variables, insufficient taxonomic resolution (i.e., responses may be clearer at species level), and/or that chironomids capable of

living in wetlands in Alberta being robust generalists that are more tolerant of environmental variation than are chironomids associated with flowing water.

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Table of Contents

List of Tables

List of Figures

Chapter 1 : General Introduction

1.1 What is biodiversity?

Biodiversity, short for biological diversity, is often defined as the variety of life on earth (Ehrlich and Wilson 1991, Sala et al. 2000). Biodiversity is commonly identified at three levels: genetic diversity, taxonomic diversity and ecosystem diversity (Sala et al. 2000). Genetic diversity refers to the very genetic make-up of each species and variation among populations. Taxonomic diversity is commonly defined as the number and relative abundance of different species or higher-level taxa in a given community. Ecosystem diversity is the variety of ecosystems, biotic communities and ecological processes.

The province of Alberta is a biologically diverse place with six distinct natural regions: Boreal, Canadian Shield, Foothills, Parkland and Rocky Mountain (Natural Regions Committee 2006). Each region is characterized by distinct geographical and climatic conditions. Alberta is home to about 91 species of mammals, 250 of breeding birds, 60 of fish, 10 of amphibians and 8 of reptiles (Alberta Environment Protection 1998). There are about 1650 species of flowering plants, 650 species of moss, a similar number of lichens, and 450 species of fungi. In addition, an estimated 20 000 insect species occurs in Alberta.

1.2. Why is biodiversity important?

Biodiversity is both essential to, and an indicator of ecosystem function (e.g., primary production). In turn, ecosystem function provides services that are essential to humans. Based on the (2005), ecosystem services can be grouped into four broad categories. Supporting services are those that “are necessary for the production of all

other ecosystem services", such as nutrient recycling, primary production, soil formation and water cycling. These services are the basis for the ecosystems to provide other services such as food supply, flood regulation and water purification. Provisioning services are the products that we harvest from ecosystems such as food (e.g. crops, seafood), water, raw materials (e.g. lumber, fibre), minerals, medicinal resources (medicines and pharmaceuticals) and energy (e.g. biofuel). Regulating services are "benefits obtained from the regulation of ecosystem processes", such as climate regulation (e.g. through storing and releasing greenhouse gas), waste decomposition, water purification and pest control. Cultural services are nonmaterial benefits people obtain from ecosystems such as recreational experiences (e.g. outdoor sports), science and education.

1.3. Threats to Biodiversity

Natural disturbances such as wildfire and insect outbreaks affect local biodiversity. However, ecosystems can often recover from natural disturbance, as such disturbances have been part of millions of years of natural selection. Today, biodiversity loss is accelerating, mainly because of disturbances caused by human activities (Hooper et al. 2005).

Habitat degradation (or loss) is considered as the greatest threat to biodiversity (Wilcove et al. 1998). It occurs when events alter a terrestrial or aquatic ecosystem so drastically that the habitat is no longer suitable for many species to live. Common human activities that cause habitat destruction are land use change and fragmentation such as deforestation and draining wetlands. Canada's boreal forest is considered to be one of the largest intact-forested ecosystems on earth, but recently industrial activities, such as oil

and gas extraction, have been rapidly increasing (Lee and Cheng 2013). Within Alberta's tar sands region, for example, habitat destruction has caused the loss of an estimated 58,000–402,000 breeding birds from the regional population (Timoney and Lee 2009).

Spread of alien species is considered as the second greatest threat to biodiversity after habitat loss (Wilcove et al. 1998). Alien species are sometimes introduced deliberately (e.g. as pets, pest control, sport), but usually they arrive by accident. They may displace native species because they often have no natural predators or parasites. In many cases, the arrival of non-native species disrupts the equilibrium of an ecosystem, threatening the diversity or abundance of native species. One alien species of great concern in Canada is Zebra Mussel (*Dreissena polymorpha*). Zebra mussels were introduced to the Great Lakes through ballast water in 1986 (Griffiths et al. 1991). Since their invasion, the native amphipod *Diporeia* has declined dramatically as *Diporeia* shares the same food source as the zebra mussels. The decline of *Diporeia* then caused the decreased growth in body sizes of lake whitefish *Coregonus clupeaformis* (Pothoven et al. 2001).

Biodiversity is also threatened when sustained overexploitation occurs (Hooper et al. 2005, Wilcove et al. 1998). Overexploitation refers to harvesting a resource at a rate faster than it can recover naturally. It can lead to dangerously low population numbers, or even extinction. For example, one of the most abundant birds in North America—passenger pigeons—went to extinction in the early 20th century, partly due to the overhunting by early European settlers (Schorger 2004). Another well-known example of overexploitation is the collapse of Atlantic northwest cod fishery. Overfishing of Atlantic

cod during the past few decades caused the cod biomass in 1992 to fall to 1% of its previous level (Hamilton and Butler 2001).

Pollution is another human-related disturbance that could affect biodiversity (Wilcove et al. 1998). Polluting substances released in the air and water can have far-reaching negative effect on biodiversity. The major air pollutant that affects biodiversity is acid deposition (Federal, Provincial and Territorial Governments of Canada 2010). It causes acidification of lakes, streams and soils that lead to reduced survival, growth and reproductive success of many species. The leading water pollutants are siltation, nutrients, bacteria, metals (primarily mercury), and oxygen depleting substances (US Environmental Protection Agency 2000). They often decrease biodiversity by directly killing organisms or by reducing their reproductive output. Nutrient loading (the release of phosphorus and nitrogen) to aquatic ecosystems from fertilizers and sewage into aquatic ecosystems is a particular concern. Nutrient enrichment (also known as eutrophication) of water bodies often leads to massive algal blooms, ultimately decreasing the amount of oxygen and light available to other plants and animals.

Climate change includes rising temperatures and more frequent extreme weather events. Climate change is important because climate affects species distributions, community structure and composition, and the nature and character of ecosystems (Sala et al. 2000, Millennium Ecosystem Assessment 2005, Federal, Provincial and Territorial Governments of Canada 2010). During the last 100 years, the average global surface temperature has increased by about 0.74°C (Federal, Provincial and Territorial Governments of Canada 2010). Global average sea level has risen at an average rate of 1.8 mm per year since 1961. In Canada, from 1950 to 2007, the mean annual air

temperature has increased by 1.4°C (Zhang et al. 2011). Both annual precipitation and the annual number of days with measureable precipitation have generally increased over Canada since 1950.

1.4 Alberta Biodiversity Monitoring Institute (ABMI)

The Alberta Biodiversity Monitoring Institute (previously known as Alberta Biodiversity Monitoring Program) was initiated in 1997 and fully implemented in 2007 in aim to provide an effective way to track biodiversity status, and provide comprehensive and scientific biodiversity information of the province for resource management (Stadt et al. 2006, ABMI 2008). The ABMI monitors more than 2000 terrestrial and aquatic species including mammals, birds, mites, vascular plants, bryophytes, lichens, and aquatic macroinvertebrates. ABMI collects extensive information of those species, their associated habitats and human footprint (i.e. land with altered natural cover by human activities) at 1656 sites evenly spaced throughout the province using the 20 km National Forest Inventory (NFI) grid.

Two of the ABMI's goals are to quantify the biological response (e.g. species abundance, community structure) to habitat elements and human footprint, and to evaluate the utility of the biological response for monitoring ecosystem health (ABMI 2012). Macroinvertebrates from wetlands are an important component of collection by ABMI. Chironomids are the most dominant organisms among the macroinvertebrates and were sorted as one of the primary organisms (Table 1.1) (ABMI 2011). However, they were not originally targeted for genus/species identification due to the procedural difficulty of identifying them more finely than subfamily. Later, interest to integrate

chironomid to their list of monitored group has been aroused in the ABMI as chironomids are so diverse and often dominate macroinvertebrate samples.

Table 1.1. Aquatic invertebrate taxa classified based on whether they are primary or secondary organisms. Secondary organisms are not considered part of the macroinvertebrate community. Primary organisms with asterisks were targeted for identification to the lowest taxonomic level possible.

Primary Organisms	Secondary Organisms
Oligochaeta (aquatic worms)	Porifera (sponges)
Hirudinea (leeches)	Hydrozoa (hydras)
Hydrozoa (hydras)	Platyhelminthes (flatworms)
Gastropoda (snails & limpets)*	Nematoda (roundworms)
Bivalvia (clams)	Cladocera (water fleas)
Hydrachnida (aquatic mites)	Ostracoda (seed shrimp)
Amphipoda (scuds)	Copepoda (copepods)
Isopoda (sow bugs)	
Decapoda (crayfish)	
Ephemeroptera (mayflies)*	
Anisoptera (dragonflies)*	
Zygoptera (damselflies)*	
Plecoptera (stoneflies)	
Hemiptera (true bugs)*	
Megaloptera (fishflies, alderflies)	
Lepidoptera (aquatic moths)	
Trichoptera (caddisflies)*	
Coleoptera (beetle adult)*	
Coleoptera (beetle larva)	
Chironomidae (midges)*	
Ceratopogonidae (no-see-ums)	
Tabanidae (horse flies)	
Tipulidae (crane flies)	
Culicidae (mosquitoes)	
Chaoboridae (phantom midges)	
Simulidae (black flies)	
Other Diptera (true flies)	

1.5 Midges (Diptera: Chironomidae)

The family Chironomidae, commonly known as non-biting midges, is one of the most abundant, diverse, and cosmopolitan groups of aquatic insects (Ferrington 2007). They occur on all continents including Antarctica and have adapted to many aquatic and semi-aquatic habitats (Andersen et al. 2013). Ferrington (2007) confirmed a total of 339 genera and 4,147 species being fully aquatic during the pre-adult stage by the review of world collection and species accounts. Local richness can be very high. For example, more than 100 species have been collected from a single stream in Alberta (Clifford 1991). Chironomids are holometabolous aquatic insects, and have four distinct life stages: egg, larva, pupa and adult. The larval stage has four instars, and may last from less than two weeks to several years depending on species and environmental conditions (Coffman and Ferrington 1996). Most of the species are restricted to freshwater habitats, and the larvae of some have adapted to anoxic conditions. For example, those that live in very deep standing deep water are often red in color due to the presence of haemoglobin, which increases the capacity for oxygen storage. Most chironomid adults do not feed, living usually only for a few days (Coffman and Ferrington 1996).

Chironomids are in the dipteran sub-order Nematocera, and so are related to other well-known fly groups such as the Ceratopogonidae (biting midges), Simuliidae (blackflies), Culicidae (mosquitoes) and Chaoboridae (phantom midges) (Walker 2001). Taxonomically, Chironomidae are broken into 11 subfamilies, seven of which occur in North America (Epler 2001). The subfamilies Tanypodinae, Orthocladiinae and Chironominae are most common. Of these, Orthocladiinae mostly occur in lotic and cold-water habitats, while Tanypodinae and Chironominae are often encountered in lentic and

warm-water systems. However, some Chironominae, especially many species from Tribe Tanytarsini, are also very common in cold-water habitats (Lindegaard 1995). Other subfamilies, such as Telmatogetoninae and Podonominae are relatively restricted in habitats (e.g. warm seashores; lentic littoral), and Diamesinae and Prodiamesinae are relatively uncommon (Oliver and Roussel 1983, Epler 2001). Table 1.2 lists the major subfamilies and tribes in Canada and their typical habitats, based primarily on publications by Oliver and Roussel (1983), Epler (2001) and Ferrington (2007).

In addition to being very diverse, chironomids are well known for their ecological roles in freshwater systems. First, they have an important role in nutrient cycling and energy flow (Ferrington 2007). Chironomid larvae are known to feed on a variety of organic substrates, such as coarse/fine detrital particles, algae and fungal spores (Oliver and Roussel 1983, Pinder 1986, Coffman and Ferrington 1996). In their larval stage, they are important food items for freshwater fish, including those of commercial interest. In both their larval and adult stages for other vertebrate species such as amphibians and insectivorous birds (Ferrington 2007, Pedro and Ramos 2009). Second, they can be used as indicator organisms to examine water quality and human impacts on freshwater ecosystems. Different species have different tolerances to pollutants, so the presence, absence and abundance of certain species may reflect changes in water quality (Saether 1979, Oliver and Roussel 1983, Pinder 1986, Epler 2001). Third, sub-fossil chironomid remains are widely used by palaeontologists to trace the past environmental and climatic changes (Walker et al. 1991, Quinlan et al. 1998, Brooks et al. 2001, Henrichs et al. 2001, Larocque et al. 2001, Porinchu et al. 2002). They have been successfully used to

reconstruct palaeo-temperatures, water depths, palaeosalinity, hypolimnetic oxygen levels, acidification and other environmental variables (Walker 2001).

Table 1.2. The major subfamilies and tribes of the Chironomidae found in Canada and their typical habitats.

Subfamily	Distribution	Tribe	Typical Habitat
Tanypodinae	worldwide, all major geographical regions except Antarctica. 4 tribes and about 25 genera found in Canada	Coelotanypodini	littoral zone of ponds & lakes (lentic)
		Tanypodini	Shallow warm still water; some flowing water and deeper parts of lakes
		Macropelopiini	streams & rives (lotic); some lentic littoral & profundal
		Pentaneurini	fast-flowing waters; lentic littoral; a few hygropetric
Podonominae	mainly in southern hemisphere. 2 tribes and 4 genera found in Canada	Boreochlini	fast-flowing and cold waters; lentic littoral
		Podonominae	fast-flowing and cold waters
Chironominae	worldwide, all major geographical regions except Antarctica. 3 tribes and at least 44 genera found in Canada	Chironomini	lentic, littoral/profundal; slow lotic; especially on sandy substrates & associated with aquatic macrophytes
		Pseudochironomini	Shallower regions of still water and quieter reaches of large bodies of flowing water.
		Tanytarsini	Lotic fast & slow water; lentic littoral; occasionally in brackish water

Continued….

Table 1.2. Continued

Subfamily	Distribution	Tribe	Typical Habitat
Orthocladiinae	worldwide distribution and widespread within Canada. 3 tribes and at least 47 genera found in Canada	Corynoneurini	Lotic fast & slow water; lentic littoral
		Metriocnemini	wide range of lentic & lotic habitats, including springs, pitcherplants, dung, interstitial, marine intertidal & semi-terrestrial
		Orthocladiini	wide range of lentic & lotic habitats, including marine intertidal
Prodiamesinae	only from the northern hemisphere and southern South America. 3 genera found in Canada		Cool adapted; still & flowing water, often in sandy substrates
Diamesinae	worldwide, occurring in the cooler parts of all major geographical regions except Antarctica. throughout Canada. 3 tribes and 8 genera found in Canada	Boreoheptagyini	cool mountain or glacial fed streams
		Diamesini	fast-flowing, cold waters; springs
		Protanypodini	deep cool lakes
Telmatogetoninae	only one genus found in Canada		Warm seashores, often in waters of low salinity

1.6 Thesis objectives and outline

This study involves the use of chironomid specimens and habitat data collected at 270 wetlands across Alberta by ABMI from 2009 to 2011. The overall goal is to explore the diversity of chironomids, assess their responses to environment, and evaluate their utility as biomonitoring tools.

One difficulty in studying chironomid ecology and diversity is caused by lack of reliable regional keys with good illustrations. So in Chapter 2, I explore the genus-level diversity of chironomid to build an inventory of chironomid taxa with a digital library (pictures of all identified chironomid taxa) in Alberta. This inventory of chironomid taxa provides a good taxonomic reference for future chironomid studies.

Chironomids have been successfully used in stream and lake monitoring in many regions across the world. In comparison, the use of chironomids for wetland monitoring is still at a young stage. The indicator value of chironomids relies on the sufficient knowledge of their ecology. So in Chapter 3 and Chapter 4, I examine how different aspects of chironomids in Alberta wetlands correlates with (and, hence, presumably are influenced by) environmental factors and the ABMI's assessment of human footprint. Specifically, Chapter 3 explores the chironomid assemblage structure and Chapter 4 explores individual chironomid taxa.

Chapter 5 provides a summary of chironomids ecology, and discuss the implication of this study to the practical utilization of chironomids to wetland biomonitoring in Alberta.

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Chapter 2 : Atlas of 4th instar larvae of common genera of non-biting midges (Diptera: Chironomidae) recorded from Albertan wetlands

2.1 Introduction

Non-biting midges (Diptera: Chironomidae) are one of the most abundant and diverse groups of freshwater macroinvertebrates (Ferrington 2008). Chironomid midges, like other Dipterans, are holometabolous and have four distinct life stages: egg, larva, pupa and adult. The larval stage is aquatic and has four instars, between hatching from the egg and becoming a pupa, shedding its exoskeleton (molting) at the end of each instar. The fourth instar is the most reliable juvenile instar for observing distinguishing features of the different genera and species. Midge larvae hold great potential for assessing the quality or "health" of freshwater ecosystems, as different species are adapted to a variety of different aquatic habitats and ecological conditions. Their extremely high diversity has a negative side, however, in that keys for large geographical areas require dauntingly large keys (e.g., the Ferrington & Berg (2008)). North American key just for the subfamily Orthocladiinae has 72 couplets) that often create uncertainty and frustration in the user, especially if there are inadequate illustrations to allow confirmation of an identification after having reached an endpoint in a key. Local checklists have great value in narrowing down candidate taxa to a manageable number. The primary purpose of this atlas is to provide beginners a checklist plus detailed pictorial record of midge larvae commonly encountered during water quality studies of Albertan wetlands.

This chapter includes a checklist of 40 genera in 4 subfamilies of chironomids identified by the author from samples collected by the Alberta Biodiversity Monitoring Institute (ABMI), a glossary describing critical features of chironomids and a description of morphological and

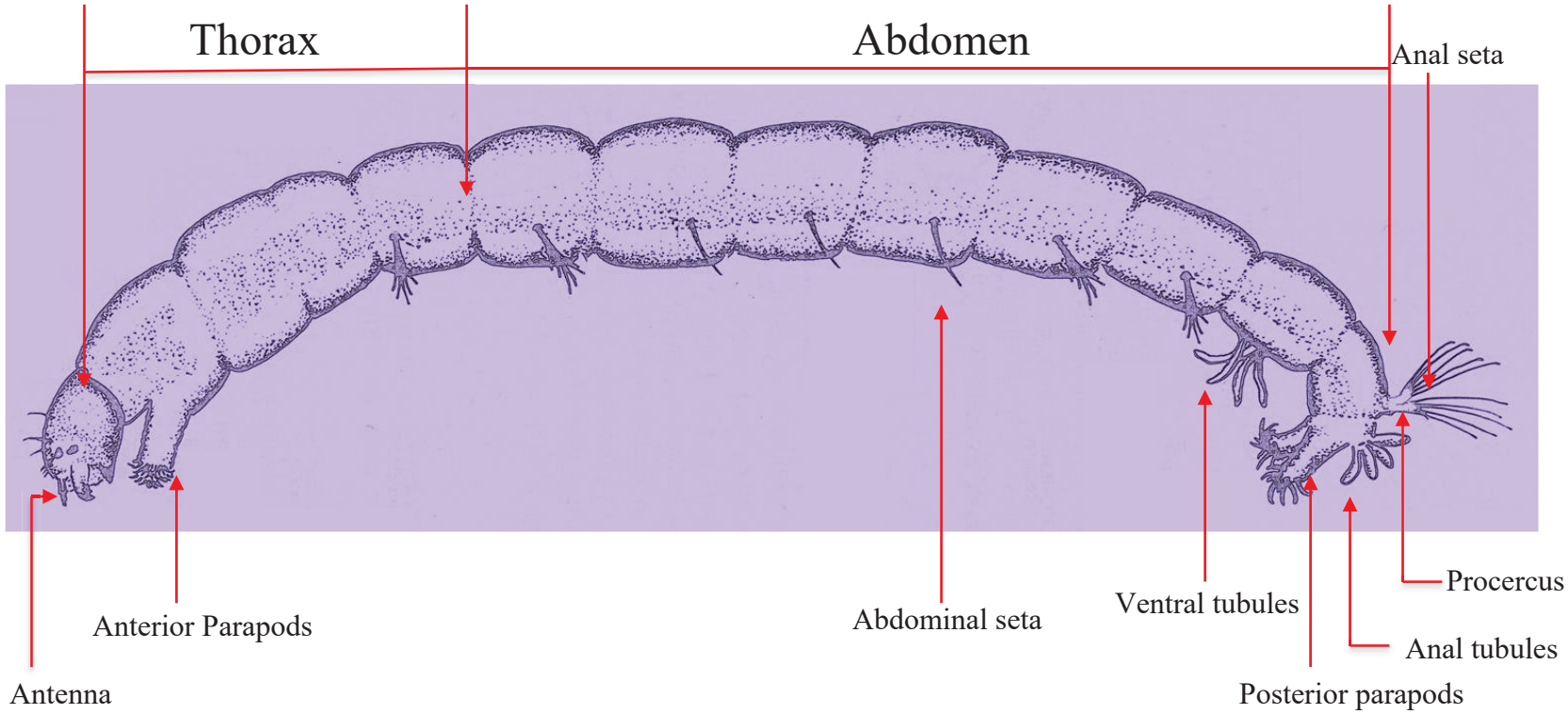
ecological features of each genus organized alphabetically by subfamily. The ABMI has reported a number of taxa not seen by the author, and no doubt over time will collect more; future version of the atlas can incorporate these additional subfamilies and genera.

Table 2.1. Checklists of Genera of Midge (Chironomidae) Larvae Reported from Alberta Wetlands

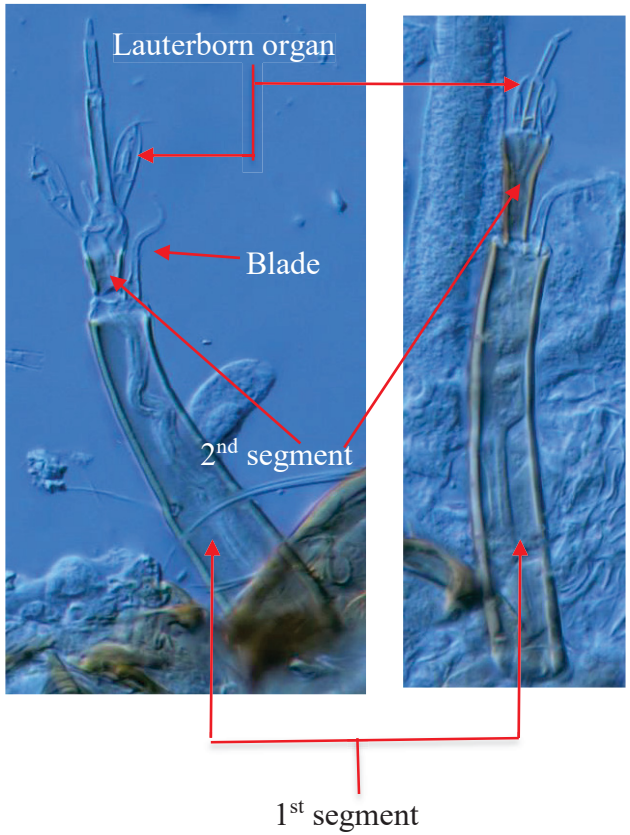
Chironominae	*Chironomus*
	Cladopelma
	Cladotanytarsus
	Cryptochironomus
	Cryptotendipes
	Dicrotendipes
	Einfeldia
	Endochironomus
	Glyptotendipes
	Lauterborniella
	Microtendipes
	Nilothauma
	Pagastiella
	Parachironomus
	Paracladopelma
	Paratanytarsus
	Phaenopsectra
	Polypedilum
	Pseudochironomus
	Rheotanytarsus
	Tanytarsus
Diamesinae	*Pothastia*
Orthocladiinae	*Acamptocladius*
	Acricotopus
	Corynoneura
	Cricotopus
	Limnophyes
	Nanocladius
	Orthocladius
	Paracladius
	Parakiefferiella
	Psectrocladius
	Zalutschia
Tanypodinae	*Ablabesmyia*
	Derotanypus
	Labrundinia
	Procladius
	Psectrotanypus
	Tanypus
	Thiemannimyia group

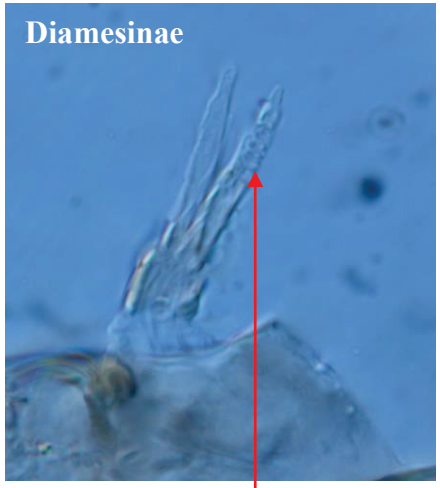
2.2 Glossary

Lateral view of a whole larva redrawn from Simpson and Bode (1980) :

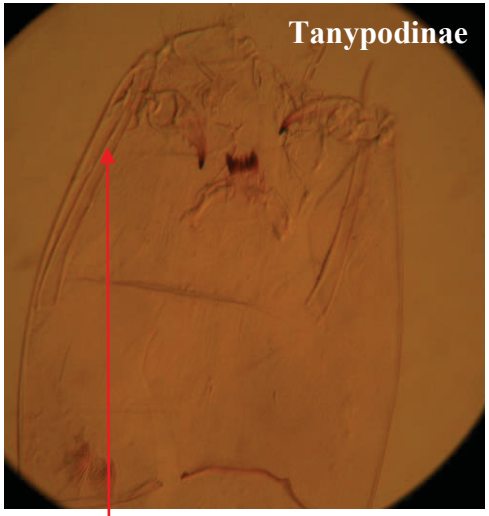


Antennae:





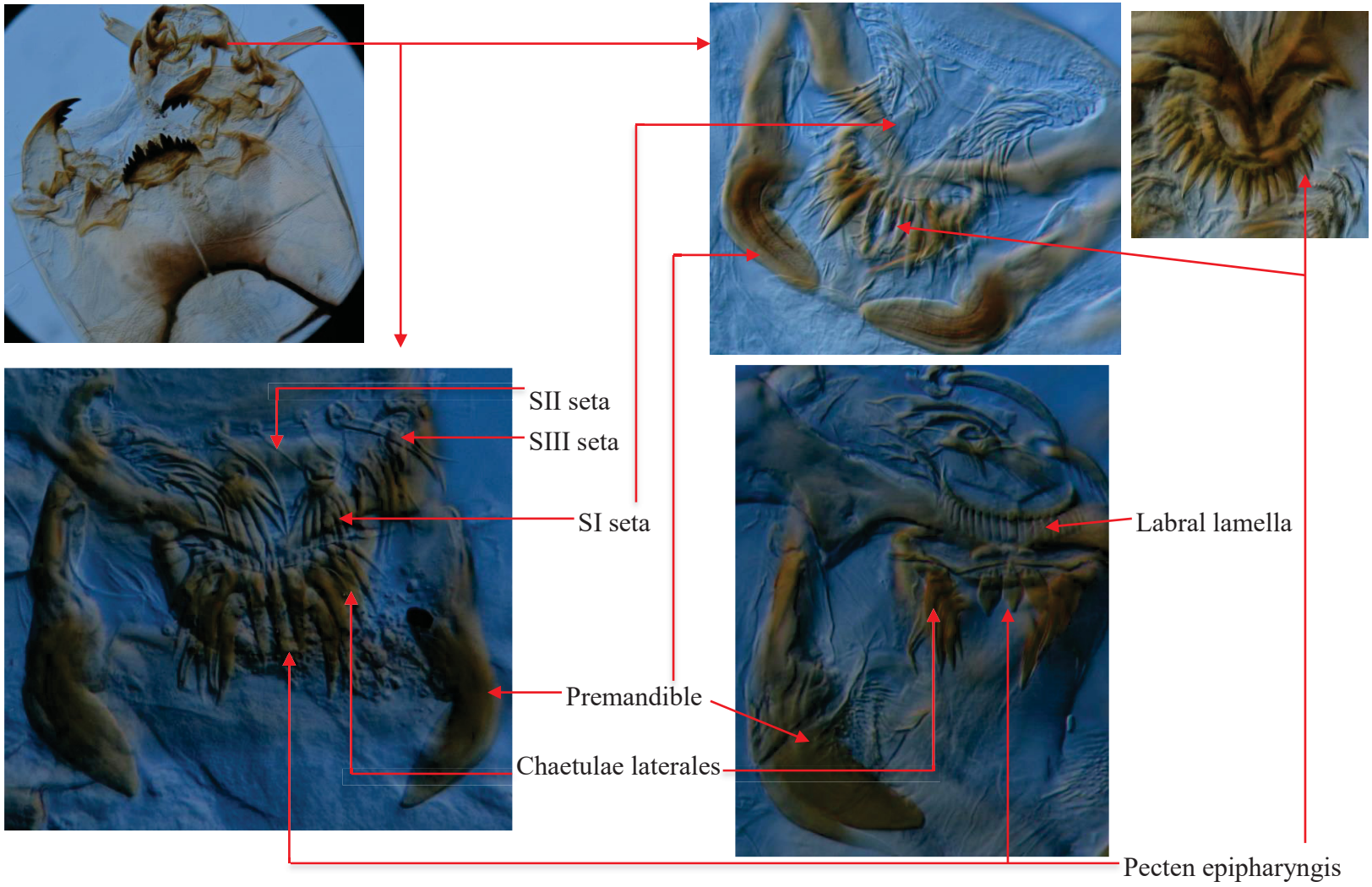
3rd segment annulated in Diamesinae



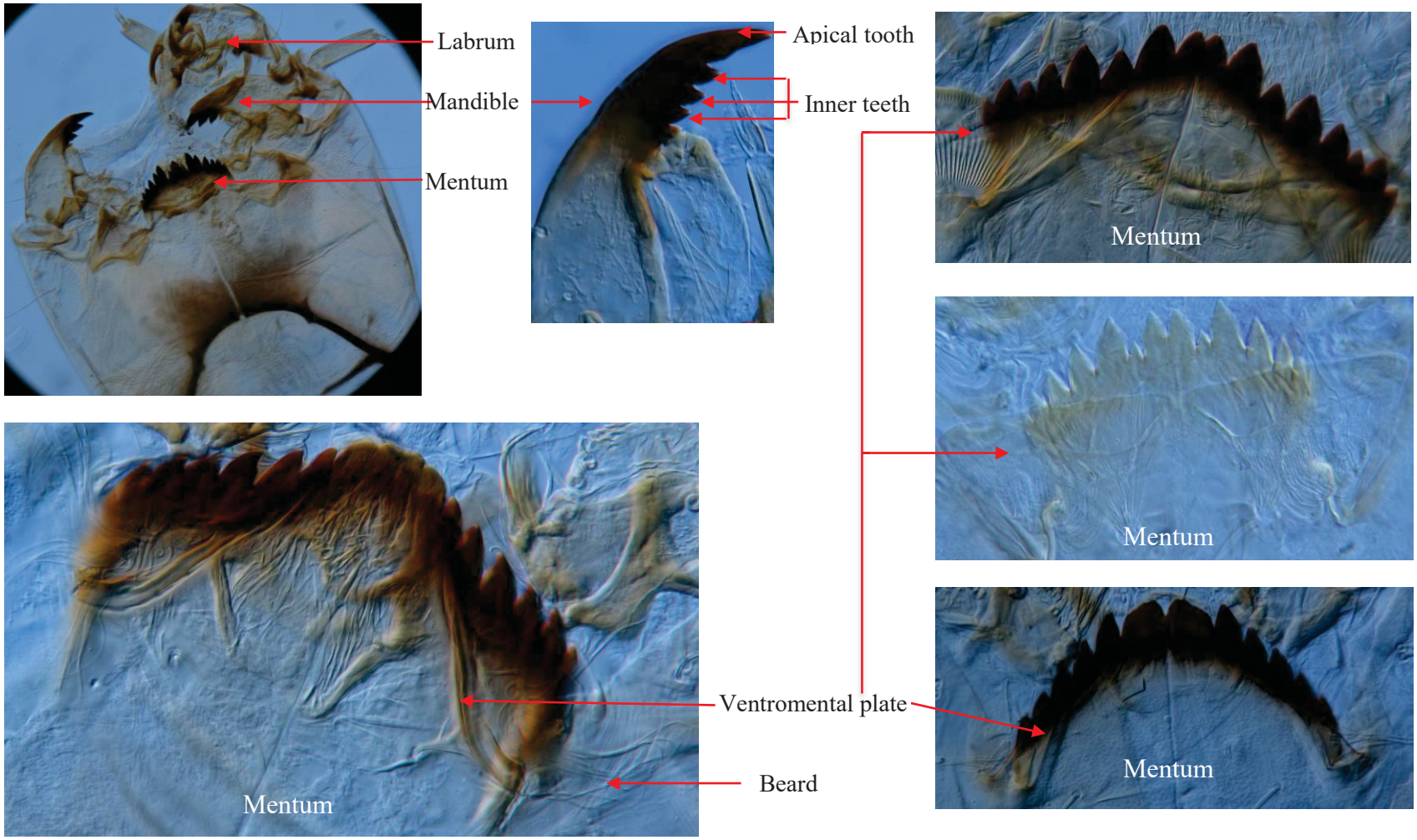
Antenna retractable in Tanypodinae

Antennal ratio refers to the ratio of the length of the first antennal segment divided by the length of the combined apical segments (i.e. the **flagellum**).

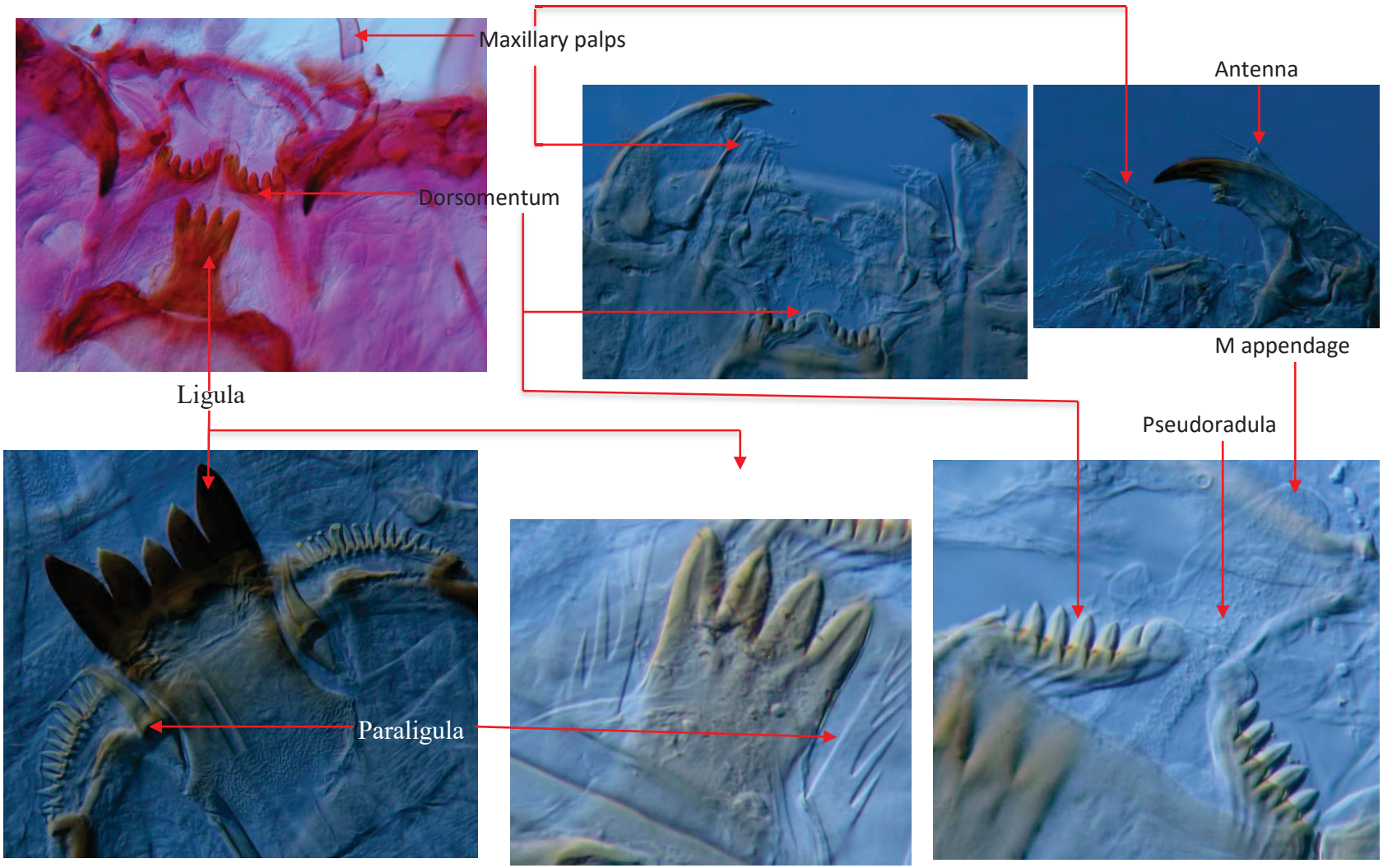
Head anterior to the mouth opening:



Head behind the mouth opening (except in Tanypodinae):



Head behind the mouth opening (Tanypodinae):



Dorsal head:

Labral sclrites

Frontal pit

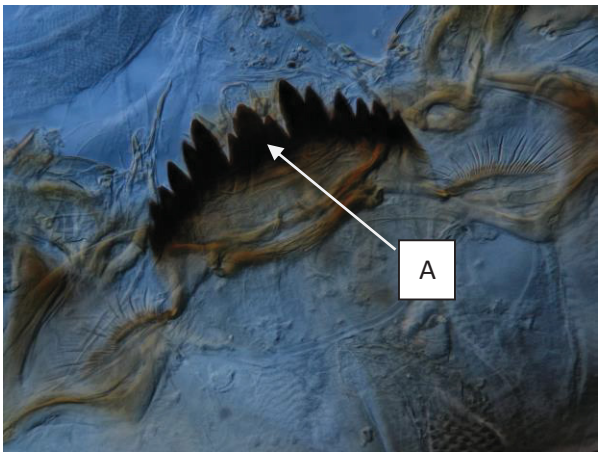
Frontal apotome

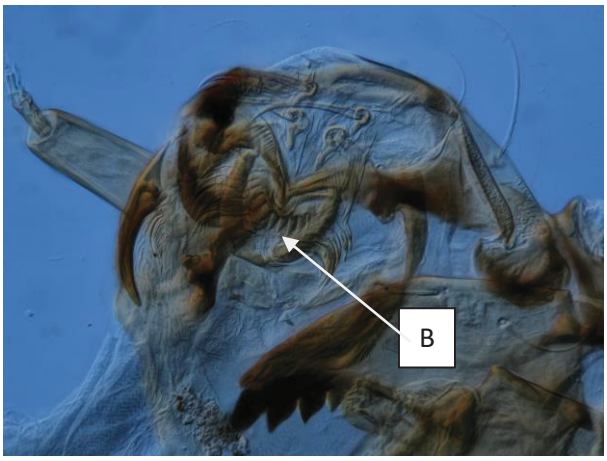
Apotomal fenestra

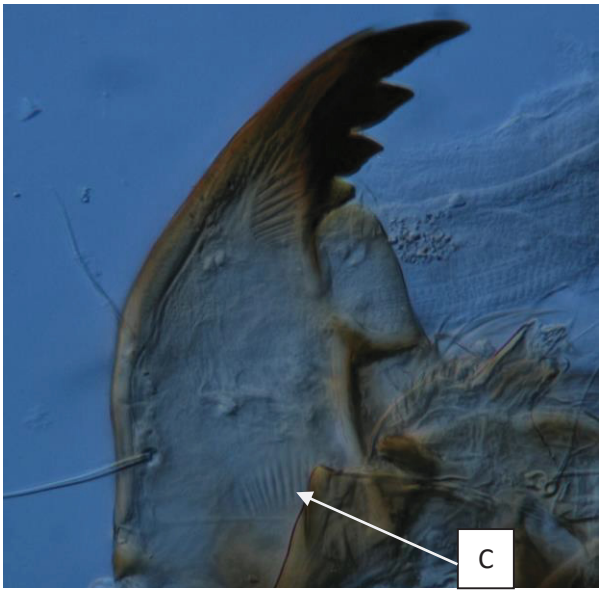
2.3 Taxonomic review

The following pages are organized alphabetically by subfamilies: Chironominae, Diamesinae, Orthocladiinae and Tanypodinae. Each genus of midge larva is illustrated on a single page. Two to four pictures of each genus are provided and labeled with arrows that point to the critical diagnostic features. The general ecology (i.e. habit trophic relationship and typical habitat) of the genus is also briefly summarized. Both descriptive features and general ecology information are derived from Oliver and Roussel (1983) , Epler (2001) and Ferrington (2008) .

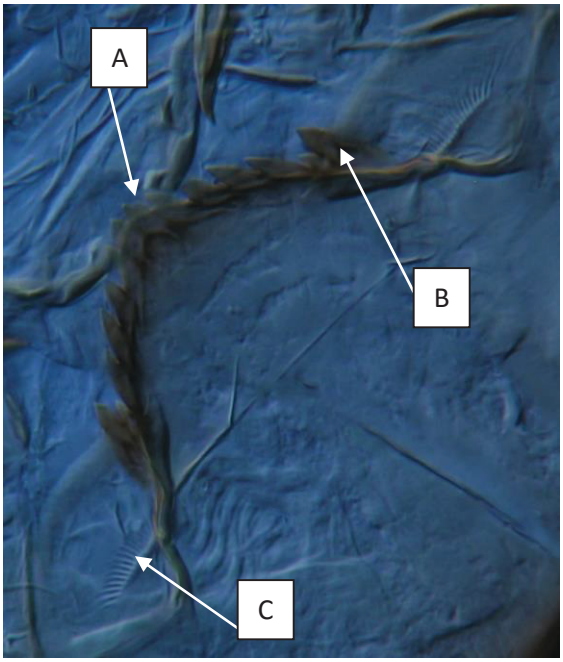
2.3.1 Subfamily Chironominae

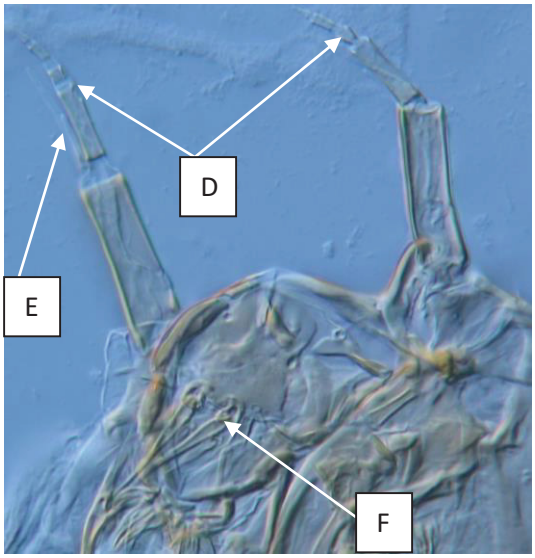


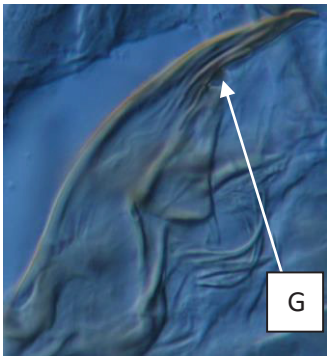




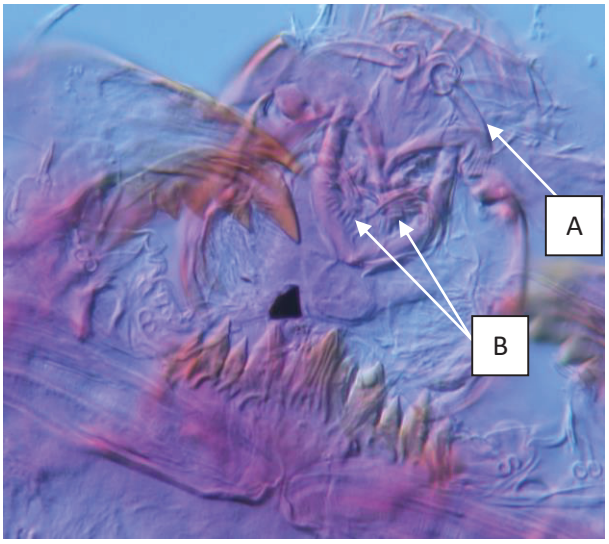
Chironominae *Chironomus sp.*	
Body and Dorsal Head	Frontal apotome present; with 2 isolated labral sclerite. Normally with 2 pairs of ventral tubules.
Antenna	5-segmented; with 3rd segment usually shorter than 4th.
Mentum	Median tooth of the mentum trifid (A); outermost teeth decreasing in size giving a convex appearance
Labrum	SI seta plumose on each side; SII simple; pecten epipharyngis simple with 15-30 well-developed teeth (B). Premandible with 2 apical teeth.
Mandible	Mandible with pale dorsal tooth, apical tooth dark, with 3 pointed inner teeth. All species basally with striae (C) on outer surface.
Ecology	Burrowers (tube builders). Collectors-gatherers; Shredders-herbivores. Prefer soft sediments of standing water.

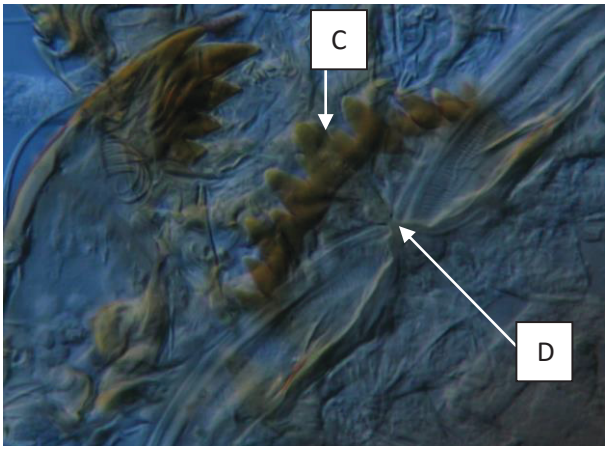


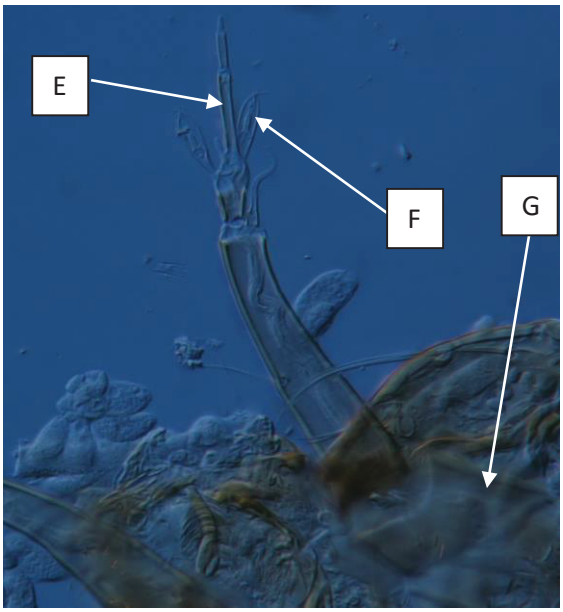




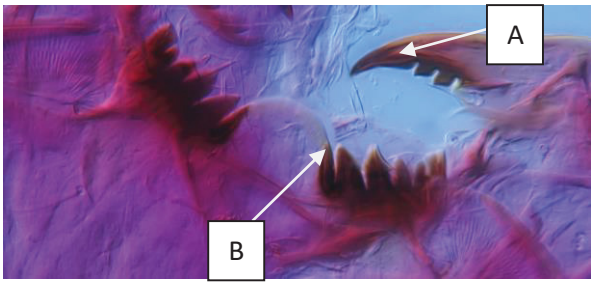
Chironominae *Cladopelma sp.*	
Body and Dorsal Head	Moderate-sized larvae, to 7 mm long, Posterior parapod claws simple.
Antenna	5 segments; basal segment longer than flagellum (E). 3rd segment somewhat reduced (D).
Mentum	Mentum with double teeth (A); outer teeth of the mentum are somewhat enlarged and set forward (B). Ventromental plates well developed with striations throughout (C).
Labrum	Premandible with 2 teeth. Brush well developed. SI seta blade-like.
Mandible	No dorsal tooth, with apical tooth and 1-2 flat inner teeth (G).
Ecology	Burrowers. Collectors-gatherers. Prefer sandy and muddy substrata.

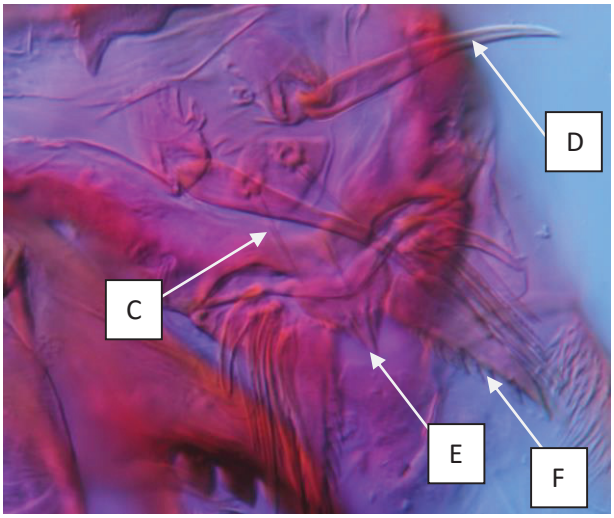


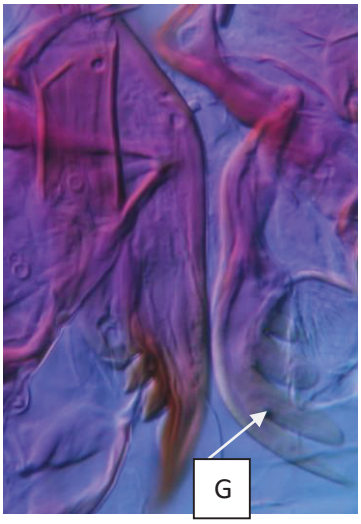




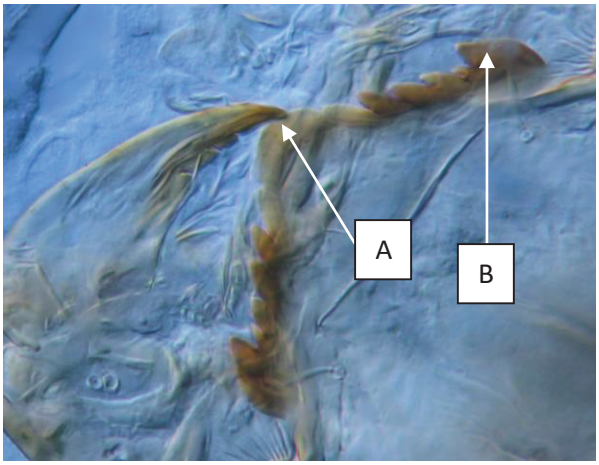
Chironominae *Cladotanytarsus sp.*	
Body and Dorsal Head	Without tubules.
Antenna	Antenna with 5 segments on a short pedestal lacking any basal tooth or spur (G). Lauterborn organs large (F), on short broad pedicels, opposite on apex of wedge-shaped 2nd segment. 3rd segment (E) usually longer than 2nd.
Mentum	Mentum with median tooth often notched (C); laterals decreasing in size or 2nd lateral much smaller than remainder. Ventromental plates close together medially (D) with fine striae.
Labrum	SI seta comb-like, fused at bases (A). Pecten epipharyngis with 3 distally serrate scales (B). Premandible with 4 or 5 apical teeth.
Mandible	Mandible with pale dorsal tooth, apical tooth and 3 pointed inner teeth dark.
Ecology	Collectors-gatherers and filters. Can be found in many types of water bodies.

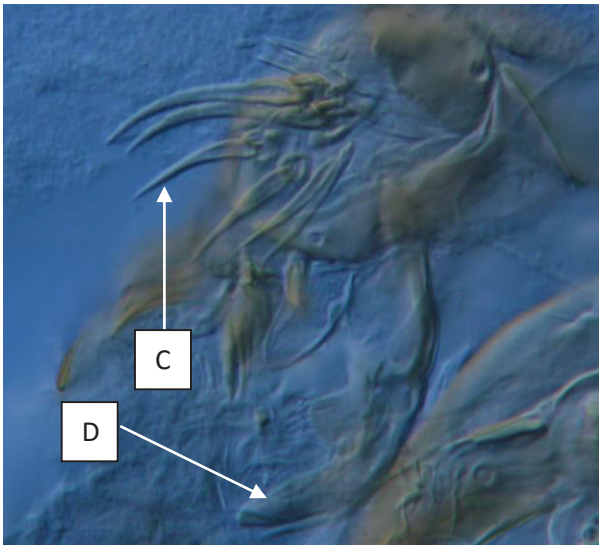


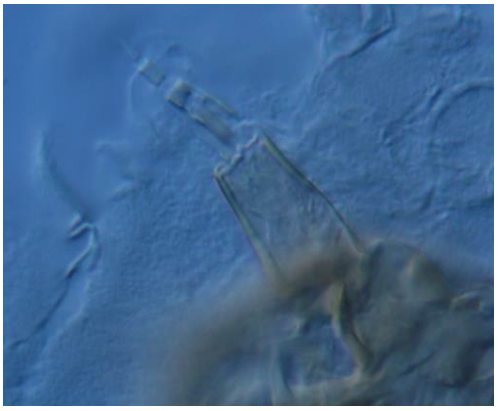




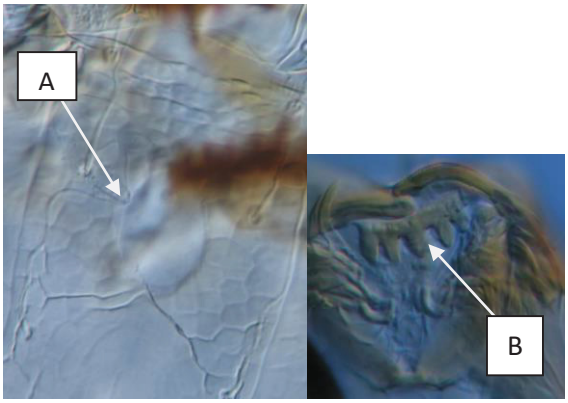
Chironominae *Cryptochironomus sp.*	
Body and Dorsal Head	Up to 15 mm long; Posterior parapod claws simple.
Antenna	5 segments, basal segment equal to or longer than flagellum. Lauterborn organs and antennal seta absent.
Mentum	With a dome-shaped pale median tooth and 6 pairs of dark lateral teeth, first fused to median (B). Lateral teeth longer than median tooth, giving the mentum an overall concave appearance.
Labrum	SI seta short (C), SII longer and robust (D); Pecten epipharyngis a triangular plate divided into 3 lobes with serrate margin (E). Premandible with 4-6 teeth (G).
Mandible	Mandible lacking dorsal tooth, with long apical tooth and 2 triangular inner teeth
Ecology	Sprawlers and burrowers; Predators; Occurs in various substrata.

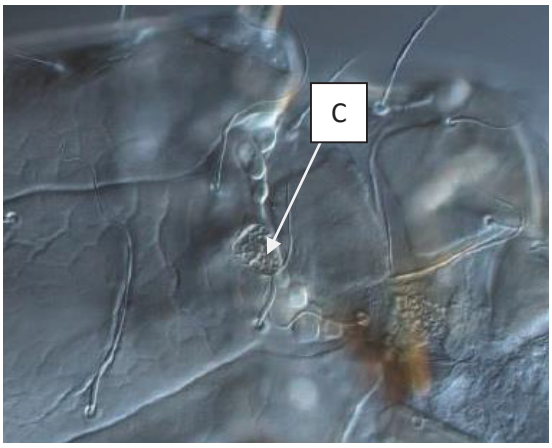


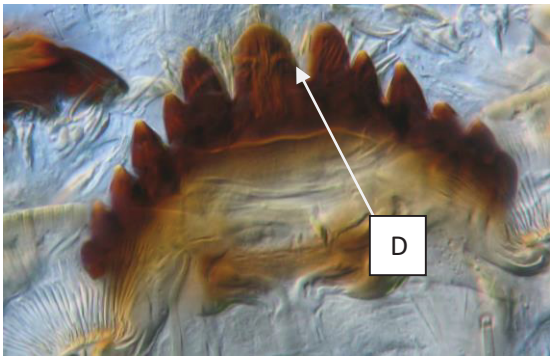


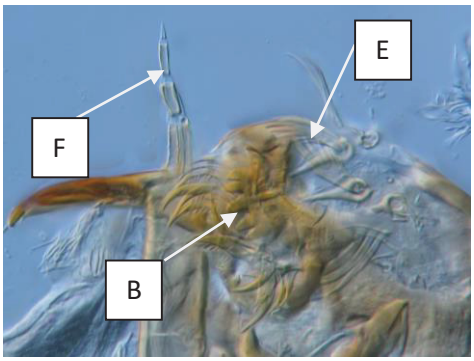


Chironominae *Cryptotendipes sp.*	
Body and Dorsal Head	Less than 6 mm long. Posterior parapod claws simple.
Antenna	5 segments. Lauterborn organs absent. Antennal blade shorter than flagellum.
Mentum	Median tooth large rounded or has lateral notches (A). Three outermost lateral teeth of mentum distinctly enlarged (B).
Labrum	SI seta broad, blade-like (C). Pecten epipharyngis a broad plate divided into 3 shallow lobes. Premandible with 2 slender teeth (D).
Mandible	Dorsal tooth absent. Apical tooth subequal in size to the two flat inner teeth.
Ecology	Sprawlers. Prefer substrata of sand and mud.

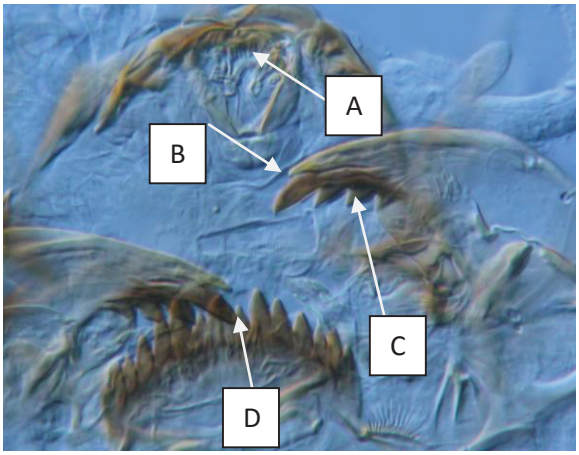


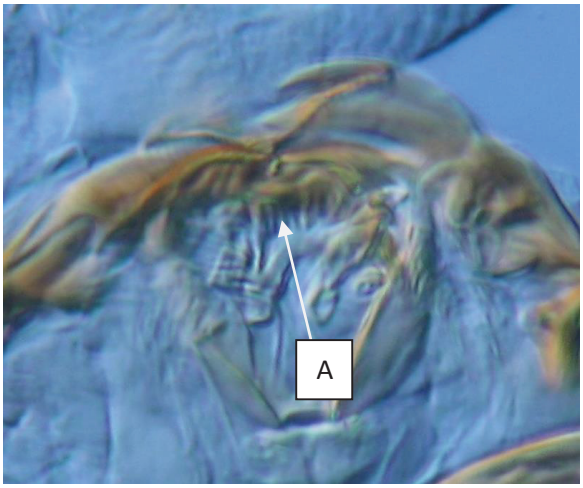


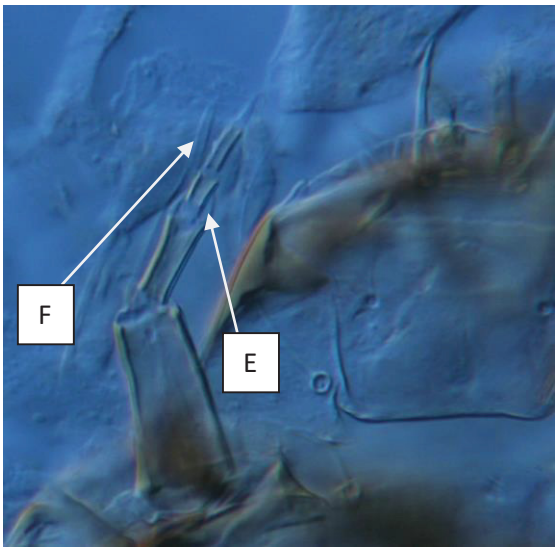




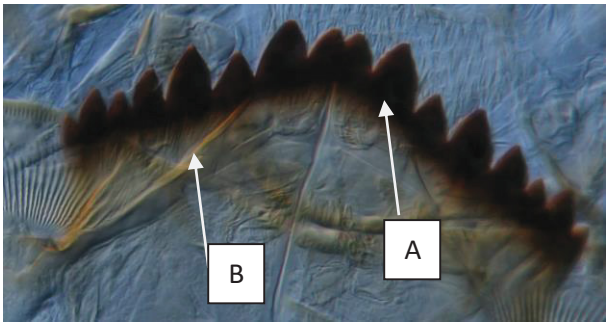
Chironominae *Dicrotendipes sp.*	
Body and Dorsal Head	Frontal apotome usually has a frontal pit (C) or an apotomal fenestra (A)
Antenna	Antenna 5 segmented with 4th (F) often obviously elongate (longer than 3rd).
Mentum	Median tooth and 1st lateral teeth enlarged and somewhat pointed (D); Ventromental plate width less than width of mentum;
Labrum	SI palmate or coarsely plumose (E). Pecten epipharyngis usually has a plate with 3-6 strong or blunt teeth (B). Premandible with 3 teeth.
Mandible	Mandible with pale, dorsal tooth.
Ecology	Burrowers. Collectors-gatherers, filterers, scrapers. Inhabit littoral zone of standing water.

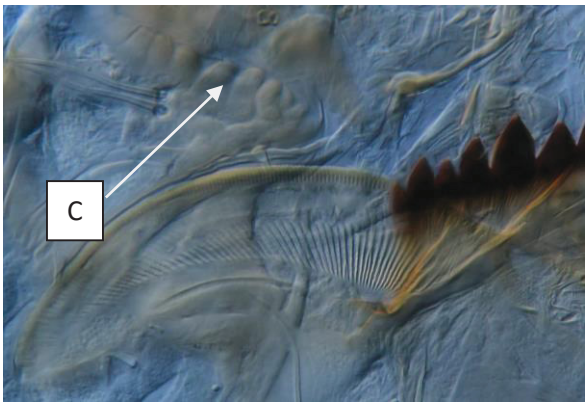


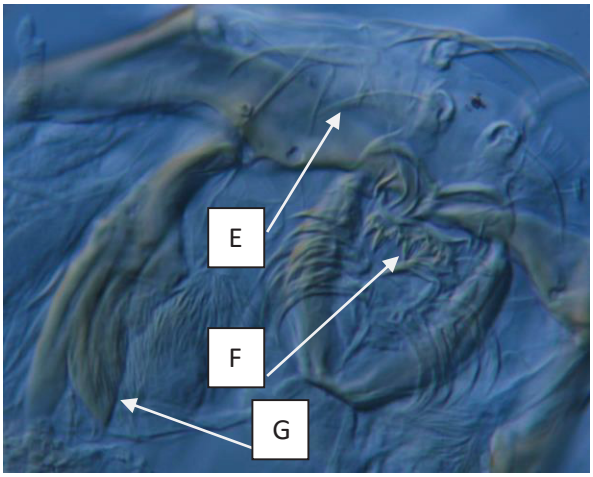




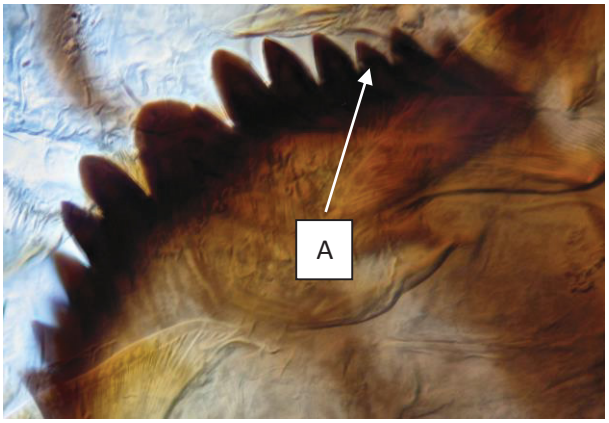
Chironominae *Einfeldia sp.*	
Body and Dorsal Head	Frontol apotome separated from labrum with frontal fenestra or not.
Antenna	5 segments, diminishing in size. Lauterborn organs moderately developed (E). Blade shorter than combination of 2-5 segments (F).
Mentum	Mentum with simple or trifid median tooth (D), 6 pairs of lateral teeth diminishing in size or 4th tooth reduced.
Labrum	SI plumose on both sides; Pecten epipharyngis a simple comb, multi-layer bearing minute teeth, or 3 lobes with minute hair-like points (A).
Mandible	Mandible with pale, prominent, dorsal tooth (B), with strong apical tooth and 2 (C) or 3 inner teeth.
Ecology	Burrowers. Collectors-gatherers. Often occurs in eutrophic standing water.

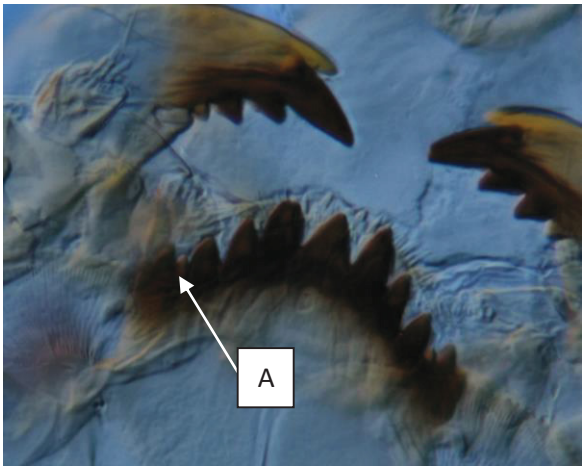


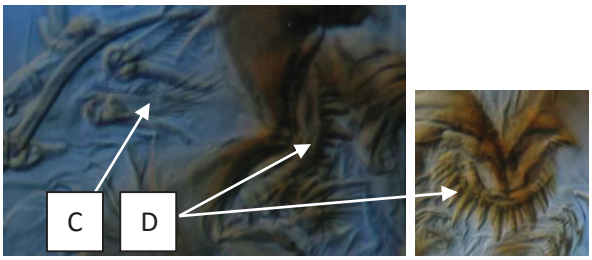


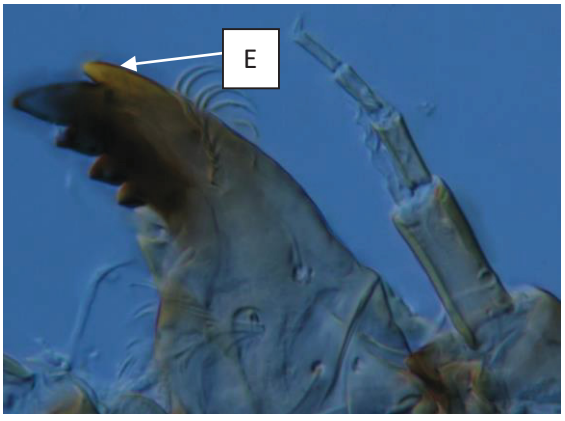


Chironominae *Endochironomus sp.*	
Body and Dorsal Head	Up to 17 mm. Frontal apotome with an equally-wide clypeus. No lateral and ventral tubules.
Antenna	5 segments, diminishing in size. Lauterborn organs about same length as 3rd segment, opposite on the apex of 2nd segment.
Mentum	With distinct 3 or 4 (common) median teeth separated from lateral teeth (A). Anterior margin of the cardo is tuberculate (C).
Labrum	SI (triangular shape) seta plumose on inner side only (E). Pecten epipharyngis divided into 3 parts, each with a few strong distal teeth and numerous minute teeth (F). Premandible with 3 teeth (G).
Mandible	Mandible with obscure pale dorsal tooth.
Ecology	Clingers (tube builders). Shredders-herbivores (miners and chewers), collectors-filters and gathers. Inhabit all types of still water or mine in leaves and stems of macrophytes.

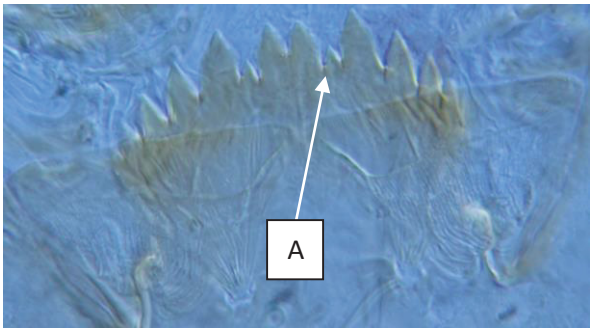


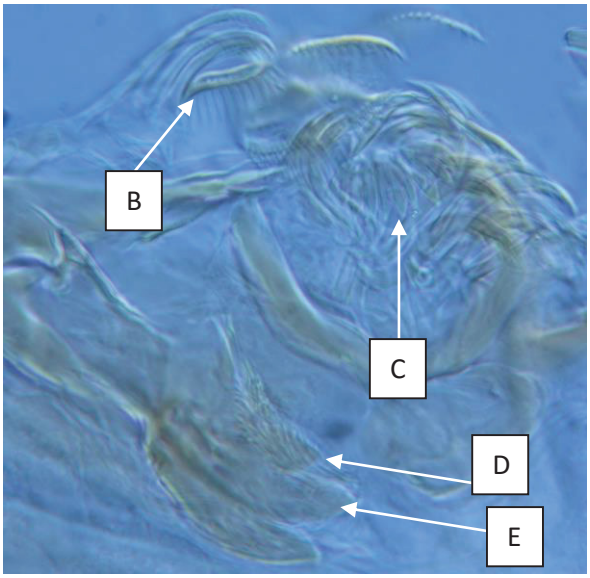


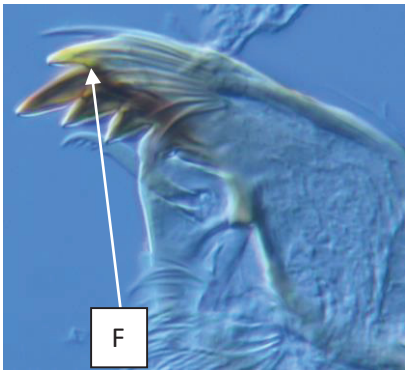


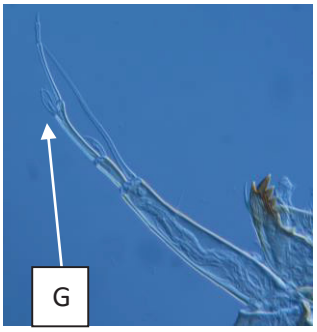


Chironominae *Glyptotendipes sp.*	
Body and Dorsal Head	Up to 18 mm; one pair of ventral tubules. Frontal apotome with anterior margin often strongly concave; two labral sclerites anterior to it.
Antenna	5 segments, diminishing in size.
Mentum	Median tooth simple with or without lateral notches; fourth lateral tooth often shorter than 2 neighbouring teeth (A). Ventromental plates often separated medially by 1.5 x width of median tooth, with smooth to crenulated anterior margin.
Labrum	Pecten epipharyngis single with many teeth of variable length (D). SI plumose to toothed on both side (C).
Mandible	One pale dorsal tooth (E); slender apical tooth followed by 3 inner teeth.
Ecology	Burrowers (miners and tube builders), clingers. Shredders-herbivores (miners and chewers), collectors-filterers and gatherers. Prefer detritus-rich littoral sediments.

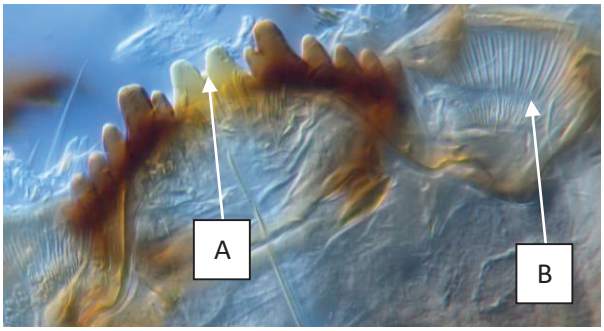


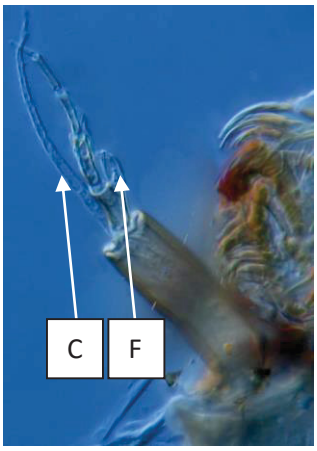


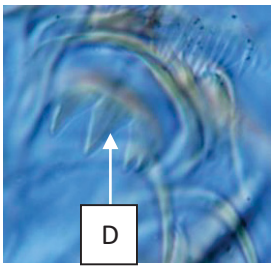


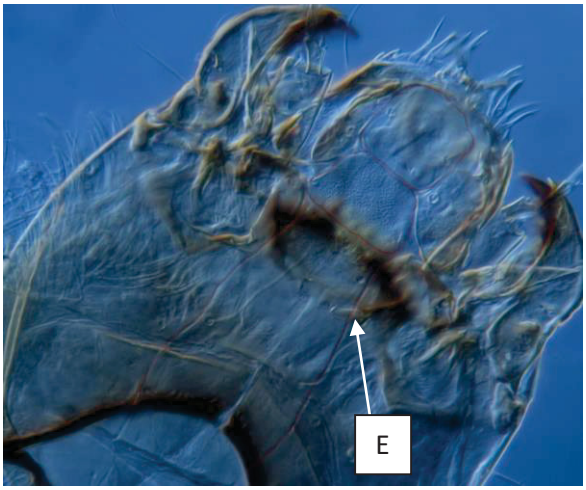


Chironominae *Lauterborniella sp.*	
Body and Dorsal Head	Small larvae, less than 4 mm. Frontal apotome with anterior margin slightly convex, two sclerites anterior to it.
Antenna	6 segments, with 3rd and 4th long. Lauterborn organs large (G), alternate at the apex of segments 2 and 3.
Mentum	Double median teeth with 6 pairs of lateral teeth, 1st pair reduced (A).
Labrum	SI plumose on one side (B), base fused. Pecten epipharyngis 3 lobes, each with several finger-like teeth (C). Premandible with 3 distal teeth (E), with beard (D).
Mandible	With one strong dorsal tooth (F).
Ecology	Climbers, sprawlers-clingers, burrowers (portable sand tube builders). Collectors-gatherers. Live in submerged vegetation of standing water.

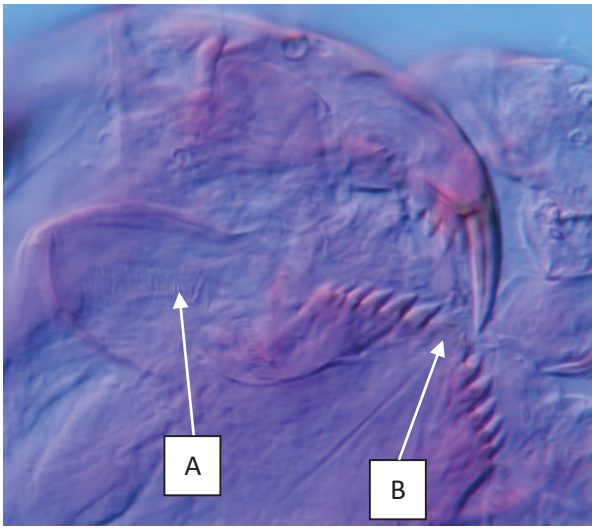


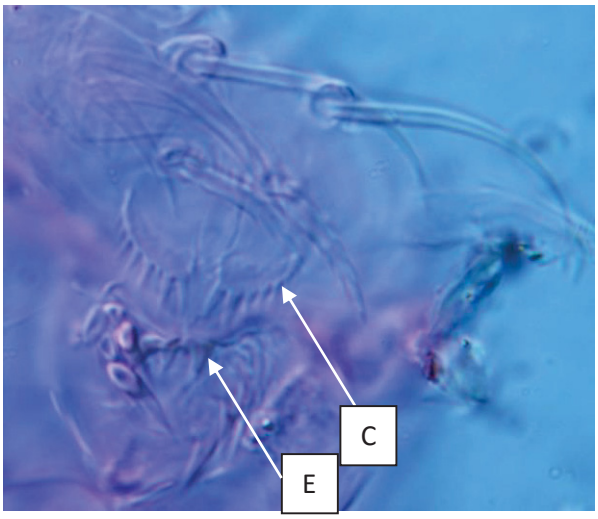


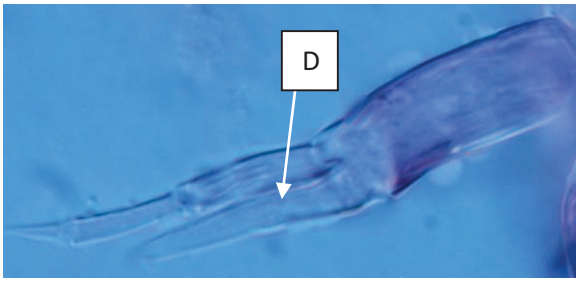




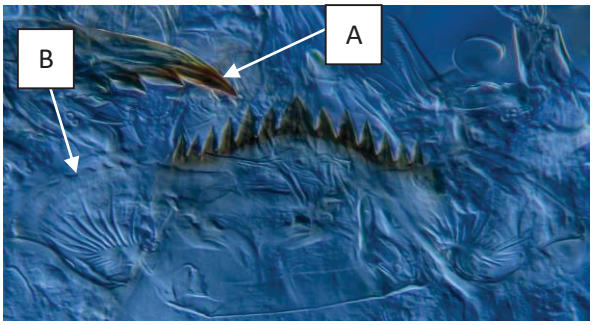
Chironominae *Microtendipes sp.*	
Body and Dorsal Head	Up to 15 mm. Lateral and ventral tubules absent. Frontal apotome separated from clypeus by a straight line (E).
Antenna	6 segments, blade extending to or beyond the apex of antenna (C). Lauterborn organs alternate at the apex of segments 2 and 3 (F).
Mentum	Pale median trifid teeth with minute median tooth (A, maybe absent). Ventromental plate coarsely striated (B).
Labrum	Pecten epipharyngis of three subequal scales (D). SI plumose, base separated.
Mandible	Pale dorsal tooth, apical tooth and 3 inner teeth.
Ecology	Clingers (net spinners). Collectors-filterers and gatherers. Often found in littoral and sublittoral sediments of large bodies of standing water.

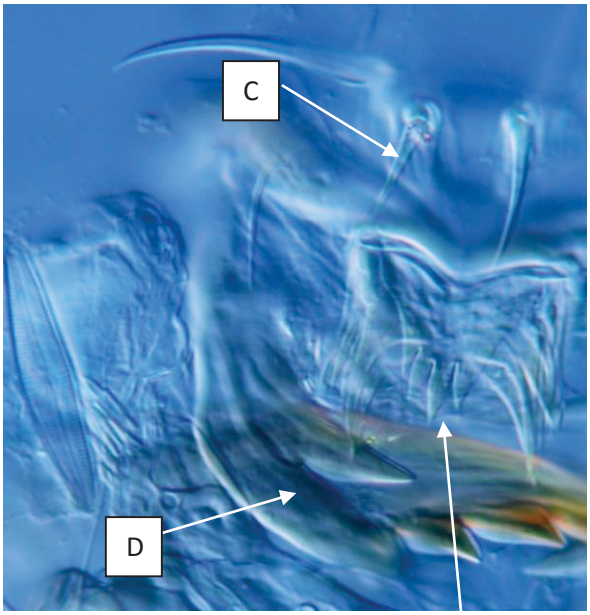


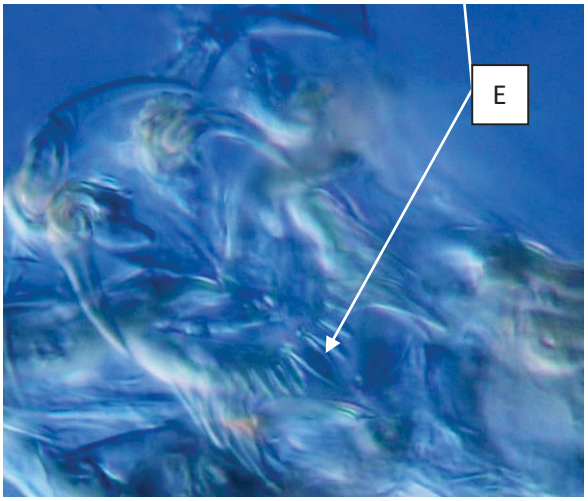




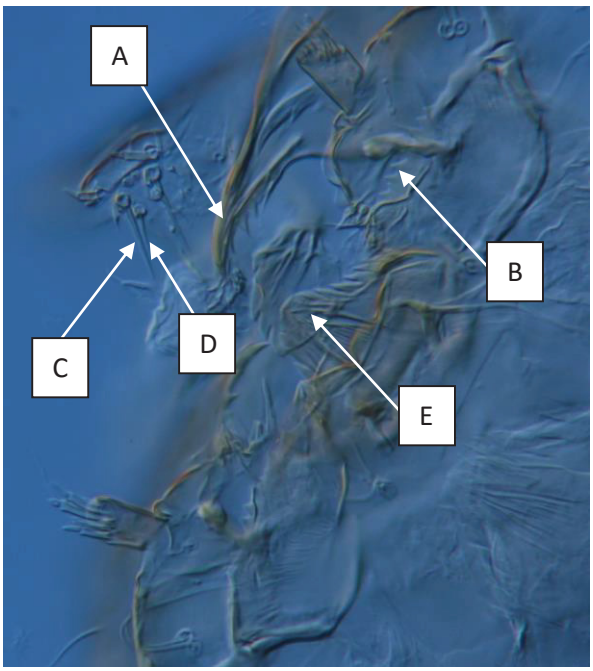
Chironominae *Nilothauma sp.*	
Body and Dorsal Head	Small, less than 5 mm. Frontal apotome separated from clypeus by a straight line.
Antenna	5 segments, basal segment short than flagellum. Blade broad, but shorter than flagellum (D).
Mentum	Pale, with median teeth shorter than 1st pair of lateral teeth (B). Striae only present in the middle of ventromental plate (A).
Labrum	Pecten epipharyngis distally trifid (E). SI plumose distally (C).
Mandible	All teeth pale. Long apical tooth on a different plane from 4 inner teeth.
Ecology	Prefer littoral and sublittoral soft sediments of large standing water.

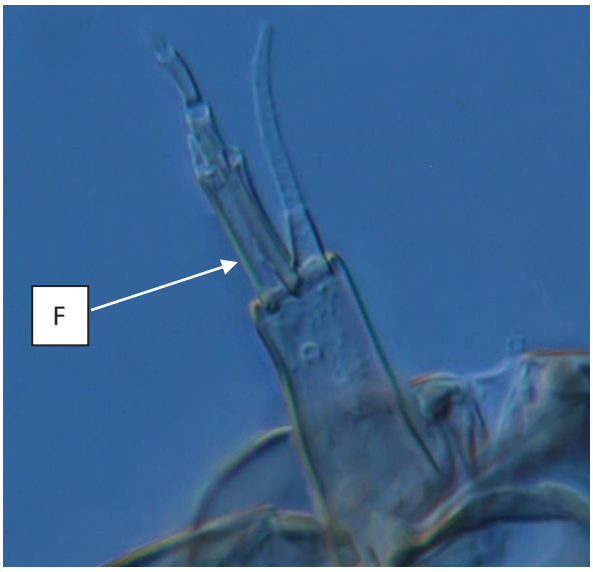




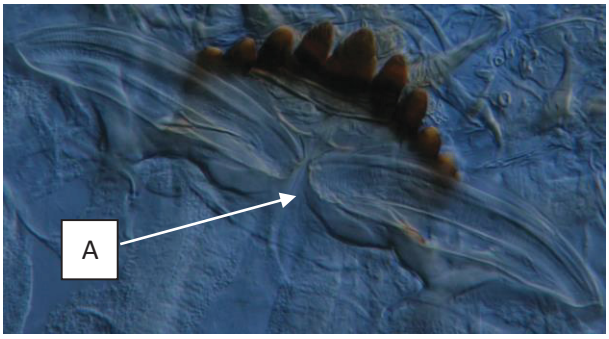


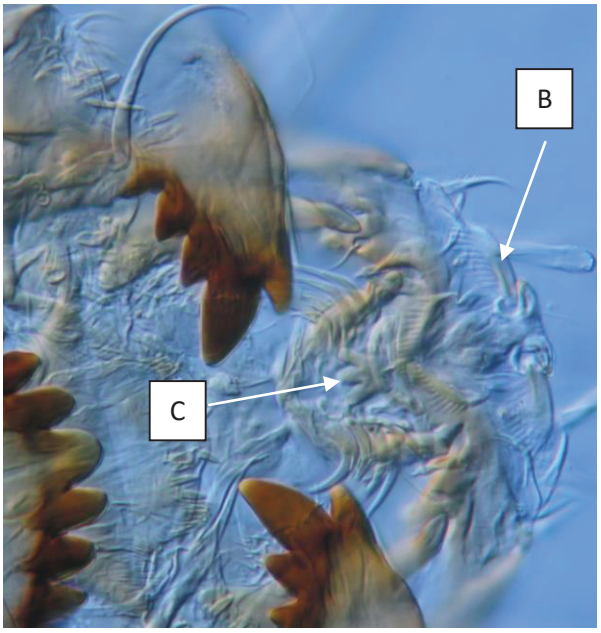
Chironominae *Parachironomus sp.*	
Body and Dorsal Head	Up to 12 mm long. Posterior parapods claws simple.
Antenna	5 segments with basal one longer than flagellum.
Mentum	Median tooth simple or with a small notch in the middle, usually 2 times wider than 1st lateral teeth. Anterior margin of ventromental plates moderately or strongly scalloped (B).
Labrum	Pecten epipharyngis a wide transparent plate with several pointy teeth (E). SI simple, blade-like (C). Premandible with 2-4 teeth (D).
Mandible	Dorsal tooth absent. Long apical tooth with 2 inner teeth (A).
Ecology	Sprawlers. Predators, collectors-gatherers, parasites. Could occur in a wide range of conditions in both standing and flowing water bodies.

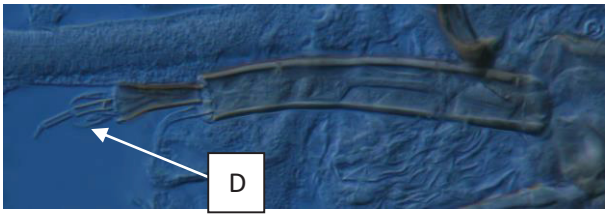




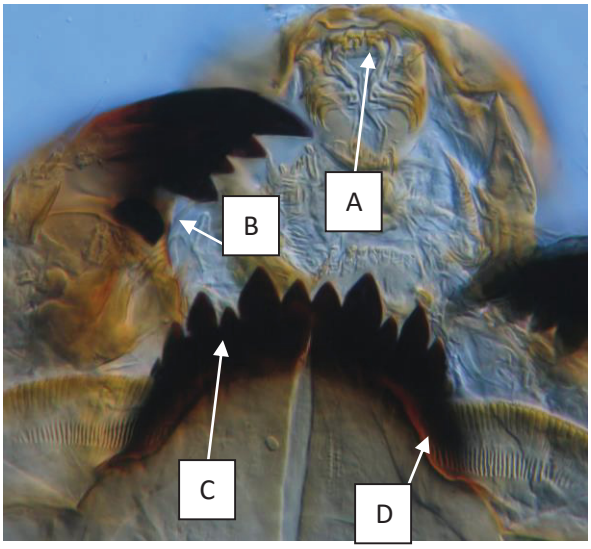
Chironominae *Paracladopelma sp.*	
Body and Dorsal Head	Up to 10 mm.
Antenna	5 segments with 2nd much longer than 3rd (F). Lauterborn organs absent.
Mentum	With double or a single dome-shaped median tooth (E). Ventromental plates coarsely striated with crenulated anterior margin (B).
Labrum	Pecten epipharyngis triangular maybe laterally notched or divided. Small, seta-like SI (D) and large SII (C). Premandible with 4 or more teeth.
Mandible	No dorsal tooth; Apical tooth long with 3 pointed inner teeth.
Ecology	Sprawlers. Often found in sandy substrata of both lentic and lotic waters. Sensitive to eutrophication.

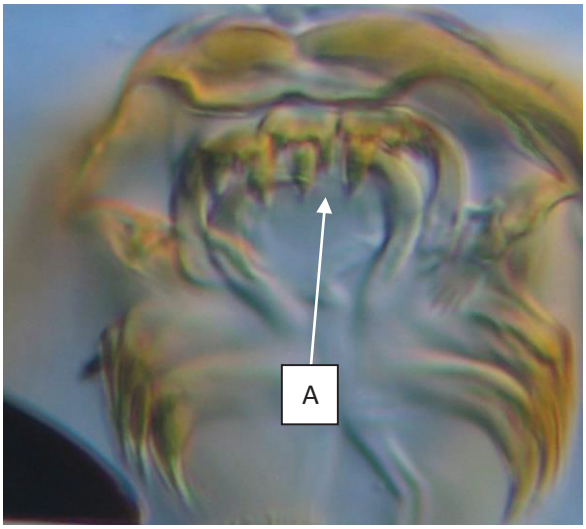




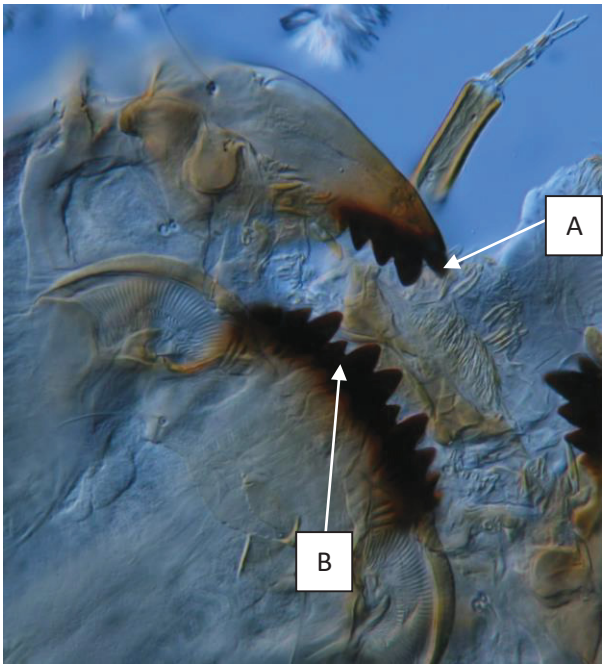


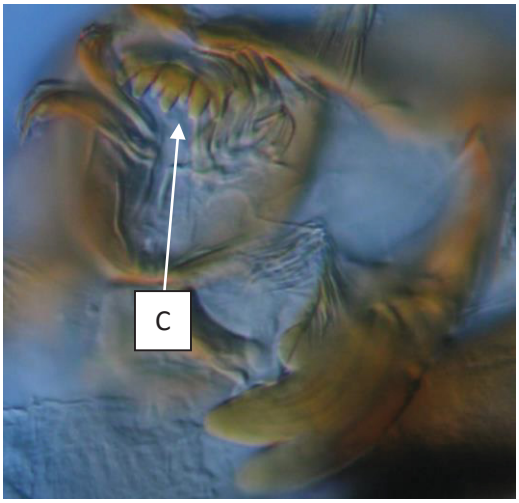
Chironominae *Paratanytarsus sp.*	
Body and Dorsal Head	Small, up to 7 mm. Lacking tubules.
Antenna	Antenna with 5 segments on a tall pedestal without spur. Lauterborn organs modest, at the apex of 2nd antennal segment (D).
Mentum	Median tooth simple with or without lateral notches. 5 pairs of lateral teeth, diminishing in size. Ventromental plates almost touch medially (A), with well-developed fine striae.
Labrum	Pecten epipharyngis single plate with 3-5 lobes (C). SI comb-like (B).
Mandible	One dark dorsal tooth, apical tooth and 2-3 pointed inner teeth.
Ecology	Sprawlers. Found in a wide range of aquatic habitats, including brackish water.

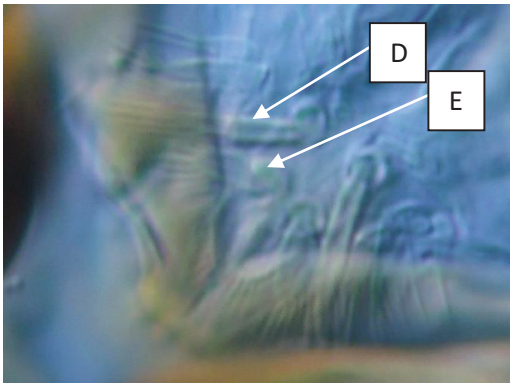




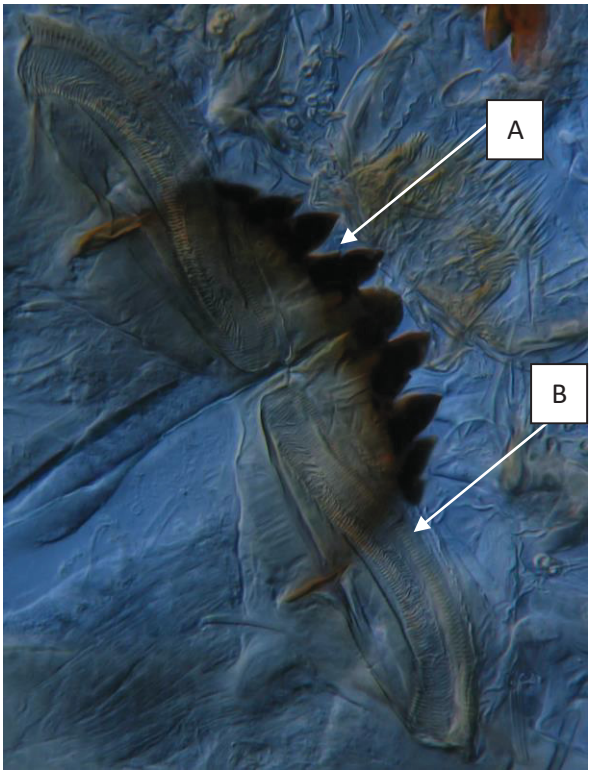
Chironominae *Phaenopsectra sp.*	
Body and Dorsal Head	Medium size, usually larger than 8 mm. Frontal apotome with a convex anterior margin, frontal fenestra absent.
Antenna	5 segments. Lauterborn organs alternate at the apex of segments 2 and 3. Blade subequal to flagellum.
Mentum	Dark black, with 4 elevated median teeth, of which inner pair is shorter than outer pair. 6 pair of lateral teeth, with first pair reduced (C). Ventromental plates medially connected to posterior margin of the outer median teeth (D).
Labrum	Pecten epipharyngis with 3 distally serrated plates (A). Premandible with 3 teeth. SI and SII plumose on both sides.
Mandible	Teeth dark with a short dorsal tooth, an apical tooth and 3 inner teeth. A deep notch between mola and the 3rd inner teeth (B).
Ecology	Clingers (tube builders); Scrapers, collectors-gatherers; Often found in sandy and muddy substrata of streams or small standing waters.

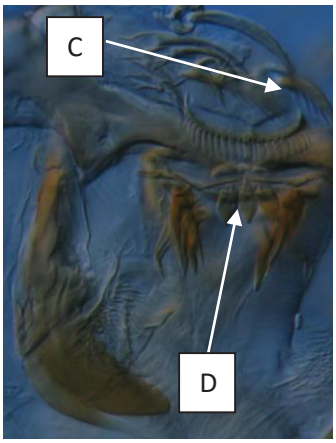


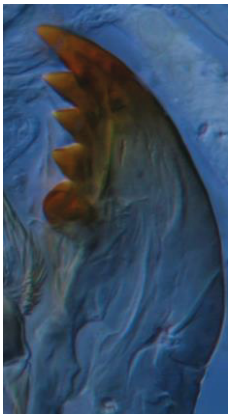




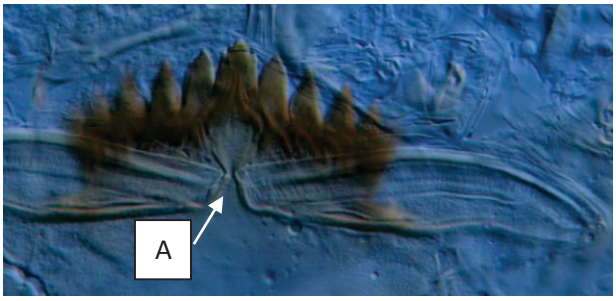
Chironominae *Polypedilum sp.*	
Body and Dorsal Head	Usually over 8 mm. Frontal apotome with anterior margin.
Antenna	5 segments. Blade subequal to flagellum. Lauterborn organs at the apex of 2nd antennal segment.
Mentum	Two median teeth and 6 pairs of lateral teeth, with first lateral tooth reduced (B). Ventromental plates separated by at least the width of median teeth, anterior margin smooth.
Labrum	Pecten epipharyngis with 3 distally serrated plates (C). Premandible with 3 teeth. SI (E) and SII (D) plumose.
Mandible	Dorsal tooth usually well developed (A). One apical tooth with 2 inner teeth.
Ecology	Climbers, clingers. Shredders-herbivores (miners), collectors- gatherers, predators. Occur in all kinds of lentic and lotic waters.

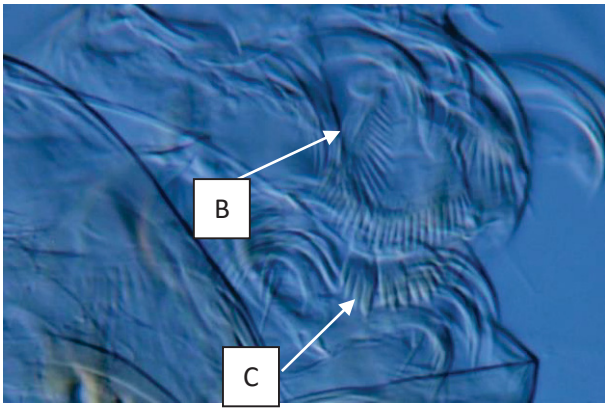


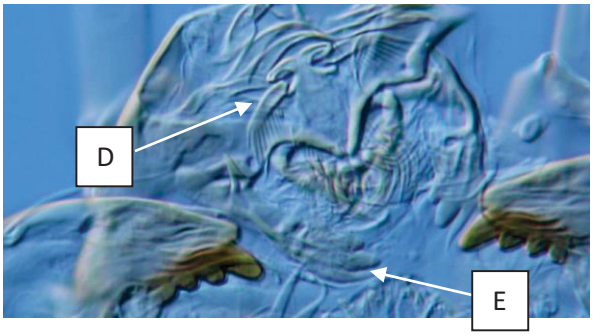




Chironominae *Pseudochironomus sp.*	
Body and Dorsal Head	Up to 11 mm. Lateral and ventral tubules absent.
Antenna	5 segments, decreasing in size. Blade as long as flagellum. Lauterborn organs at the apex of 2nd antennal segment.
Mentum	With a broad, rounded median tooth, 1st pair of lateral teeth extended as long as median tooth, 2nd pair of lateral teeth reduced or fused to 1st (A). Large-bar like ventromental plates close together medially, densely striated (B).
Labrum	Pecten epipharyngis of three simple lobes (D). SI plumose (C), not fused at base.
Mandible	Without dorsal tooth, with pale apical tooth and 4 dark inner teeth
Ecology	Burrowers. Collectors- gatherers. Prefer sandy and gravelly substrata of lakes and rivers.

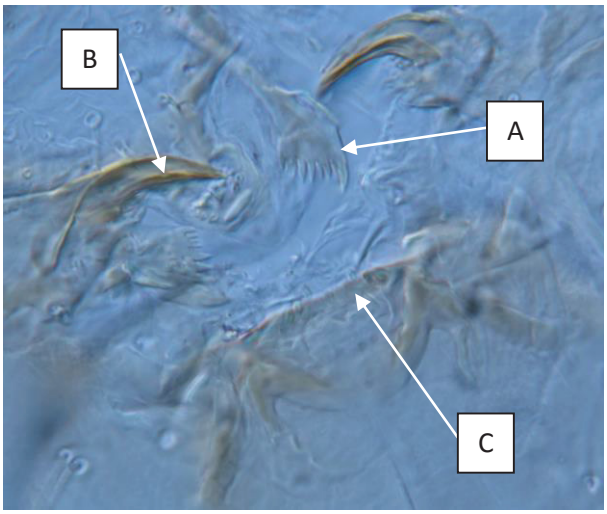


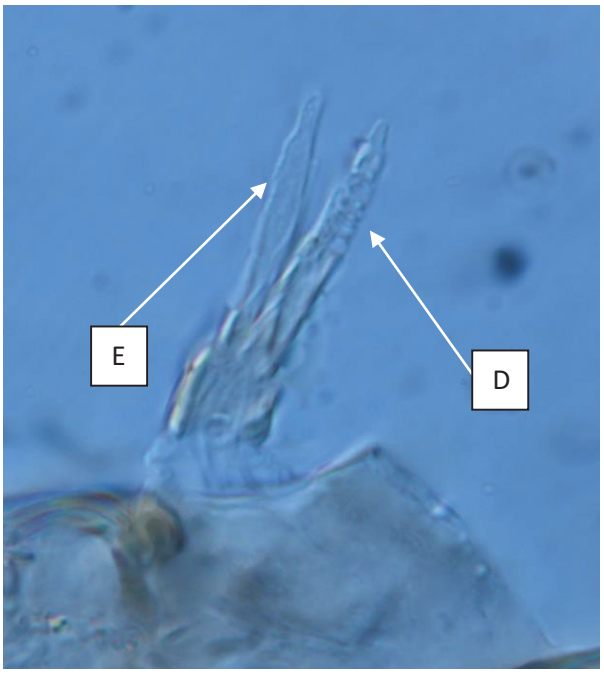




Chironominae *Tanytarsus sp.*	
Body and Dorsal Head	Up to 9 mm without tubules.
Antenna	5 segments on a tall tubercle with or without spur. Lauterborn organs usually on long stalks at the apex of 2nd segment (F). Stalks maybe annulated or not.
Mentum	Median tooth rounded or with lateral notches. Ventromental plates almost touch medially (A), with fine striae.
Labrum	Pecten epipharyngis 3 distally serrate lobes (C). SI comb-like (B), SII on large pedestal.
Mandible	With 1-2 dorsal teeth, apical tooth and 2-3 pointed inner teeth.
Ecology	Climbers, clingers (net spinners). Collectors-filterers and gatherers, a few scrapers. Common, could occur in all types of aquatic habitat.

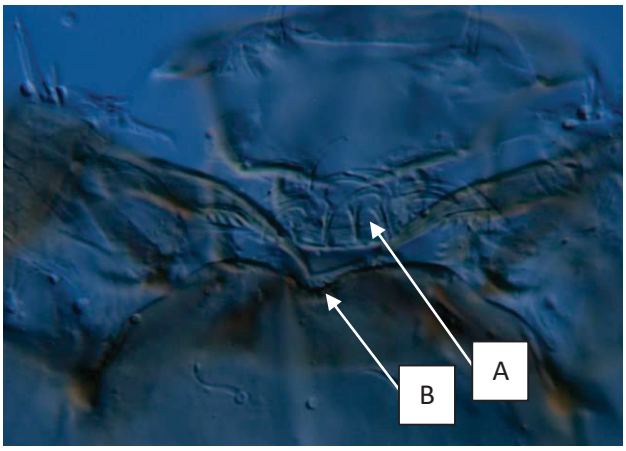
2.3.2 Subfamily Diamesinae

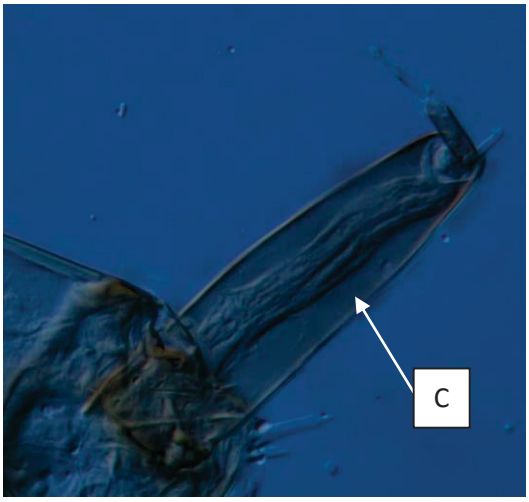


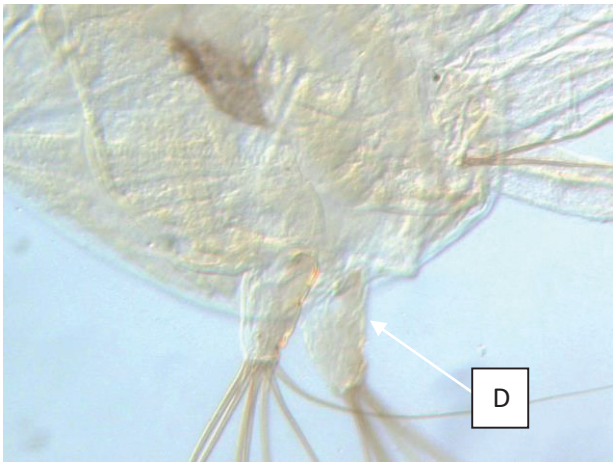


Diamesinae *Pothastia sp.*	
Body and Dorsal Head	Medium size, up to 11 mm long.
Antenna	5 segments with 3rd annulated (D), 5th longer than 4th. Blade as long as combined lengths of segments 2-5 (E).
Mentum	Median area lacking teeth (C), with all lateral teeth covered by ventromentum.
Labrum	Pecten epipharyngis of 3 narrow, pointed scales. Premandible with 5-6 teeth (A). SI seta-like.
Mandible	Apical tooth longer than combined width of all inner teeth (B).
Ecology	Prefer lentic habitat.

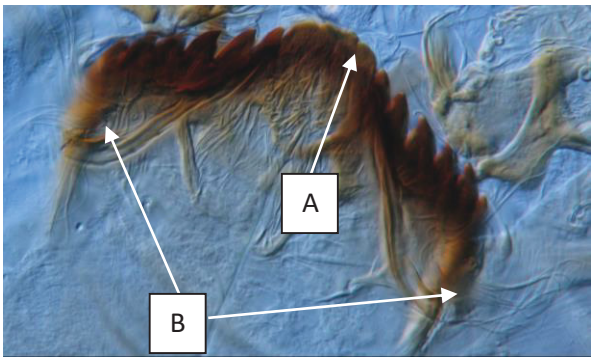
2.3.3 Subfamily Orthocladiinae

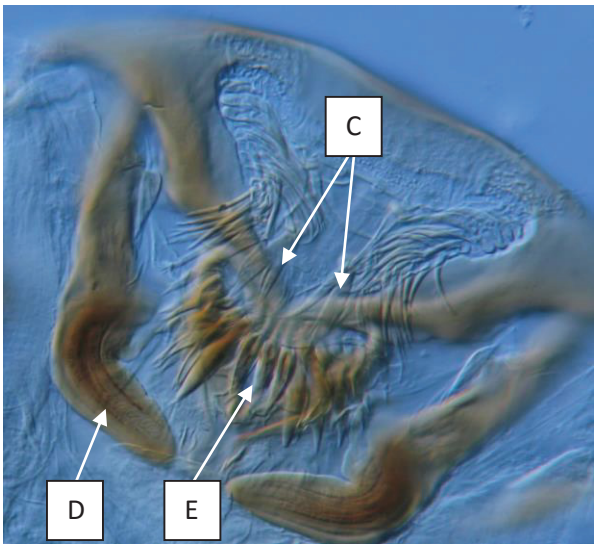


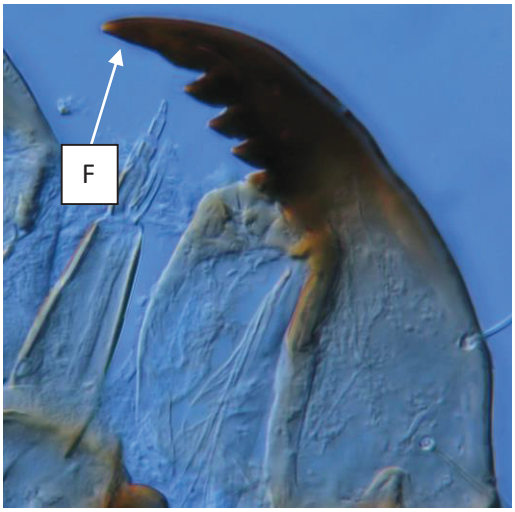




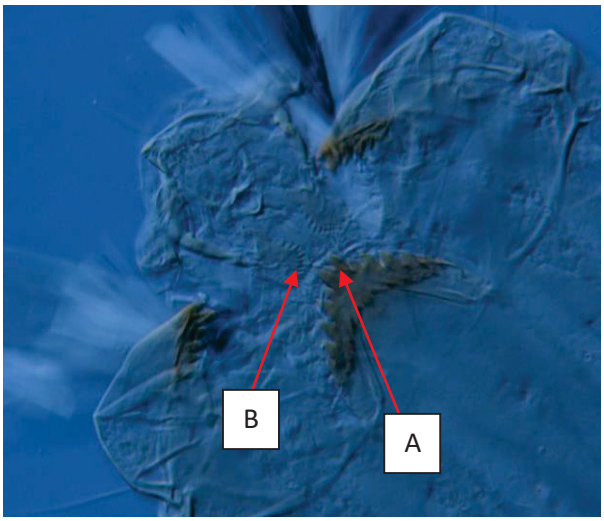
Orthocladiinae *Acamptocladius sp.*	
Body and Dorsal Head	Small, up to 4.5 mm long.
Antenna	5 segments with 3rd and 4th segments hard to distinguish. Blade as long as combined lengths of segments 2-5.
Mentum	Ventromentum extending over dorsomentum; 3 small ventromental teeth (B), 12-18 pairs of small dorsolmental teeth arranged on lateral sides.
Labrum	Pecten epipharyngis of 3 narrow, pointed scales. Premandible with 3 distinct apical and 1 inner and 1 outer tooth; All setae simple and fine.
Mandible	Apical tooth much longer than combined width of all inner teeth.
Ecology	Collectors-gatherers.

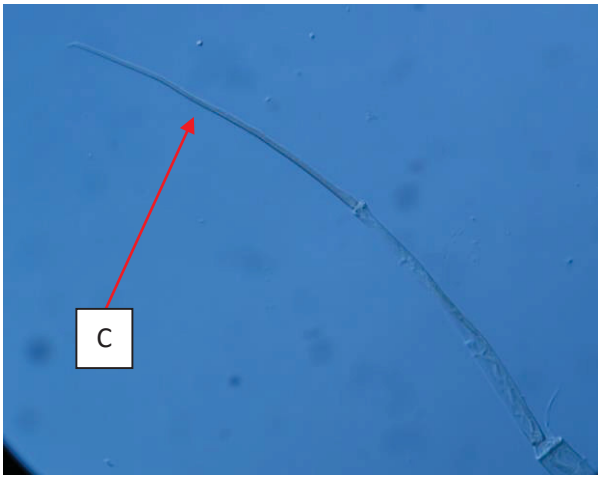




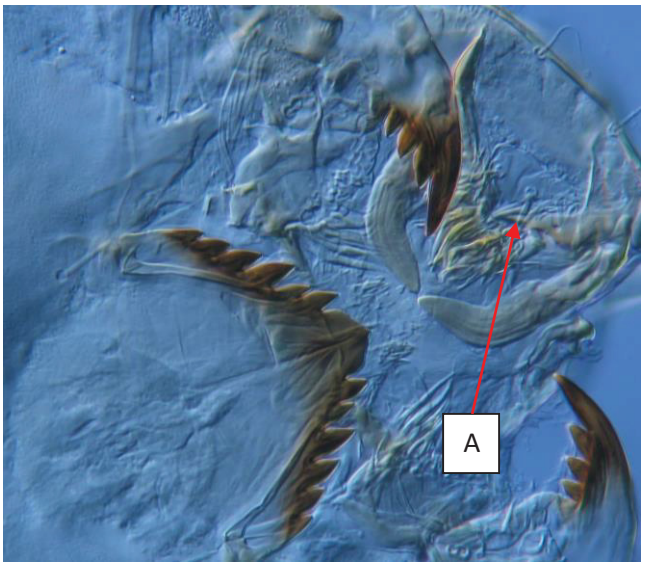


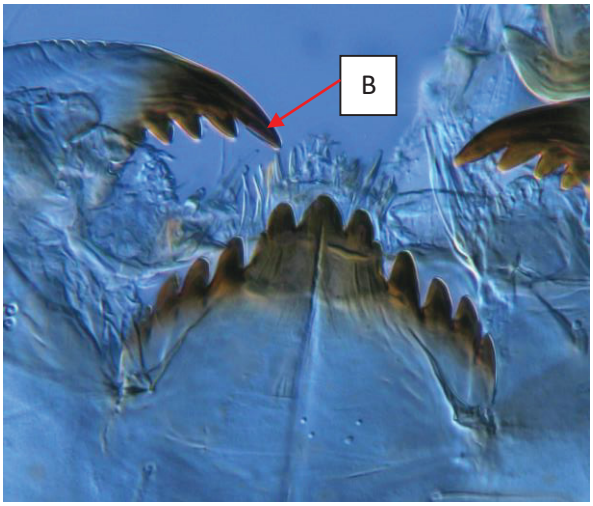
Orthocladiinae *Acricotopus sp.*	
Body and Dorsal Head	Medium size, up to 8 mm long.
Antenna	5 segments, diminishing in size.
Mentum	1 broad median tooth often notched dividing it into 4 parts (A). Ventromental plate with well developed long beard (B).
Labrum	Pecten epipharyngis of 3 scales (E). Premandible with 1 long apical tooth (D) and 1 broad inner tooth. SI bifid with secondary feathering (C).
Mandible	Apical tooth slightly longer than combined width of 3 inner teeth.
Ecology	Sprawler. Occur in a variety of freshwater habitats.



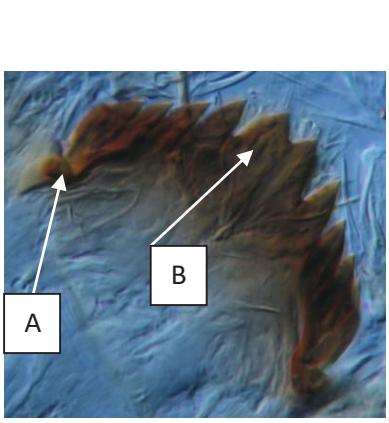


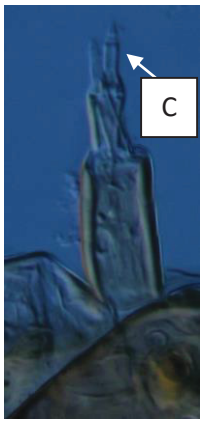
Orthocladiinae *Corynoneura sp.*	
Body and Dorsal Head	Small, less than 3 mm. Both anterior and posterior parapods elongate.
Antenna	4 segments, often longer than head capsule. Segments 3 (C) usually darkened and longer than second; segment 4 minute.
Mentum	Triangular-shaped, with 2 or 3 median teeth (A) and 5 pairs of lateral teeth; 1st lateral smaller than 2nd lateral. Ventromental plate vestigial; beard absent.
Labrum	Pecten epipharyngis of 3 scales. Premandible apically serrated. SI simple.
Mandible	Apical tooth with 4 inner teeth; apical tooth usually shorter than any of the inner teeth.
Ecology	Sprawlers. Collectors-gatherers. Prefer lentic habitat.

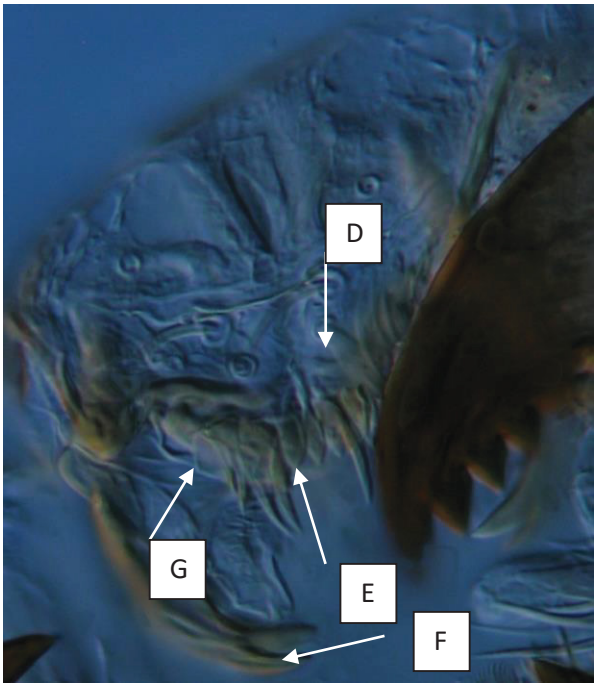


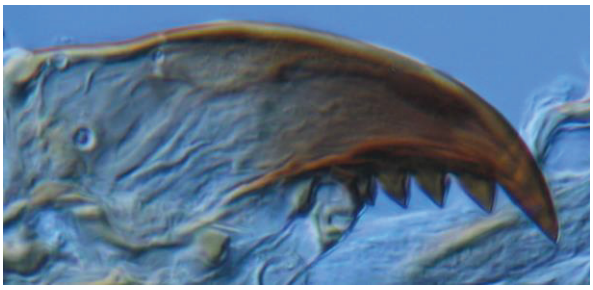


Orthocladiinae *Cricotopus sp.*	
Body and Dorsal Head	Medium size up to 8mm. Abdominal segments usually with 1 pair of tufts of seta (C).
Antenna	Usually 5 segments, decreasing in length. Occasionally antenna is very short. Lauterborn organ usually modestly developed.
Mentum	One median tooth usually with 6 pair of lateral teeth. Ventromental plate narrow, beard often absent.
Labrum	Pecten epipharyngis of 3 scales or 1 scale. SI usually bifid (A), with remaining S setae simple.
Mandible	Apical tooth shorter than combined width of 3 inner teeth (B).
Ecology	Shredders (herbivores) or collectors-gatherers. Often associated with aquatic vegetation

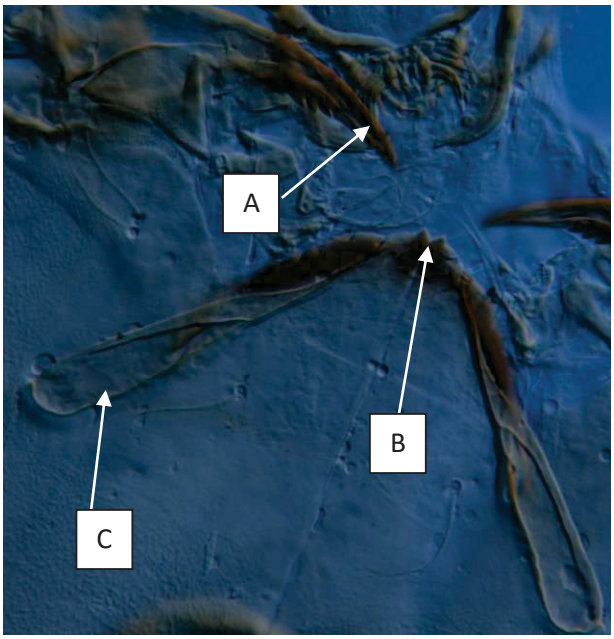


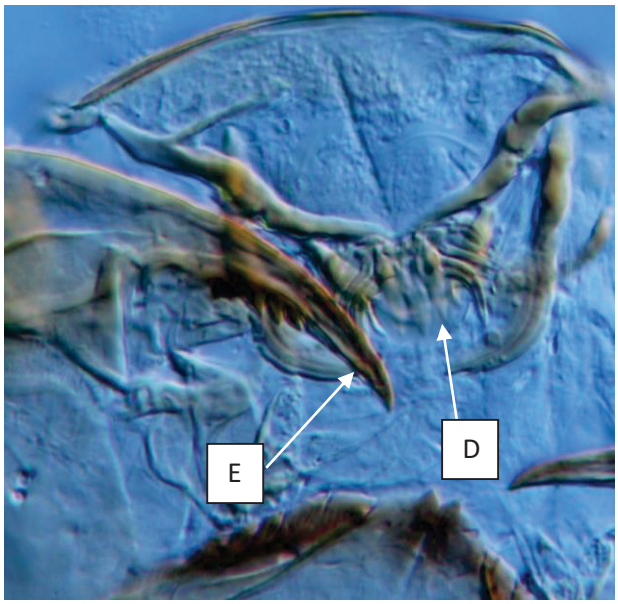




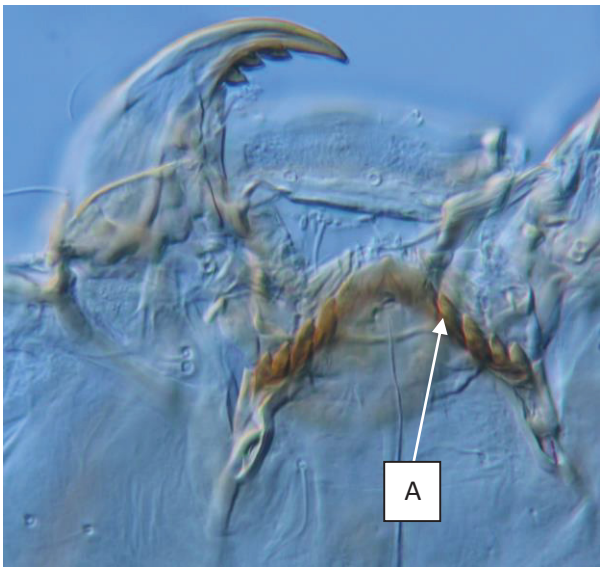


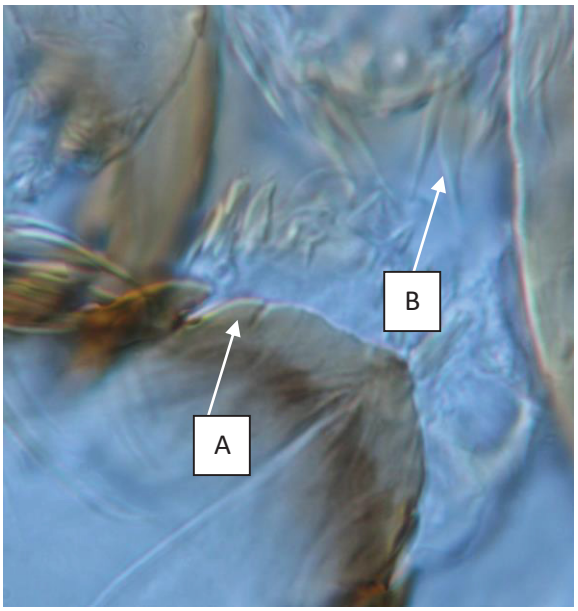
Orthocladiinae *Limnophyes sp.*	
Body and Dorsal Head	Small size, up to 6 mm long. Anal tubules usually shorter than posterior parapods.
Antenna	5 segments, with 4th (C) longer than 3rd segment. Antenna short, usually less than 1/2 length of mandible. Blade as long as flagellum.
Mentum	2 median teeth (B) usually broader and higher than the first pair of lateral teeth. Base of mentum with a rounded tooth, likely an extension of the ventromental plate (A). Beard absent.
Labrum	Pecten epipharyngis of 3 scales (E), often difficult to distinguish from chaetulae laterals (G). Premandible apically bifid (F). SI serrate, sometimes branches reduced, such that SI is simple (D).
Mandible	Apical tooth shorter than combined width of 3 inner teeth.
Ecology	Sprawlers. Collectors-gatherers. Could occur in many types of habitat.

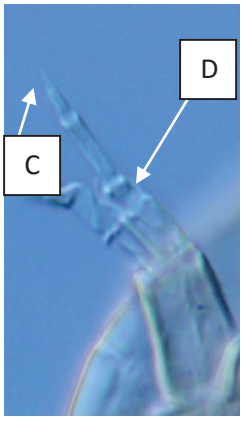




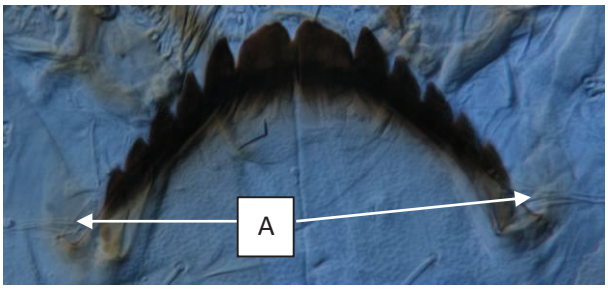
Orthocladiinae *Nanocladius sp.*	
Body and Dorsal Head	Small larvae, up to 5 mm long.
Antenna	5 segments, diminishing in size with 5th hair-like and vestigial. Blade shorter than flagellum. Lauterborn organ usually distinct.
Mentum	One broad median tooth, usually with two nipple-like projections in the middle (B). Ventromental plate well developed, usually extend beyond the lateral margin of mentum (C). Beard absent.
Labrum	Pecten epipharyngis consisting of 3 pointed scales (D). Premandible with 1-5 apical teeth. SI-SIII weak and simple.
Mandible	Apical tooth (E) longer than combined width of 3 inner teeth.
Ecology	Sprawlers. Collectors-gatherers. Occur in a variety of habitats.

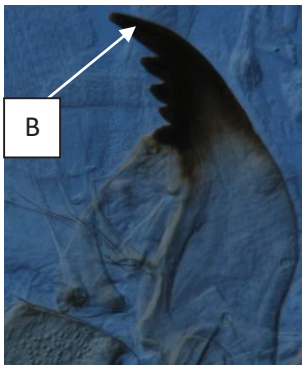


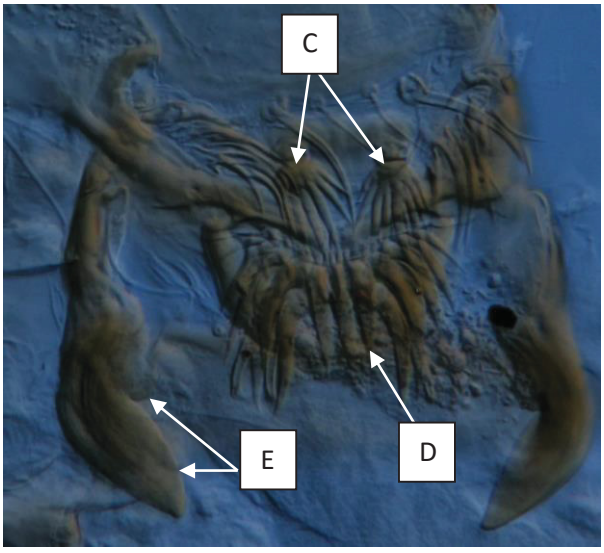




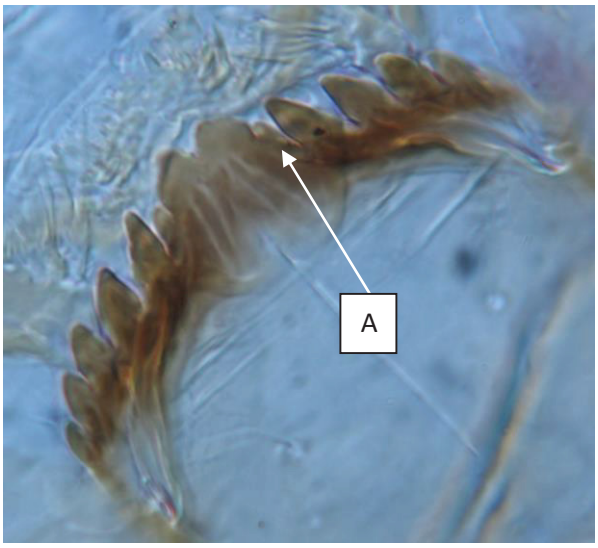
Orthocladiinae *Parakiefferiella sp.*	
Body and Dorsal Head	Small larvae, less than 4 mm long.
Antenna	6 segments with 6th segment (C) hair-like and vestigial, making it hard to see. Segment 3 (D) shorter than 4. Blade usually extending beyond segment 3.
Mentum	1 or 2 median teeth with 6 pairs of lateral teeth; first lateral teeth (A) often appressed to median tooth. Ventromental plate well developed. Beard absent.
Labrum	Pecten epipharyngis consisting of 3 pointed scales. Premandible apically simple or rarely bifid, and a broad inner tooth. SI bifid (B) or with several branches.
Mandible	Apical tooth subequal to or longer than combined width of 3 inner teeth.
Ecology	Sprawlers. Collectors-gatherers. Could occur in all types of aquatic habitats.

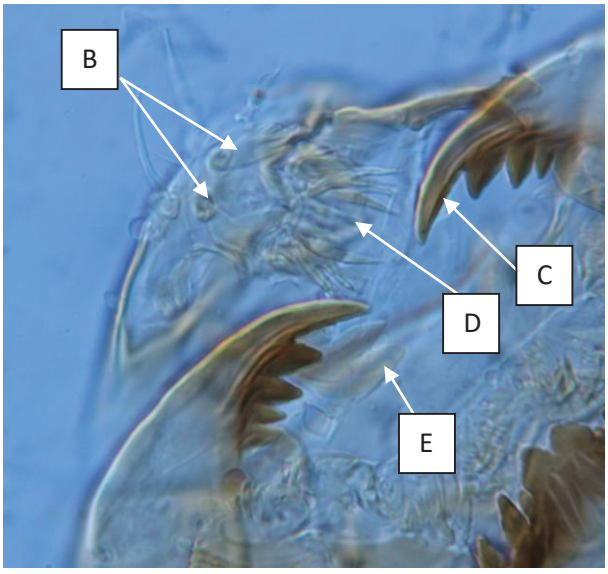






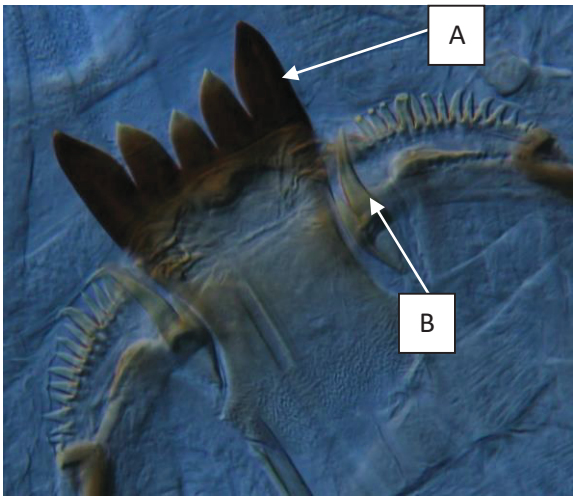
Orthocladiinae *Psectrocladius sp.*	
Body and Dorsal Head	Medium size, up to 11 mm long.
Antenna	5 segments, diminishing in size. Blade shorter than combined lengths of segments 2-5.
Mentum	Mentum with 1-2 median teeth; when 1, then either with median or lateral low projections, with triangular median point, trifid, or with pair of nipple-like median projections; 5 pairs of lateral teeth present. Ventromental plate with well developed long beard (A).
Labrum	Pecten epipharyngis of 3 scales (D). Premandible apically simple (E). SI distinctive (C), palmate with 3-10 lobes, either equal in size or outer lobes smaller.
Mandible	Apical tooth (B) longer than combined width of 3 inner teeth.
Ecology	Sprawlers, burrowers. Collectors-gatherers, shredders-herbivores. Almost exclusively lentic.

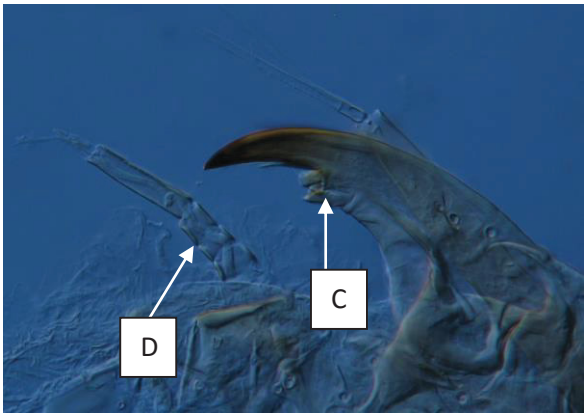




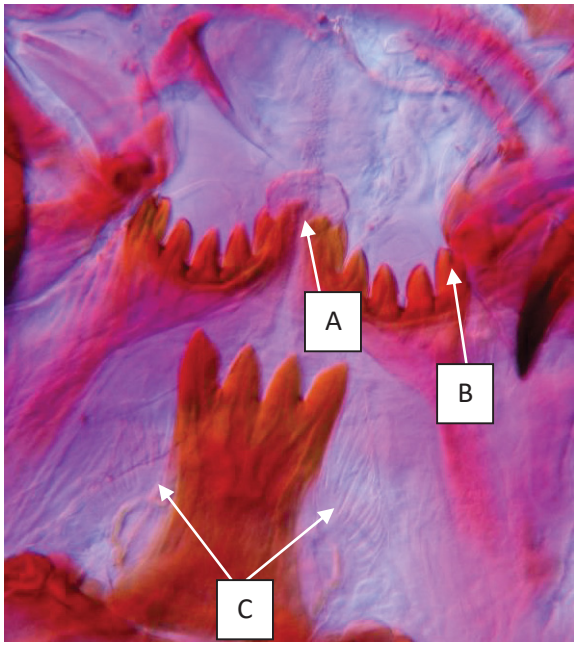
Orthocladiinae *Zalutschia sp.*	
Body and Dorsal Head	Medium size, up to 7 mm long.
Antenna	6 segments with 6th segment hair-like and minute, making it hard to see. Lauterborn organs distinct, shorter than segment 3.
Mentum	Two median teeth and 6 pairs of lateral teeth; first lateral tooth reduced (A). Ventromental plate well developed, with a few fine beard present.
Labrum	Pecten epipharyngis consisting of 3 simple scales (D). Premandible apically bifid (E). SI finely or coarsely plumose (B).
Mandible	Apical tooth shorter than combined width of 3 inner teeth (C).
Ecology	Mainly found in lakes and ponds, occasionally in streams.

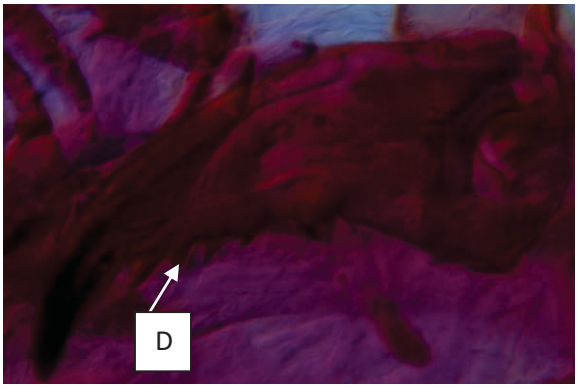
2.3.4 Subfamily Tanypodinae



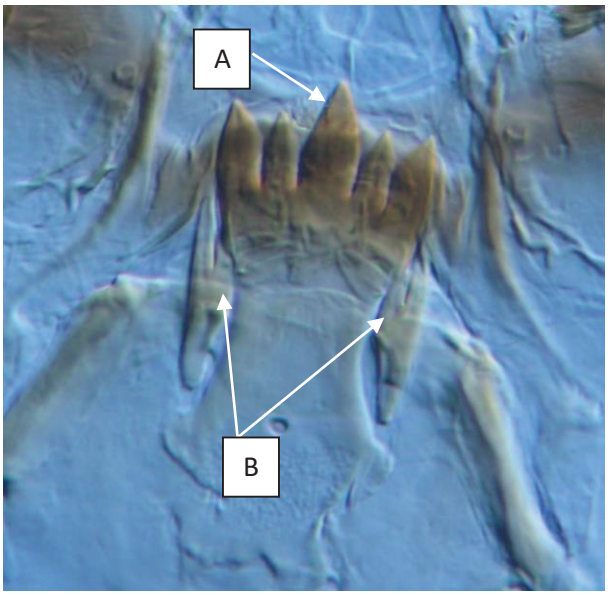


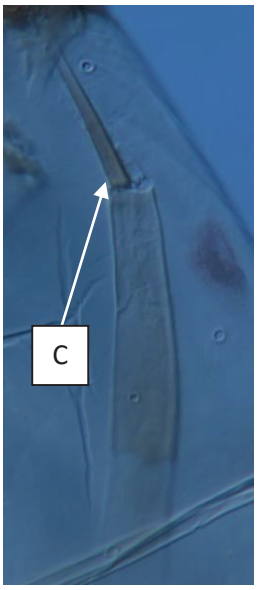
Tanypodinae *Ablabesmyia sp.*	
Body	Large larvae, up to 11 mm long. Head yellow brown, dark claws of posterior parapods.
Antenna	About 1/2 as long as head, 3x as long as mandible Antennal ratio (length of 1st segment/rest of segments) 3.8-12.0.
Mentum and M appendage	Dorsomentum without teeth. Pseudoradula widest near middle and with granules arranged in parallel, longitudinal rows.
Maxilla	Maxillary palpus subdivided into 2-6 segments (D). Ring organ much smaller than width of palp, located between 2 apical segments.
Ligula and Paraligula	Ligula 5 teeth, row of teeth moderately concave (A). Paraligula bifid, ½ as long as ligula (B).
Mandible	With well developed inner and accessory teeth (C).
Ecology	Sprawlers. Predators (engulfers and piercers), collectors-gatherers (early instar). Quite common, live in small and large standing and flowing waters.

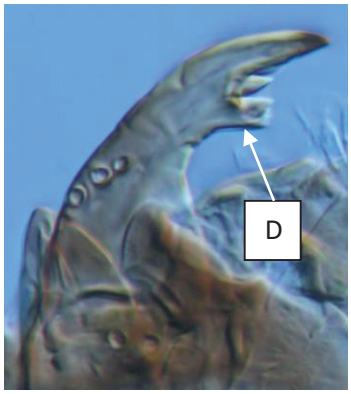




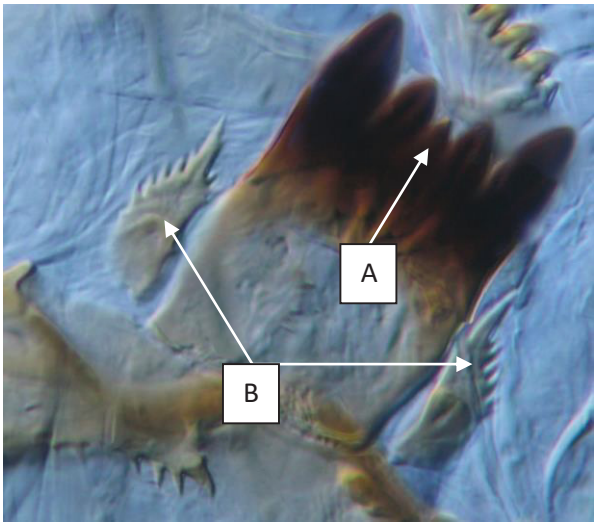
Tanypodinae *Derotanypus sp.*	
Body	Large larvae, up to 13 mm long. Head capsule rounded-oval.
Antenna	Slightly longer than mandible. Antennal ratio 6.0-7.5.
Mentum and M appendage	Dorsomentum (A) with 4-7 teeth on each side in strongly concaved row, with the 2 outer teeth fused (B).
Maxilla	Basal segment about 2.5X as long as wide, with ring organ located at base of distal 1/3
Ligula and Paraligula	Ligula 4 teeth. Paraligula pectinate (C).
Mandible	With more than 4 inner teeth (D).
Ecology	Prefer small, cold, standing and flowing water bodies, able to tolerate high salinities.

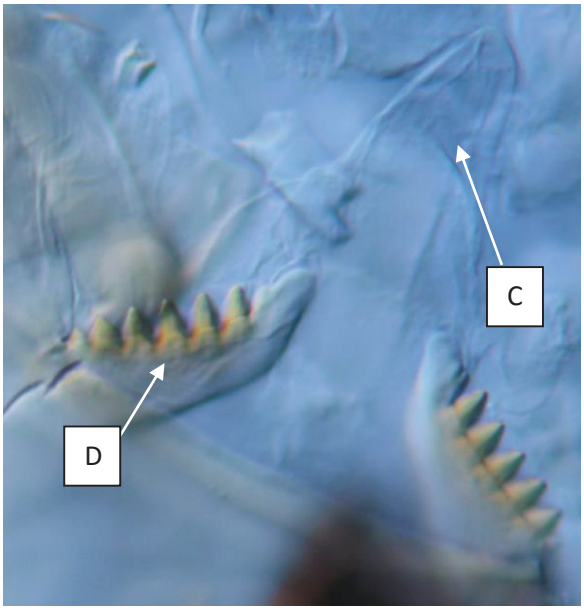


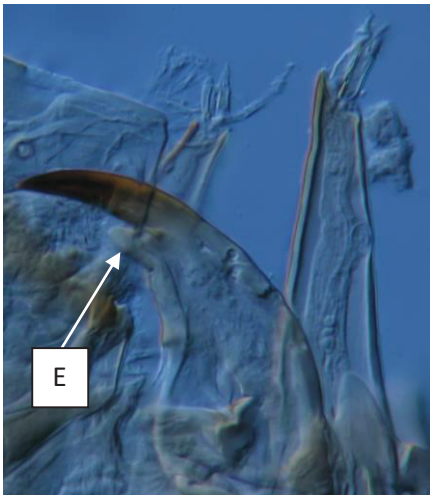




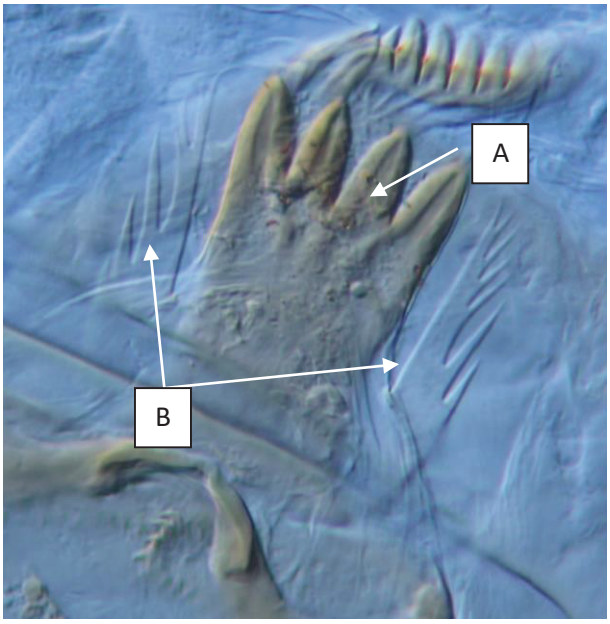
Tanypodinae *Labrundinia sp.*	
Body	Small larvae, up to 5 mm long. Head capsule sometimes marked with brown or black.
Antenna	Relatively long, 3/5 length of head, 3.5X as long as mandible. 2nd segment often more darkly pigmented (C).
Mentum and M appendage	Dorsomentum without teeth.
Maxilla	Basal segment about 2-4 times as long as wide, with ring organ located at middle.
Ligula and Paraligula	Ligula strongly constricted in the middle with 5 teeth; middle tooth longer than lateral teeth (A). Paraligula bifid (B).
Mandible	With well developed inner and accessory teeth (D).
Ecology	Sprawlers. Predators (engulfers and piercers). Inhabit small standing waters as well as in slow moving waters.

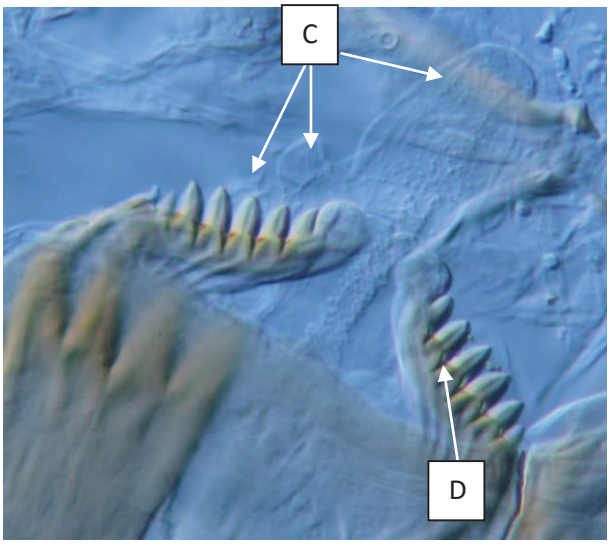


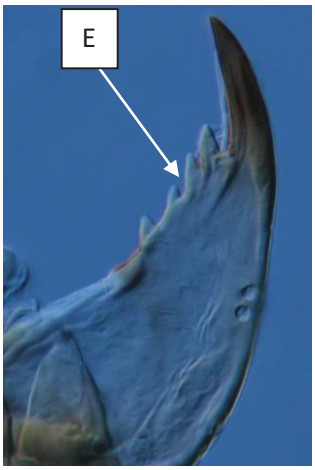




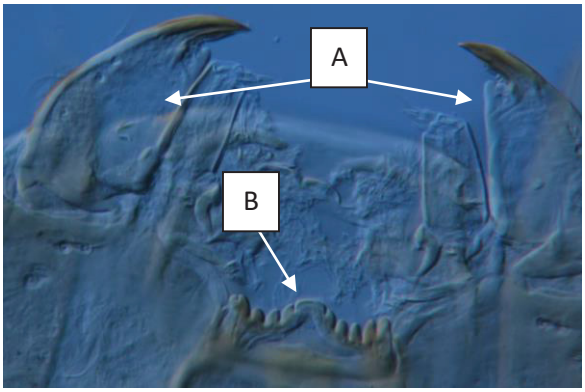
Tanypodinae *Procladius sp.*	
Body	Medium larvae, up to 11 mm long. Head capsule oval.
Antenna	As long as mandible.
Mentum and M appendage	Dorsomentum with 6-8 teeth on each side (D). Pseudoradula (C) widest near base and with distinct, uniformly granulose band.
Maxilla	Basal segment about 2.5 times as long as wide, with ring organ located at middle.
Ligula and Paraligula	Ligula with 5 teeth in a concave row with middle tooth shorter than lateral teeth (A). Paraligula with 5-10 teeth outer side and 1-3 teeth inner side (B).
Mandible	With a large basal tooth (E).
Ecology	Sprawlers. Predators (engulfers and piercers), collectors-gatherers (early instar). Prefer muddy substrata of standing or slowly flowing waters.

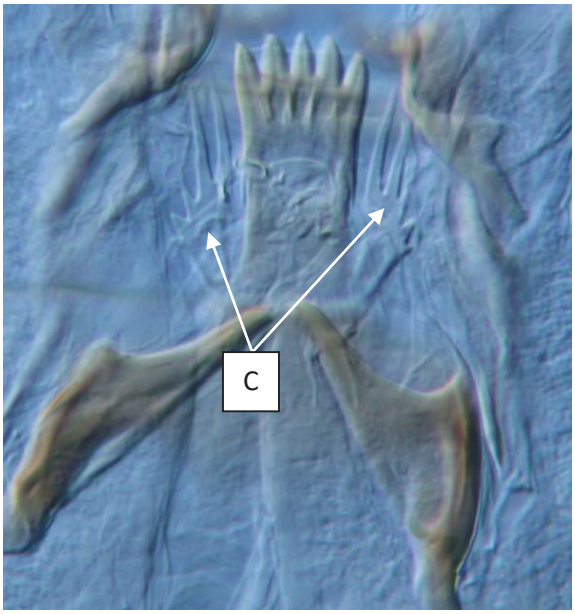






Tanypodinae *Psectrotanypus sp.*	
Body	Medium to large larvae, up to 11 mm long. Head capsule rounded-oval.
Antenna	As long as mandible.
Mentum and M appendage	Dorsomentum with 6-8 teeth on each side (D). M appendage divided into 5 lobes (C): a median lobe, two bladder-shaped lobes and above the dorsomental plate, a pair of rounded lobe.
Maxilla	Basal segment about 2.5 times as long as wide, with ring organ located at distal or middle.
Ligula and Paraligula	Ligula with 4 teeth equal in size (A). Paraligula multi-branched on outer side (B).
Mandible	With a slender apical tooth and 4-5 inner teeth (E).
Ecology	Sprawlers. Predators (engulfers). Live in small water bodies and slow-flowing streams.





Tanypodinae *Tanypus sp.*	
Body	Large larvae, up to 11 mm long. Head capsule rounded-oval.
Antenna	Slightly longer than mandible.
Mentum and M appendage	Dorsomentum with 6-8 teeth on each side (B). M appendage divided into 5 lobes: a median lobe, two bladder-shaped lobes and above the dorsomental plate, a pair of broad lobe.
Maxilla	Basal segment about 2.5 times as long as wide, with ring organ located at base or middle.
Ligula and Paraligula	Ligula with 5 teeth, tooth row usually convex. Paraligula large, with 5 or more long branches on outer side, inner side usually smooth (C).
Mandible	Base enlarged (A), with 2-3 inner teeth.
Ecology	Sprawlers. Predators (engulfers and piercers), collectors-gatherers. Live in sediments in standing and slowly flowing waters.

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Chapter 3 : Correlations between Spatial, Environmental and Human Footprint Factors and the Assemblage Structure of Chironomid Midges in Wetlands in Alberta

3.1 Introduction

One of the basic goals in ecology is to understand what factors influence the distribution of organisms. For freshwater invertebrates, environmental filtering is assumed to be important in regulating the community structure (Poff 1997, Heino et al. 2007, Gerth et al. 2013). Members of a regional species pool must pass through multiple landscape filters operating at different levels ranging from broad scale (e.g. watershed) to fine scale (e.g. microhabitat). Thus, the relative importance of determinants of community composition is scale-dependent. On the other hand, similarity in structure of nearby communities could arise from neutral processes such as dispersal and ecological drift (i.e. chance event) (Legendre 1993, Dray et al. 2006, Thompson and Townsend 2006). Therefore, the current structure of any given community could be explained by a multi-scale interaction of environmental conditions (both abiotic and biotic) and random processes that have occurred over time (Thompson and Townsend 2006, Gerth et al. 2013).

Wetlands are areas that are saturated with water, either permanently or seasonally, and are characterized by poorly drained soils and water-loving plants. In Alberta, wetlands cover about 21% of the province (Wray and Bayley 2006). They are important as they not only support unique communities, but also influence the adjacent terrestrial ecosystem (Davis et al. 2006). They provide a wide range of functions, including improving water quality through filtration, providing habitats for wildlife, and storing

floodwaters (Gibbs 2000). However, wetlands are often impacted, degraded, or even destroyed by human activities (e.g., agriculture, forest harvesting, etc.) (Gibbs 1993, Gibbs 2000). An effective monitoring strategy is essential for proper wetland management. Development of a biological monitoring (biomonitoring) strategy requires two steps: evaluation of the effect of environmental factors on biological responses (e.g., species richness), and then using the biological responses to identify and monitor changes in the environment (Barbour et al. 1999, Reece and Richardson 1999). Aquatic macroinvertebrates have been widely used as indicators for health of freshwater habitats, because of their broad range of responses to various environmental factors (Barbour et al. 1999). However, freshwater biomonitoring has historically been focused on lotic habitats (i.e. rivers and streams) and large lentic systems (i.e. permanent lakes); more research is needed to further evaluate the use of macroinvertebrates for monitoring small wetlands (Davis et al. 2006).

The family Chironomidae (commonly known as non-biting midges) is one of the most abundant and diverse groups of insects in freshwater environments(Pinder 1986, Ferrington 2008). Chironomids occur on all continents including Antarctica and have adapted to all kinds of aquatic and semi-aquatic habitats. They are considered important components of freshwater communities for several reasons. First, they play an important role in nutrient cycling and energy flow in freshwater systems. They are a valuable food source for freshwater fish, and for other vertebrate species such as amphibians and insectivorous birds (Pedro and Ramos 2009). Second, they have been used as indicator organisms to examine water quality and human impacts on freshwater ecosystems (Saether 1979, Pinder 1986). Third, midge remains or fossils are widely used by

paleolimnologists to trace past environmental and climatic changes (Pinder 1986, Walker et al. 1991). However, the diversity of this abundant and ecologically important family is often neglected in freshwater studies due to the extra effort required to identify them more finely than subfamily (Epler 2001). So the ecology of chironomids and value as indicators in wetlands need to be evaluated.

The goal of this study is to determine how chironomid assemblages in Alberta wetlands correlate with (and, hence, presumably are influenced by) environmental variables including anthropogenic influences at different spatial scales. Identifying the important factors of chironomid species turnover would help us to evaluate the use of including chironomids in wetland biomonitoring.

3.2 Method

3.2.1 Study Sites

The study was conducted at wetland sites in Alberta maintained by the Alberta Biodiversity Monitoring Institute (ABMI) (http://www.abmi.ca/home.html) (Figure 3.1). The basic design of ABMI contains 1656 sites evenly distributed throughout Alberta using the 20 km National Forest Inventory (NFI) grid (ABMI 2008). Near each NFI site, a wetland is chosen randomly from a pool of suitable wetlands which meet the following criteria: 1) be permanent; 2) have >1.0 ha of open water and be >0.5 m deep during July; 3) have a well-developed zone of vegetation (ABMI 2013). However, the selection criteria did not exclude human created wetlands. Data and specimens used in this study were collected at 270 wetlands across the province by ABMI from June 15 to July 31 between 2009 and 2011.

3.2.2 Potential Explanatory Variables

3.2.2.1 Water Quality

Chemical and physical variables in each sampled water body were measured by ABMI personnel at the deepest point of the wetland and at 2 additional points located at 25-m intervals moving toward the geometric center of the wetland (ABMI 2013). Water temperature, pH, dissolved oxygen, conductivity and salinity were determined at each point using a Hydrolab multi-probe metre in the field. A one litre water sample was collected at each of the three points and processed in the laboratory using standard protocols to determine total nitrogen (TN), total phosphorus (TP), and total dissolved organic carbon (DOC). Water samples and physiochemistry data were taken between 1:00 and 2:00 pm to avoid variation caused by time of day.

3.2.2.2 Climate data

To characterize the local climatic conditions of each site, mean annual temperature (MAT), mean annual precipitation (MAP), frost-free period (FFP) and potential evapotranspiration (PET) were derived by overlaying the wetland location with the climate layer, which is based on interpolated climate data (Daly et al. 2002, Hijmans et al. 2005) from weather stations for the period 1961–1990. The western North American portion of these data is described by Wang et al. (2012).

3.2.2.3 Habitat Characteristics

Vegetation height above substrate and density were surveyed by ABMI personnel at three transects (10 x 2 m) in the open water at each wetland (ABMI 2013). First, the vegetation transects were categorized based on vegetation height as Non-Vegetated (floating or submerged plants <10% cover), Short Submerged (>50% vegetation extending 0.0-0.3 m above the substrate), Medium Submerged (>50% vegetation extending 0.3-1.3 m above the substrate), Tall Submerged (>50% vegetation extending >1.3 m above the substrate), and Floating (>50% vegetation with floating leaves on the water surface). Second, the vegetation transects were categorized based on vegetation density as Non-Vegetated (floating or submerged plants <10% cover), Sparse (aquatic vegetation covering <25% of the substrate, Moderate (aquatic vegetation covering 25-75% of the substrate), and Dense (aquatic vegetation covering >75% of the substrate). Then the categorical variables of vegetation height and density were coded as ordinal variables from 0-4 and 0-3, respectively. Wetland bathymetry was characterized using 1 primary and 2 secondary axes (ABMI 2013). Water depth measurements were taken at 12 points equally spaced along the first axes and at 8 points equally spaced along the two additional axes.

Human footprints and surrounding vegetation types were characterized by ABMI personnel by manually interpreting 1:30,000 air photos and existing geographic information system (GIS) data layers (ABMI 2012). Human footprints are defined by ABMI (2012) as "the geographic extent of areas under human use that have either lost their natural cover (e.g., roads, agricultural land) or whose natural cover is periodically or temporarily replaced by resource extraction activities (e.g., forestry, surface mining)."

Human footprints were categorizes into six broad types (Table 3.4): Agriculture, Forestry, Hard Linear Features (e.g., roads, rails), Soft Linear Features (e.g. pipelines, seismic lines), Human-Created Water Bodies and Urban Industrial Features. Surrounding vegetation was categorized into 10 broad types (Table 3.4): Deciduous, Mixedwood, White Spruce, Pine, Black Spruce, Larch Fen, Bog Fen, Marsh, Swamp and Grass Shrub. The percentage area of these human footprint and vegetation types were quantified within a 250 m wide buffer around the open water of the wetland using ArcGIS and ArcView software. In addition, the area of the open water and riparian zone of each wetland, and the natural ecoregion and subecoregion in which each wetland is located were also determined by ABMI personnel from existing GIS data layers.

3.2.2.4 Spatial Variables

Wetlands in this study were sampled across the province. At such a large spatial scale, I expected some spatial structures in chironomid assemblages, which could be either caused by spatially structured environmental variables or autocorrelation. To better understand how chironomid assemblages were structured in space, I added spatial variables that represent different spatial scales to account for potential spatial patterns. I created the spatial variables through principle coordinates of neighbour matrices (PCNM) (Borcard et al. 2004, Dray et al. 2006). The PCNM approach first calculated the distance among all sites from their latitude and longitude. Then a truncated matrix of their distances among sites were used in a principal coordinates analysis to generate the spatial descriptors representing a spectral decomposition of the spatial relationships among all

sites. Low-order PCNM variables represent broad spatial scales while high-order PCNM variables represent fine spatial scales.

3.2.3 Aquatic invertebrates

Aquatic invertebrates were sampled by ABMI personnel at 10 locations in each wetland using a modified D-ring dip net with a mesh size of 500 μm (ABMI 2013). Samples were preserved using 10% buffered formalin in the field. Once back to the lab, the preservative was changed to 70% ethanol (ABMI 2011).

In the lab, all ten samples from the same wetland were first put through a 500 μm sieve and combined to create a composite sample (ABMI 2011). Then the composite sample was elutriated to go through 2.8 mm and 500 μm sieves. The elutriated material retained by both the 2.8 mm and 500 μm sieves was rinsed into a Marchant box sub-sampler (Marchant 1989) to fill each of the 100 separate cells. The Marchant box was closed, inverted and gently swirled to evenly distribute the elutriated materials in the Marchant box cells. A random-numbers table was used to select a cell whose contents were transferred to a Petri dish. ABMI target taxa (Table 1.1) were sorted into separate vials filled with 70% ethanol using a 10-40x microscope with a fiber-optic light source. The goal was to collect at least 350 undamaged specimens of all target taxa combined. If this was not reached from the first cell, another cell was randomly selected and sorted until there were 350 target organisms or all 100 cells were sorted.

I then picked through the chironomid samples to identify the specimens more finely. If there were more than 100 specimens in a single sample, it was subsampled. For 2009 and 2010 samples, I randomly selected 100 chironomids for identification from

each sample. To do this, I placed the chironomid larvae in a Petri dish overlying a grid of 100 cells and used a random number generator to randomly sample cells until a total of 100 chironomids were reached. To identify the chironomid specimens I first put them into lactic acid for 12-24 hours to clear before slide-mounting them in polyvinyl alcohol mounting medium (PVA, cat. #6371A from BioQuip, Rancho Dominguez, California). Slides were then left on slide warmers for 2-3 days at ~45°C before they were examined under a compound light microscope. I used various taxonomic keys (Oliver and Roussel 1983, Coffman and Ferrington 1996, Epler 2001) to identify the chironomids to the finest possible taxonomic level. Originally I tried to identify all chironomids to morphospecies, however, verification of the morphospecies identification by other chironomid taxonomy expert (Robert Hinchcliffe from Royal Alberta Museum) showed a large number of chironomids couldn't be reliably identified at morphospecies. So in all my analysis, I only used the chironomid data at genus-level. For 2011 samples, chironomids were subsampled and identified by ABMI personnel. Their subsampling strategy was to randomly select no more than 100 chironomid specimens (instead of exact 100 as I used) for fine identification. To determine the number of chironomids that needs to be identified in a sample with more than 100 specimens, the number of chironomid larvae in the entire sample was divided by the number of Marchant box cells sorted, which was then multiplied by a whole number to get a number as close to 100 without going over.

Finally, the true abundance of each taxon in the original (un-subsampled) sample was estimated as (relative abundance of this taxon * the total number of chironomids sorted)/proportion of Marchant box cells sorted. For example, if 250 chironomids were sorted from a site from 50 Marchant box cells, and 40 out of the 100 identified

chironomids were from the genus *Chironomus*, then the relative abundance of *Chironomus* is 40/100=0.4, the proportion of Marchant box cells sorted is 50/100=0.5, and the abundance of *Chironomus* in the complete sample is estimated as (0.4*250)/0.5=200.

3.2.4 Data Analysis

Although the wetland sites were from 6 natural ecoregions, Canadian Shield and Boreal ecoregion were pooled together as a single region (CB); Parkland and Grassland were pooled together as a single region (PG); and Rocky Mountain and Foothills were pooled together as a single region (RF) in the final analysis because of their similarities on the measured environmental conditions with each other as well as only a few sites were surveyed in Canadian Shield and Rocky Mountain ecoregions.

All analyses were performed in the R environment (R Development Core Team 2013) using the vegan (Oksanen et al. 2015) and packfor (Dray et al. 2011) packages. Tests of homogeneity of multivariate dispersions (PERMDISP) were used to see if there was a difference in environmental heterogeneity among the three-pooled regions (Anderson 2006). Heterogeneity differences refer to environment variation within each region and how this variation differs among the regions. Nonparametric multivariate analysis of variance (npMANOVA) was used to test whether the average environmental characteristics differed between regions (Anderson 2001a, Anderson 2001b), as a measure of the overall differences between regions. All the environmental data were either arcsine-transformed (data in percentage) or log-transformed (except pH which is the log of hydrogen ion concentration) prior to analysis. Afterwards, all variables were

standardized to mean zero and unit variance. Euclidean distance matrix was used in the PERMDISP and npMANOVA. Comparisons were also made for differences in average taxon composition (centroids of the data clouds) and in the heterogeneity of taxa composition (i.e. beta-diversity) among regions as described above for environmental data (PERMDISP and npRMANOVA). However, the distance (i.e. assemblage dissimilarity) matrix for the biotic analyses was based on a Bray-Curtis measure (rather than Euclidian distance as used with environmental data), since Bray-Curtis is considered as a more appropriate distance measure for community data (Anderson 2006, Faith et al. 1987). All the taxon abundance data were log (x+1)-transformed before analysis. These analyses were performed to provide background information for the interpretation of variation in chironomid assemblages explained by different sources of explanatory variables.

To evaluate the relationship between the chironomid assemblages and explanatory variables, I first used the detrended correspondence analysis (DCA) to determine the appropriate response model (i.e., linear, unimodal). The DCA performed on the chironomid data yielded gradient lengths less than three standard deviations; therefore, I chose redundancy analysis (RDA), the constrained ordination of linear model. Next I used RDA separately for different explanatory variable groups: spatial variables, human footprint (HF) variables and non-human-footprint (NHF) environmental variables. In the case where the global model was statistically significant ($p < 0.05$ from 9999 Monte Carlo permutations), a forward selection procedure (Blanchet et al. 2008) was performed to retain the most important variables in explaining the chironomid assemblages. The retained variables were then used in the partial redundancy analysis (pRDA) to assess the

relative contributions of different explanatory variable groups to the assemblage patterns (Borcard et al. 1992, Peres-Neto et al. 2006). By using pRDA, the variance in the chironomid assemblages was partitioned into 8 components: pure spatial effect [a], pure NHF environment effect [b], pure HF effect [c], interaction between space and NHF environment [d], interaction between space and HF [f], interaction between NHF environment and HF [e], interaction among all three data sets [g] and unexplained [U].

RDA, pRDA and variance partitioning were run for data from all 6 regions pooled and separately for three pooled pairs of regions. The variance explained by each variable group was calculated using adjusted R^2, which provides unbiased estimates of the explained variance (Peres-Neto et al. 2006). All the environmental data were either arcsine-transformed (data in percentage) or log-transformed (except pH which is the log of hydrogen ion concentration). All variables were then standardized to mean zero and unit variance. The chironomid abundance data were Hellinger-transformed (i.e. square root of the relative abundance at each site) so that they are appropriate for a Euclidean distance-based ordination analysis(e.g., RDA, Legendre and Gallagher 2001).

3.3 Results

3.3.1 General Environmental Pattern

Sites of the three-pooled regions formed three distinct groups in the PCA ordination plot based on the measured environmental variables (Figure 3.2), and were significantly different (npMANOVA; $p<0.001$; Table 3.1). Wetlands at CB (Canadian Shield-Boreal) region were mainly characterized by their surrounding vegetation (i.e., Larch Fen, Black Spruce, Bog Fen, and Swamp) and larger riparian/open water area ratio

(R), while those at RF (Rocky Mountain-Foothills) region were associated with pine, mixedwood and lower mean annual temperature (MAT). In PG (Parkland-Grasslands) region, wetland sites were defined by their climatic conditions (i.e., higher potential evapotranspiration (PET) and longer frost-free period (FFP)), water physiochemstry (i.e. higher level of phosphorus (Phos), conductivity (Con), nitrogen (Nitro) and salinity (Sali)), and agriculture land use (Ag). The PERMDISP analysis indicated that there were significant differences in overall environmental heterogeneity among regions ($p=0.009$). RF has the largest environmental variability (average distance to the group centroid), and is significantly different from CB (Tukey's *post hoc* tests, $p<0.001$) and PG ($p<0.001$). However, CB and PG were not significantly different from each other, indicating similar environmental heterogeneity.

3.3.2. General chironomid assemblage pattern

I identified a total of 40 genera of chironomids from 2009 and 2010 samples and ABMI identified another 9 genera from 2011 samples, with 43 from CB, 36 from RF, and 28 from PG (taxonomic details in Chapter 2). The mean genus richness per site was 7.6, ranging from 1 to 16. On average, richness was highest in CB (9.5 ± 3.6 SD) followed by RF (7.5 ± 3.6 SD) and PG (5.8 ± 3.0 SD) (Figure 3.1). Average taxon composition (centroid of the data clouds) differed among regions (npMANOVA, $p<0.001$; Table 3.1). However, there was considerable variation within regions resulting in large overlap between regions in composition (Figure 3.3). The PERMDISP analysis showed uneven dispersion of taxon composition (i.e. beta-diversity) among regions (Table 3.1). Chironomid assemblages in RF have the highest beta-diversity followed by PG and CB.

However, only RF and CB were significant different from each other in Tukey's *post hoc* test ($p<0.001$).

3.3.3. Variation partitioning

3.3.3.1 Provincial extent

The full NHF environmental, full spatial and full human footprint models were all significant (9999 Monte Carlo permutations, $p = 0.005$ for all three models). The reduced models using forward selection procedure retained 7 NHF (non-human-footprint) environmental variables, 2 human-footprint variables and 11 spatial variables (Figure 3.4, Table 3.2). At the provincial extent, 13.1 % of the variance was explained by the explanatory variables (Figure 3.5). The pure NHF environment effect [a] and pure spatial effect [b] each explained 4% of the variance, while pure HF effect [c] was near zero. The shared fraction between NHF environment and space [d] was 2.0 %, whereas the joint effect of space and HF [f], NHF environment and human footprint [e] were both near zero. Finally the interaction among all three predictor datasets [g] explained 3% of the variance.

3.3.3.2 Regional extent

For the RF pooled region, all the three RDA models were non-significant, showing that no variance in the chironomid assemblages could be explained by the measured variables (Table 3.2). Therefore, I did not perform forward selection and variance partitioning for this region. For the PG region, the full spatial and NHF environmental models were significant ($p = 0.025$ and 0.005), while no significant human footprint

effect was found ($p = 0.14$). So, the forward selection and variance partitioning were only made for spatial and NHF environmental variables. The reduced models retained 5 NHF environmental variables and 4 spatial variables (Table 3.2). The fractions of variance accounted for by the pure NHF environmental [a] and pure spatial [b] effect were 3% and 5% respectively. The fraction explained by spatially structured NHF environmental variables [d] was only 1%. Therefore, a total of 9% of the variance was explained by all predictor variables (Figure 3.7). At CB region, all three full RDA models were significant ($p<0.05$). A total of 11.4% of the variance was explained (Figure 3.6). In the reduced RDA model, 5 NHF environmental variables, 4 spatial variables and 1 human footprint variable were selected (Table 3.2). Pure NHF environmental effect [a] and pure spatial effect [b] each explained 4% of the variance, while pure human footprint effect [c] explained only 0.5% of the variance. The spatially structured NHF environmental [d] variables explained 1%. However, there is no joint effect between human footprint and space [f]. The interaction between NHF environmental variables and human footprint variables [e] explained 1% of the variance. The joint effect of all three datasets [g] accounted for 1% of the variance as well.

3.4 Discussion

3.4.1 General environmental and chironomid assemblage pattern

Both environmental characteristics and chironomid assemblage compositions were different among the three-pooled regions. The regions also differed in environmental variability, spatial extent and beta-diversity (taxon-composition dispersion) (Table 3.1). It has been proposed that beta diversity, as measured by Bray-Curtis dissimilarities, is in

general positively correlated with environmental heterogeneity and spatial scale (Anderson 2006, Warwick and Clarke 1993, Landeiro et al. 2012). However, I did not see such a pattern in my study. In fact, when one compares the CB (Canadian Shield-Boreal) region with the PG (Parkland-Grassland) region, the opposite pattern is observed. The CB region has larger dispersion in both environmental variables and spatial extent than PG region, whereas the beta diversity at CB is smaller than at PG. A possible explanation is that the association is mediated by the relative importance of measured explanatory variables between regions, which is supported by our RDA analysis. The total variance explained by the measured explanatory variables for the CB region was higher than for the PG region, indicating our measured explanatory variables are more important in affecting the biological community at CB region. RF (Rocky Mountain-Foothill) region has the smallest spatial extent, while it has the largest beta diversity and environmental heterogeneity. However, at the RF region, no variance could be explained by the measured variables, thus attempting to interpret the association between beta diversity, environmental heterogeneity and spatial extent is meaningless.

3.4.2 Variance partitioning

Variance partitioning has often been used to tease apart the roles of spatial structuring and environmental control in the context of metacommunity (Thompson and Townsend 2006, Diniz-Filho et al. 2012, Meynard et al. 2013). It was expected that both spatial structuring and environmental filtering were important at broad extent, and environmental filtering will become more important as the spatial extent gets smaller. This is because at the large scale, dispersal becomes a limitation for a lot of organisms.

3.4.2.1 Provincial extent

I found that pure spatial and pure environmental effects (including both NHF environment and human footprint) were similarly important in explaining the variance in the provincial extent. Spatial and environmental effects are often linked to dispersal processes and species sorting (Flinn et al. 2010, Landeiro et al. 2012, Meynard et al. 2013, Rezende et al. 2014). In the reduced spatial RDA model, most of the spatial variables retained (10 out of 11) represent broad spatial scales indicating broad spatial structure in chironomid assemblages. At such a large scale, dispersal can be expected to be limiting for chironomids as adult chironomids are poor fliers and short-lived, and normally disperse over short distances (< 1 km) (Delettre and Morvan 2000), although passive dispersal of adults via wind (Armitage et al. 1995) and of larvae via migratory birds (Green and Sanchez 2006) could take them much further. However, interpretation of the pure spatial effect has to be made with caution as observed spatial structure could be always due to a lack of knowledge of unmeasured yet spatially structured environmental variables or processes(Borcard et al. 1992, Meynard et al. 2013). Beside the pure effect, there is a large amount of variance (5%) shared by spatial and environmental components, indicating that some environmental variables are structured in space. This is also supported by the results of PCA analysis and npMANOVA test of the environmental data, which showed that environment characteristics were different among regions (i.e. vary in space).

Many authors have documented water quality as an important determinant of chironomid assemblage structure (Saether 1979, Pinder 1986, Armitage et al. 1995,

Quinlan et al. 1998, Brooks et al. 2001, Porinchu et al. 2002). For example, nutrients (especially phosphorus and nitrogen) were found to affect chironomid composition (Brooks et al. 2001, Luoto 2011) by altering the physical and chemical properties of lakes (Porinchu et al. 2002, Smith et al. 2006). This reflects the established use of chironomids to characterize the trophic status in lakes (Saether 1979, Brooks et al. 2001, Porinchu et al. 2002). Increased input of phosphorous and nitrogen tend to increase the productivity of primary producers. Extreme eutrophication can cause massive algal blooms, and subsequent death and decomposition of the algae can lower dissolved oxygen (DO) concentrations to lethal levels. Salinity (or conductivity) has also been identified important in affecting chironomid assemblages (Rawson and Moore 1944, Henrichs et al. 2001, Walker 2001). Most chironomids can only tolerate moderate salinity with a limited number of taxa associated with saline environments (Rawson and Moore 1944, Pinder 1986). In addition, most chironomids are adapted to the range of pH between 6.0 and 9.0 (Pinder 1986, Woodcock et al. 2005). pH's outside of this range will result in the occurrence of fewer species, as calcium and sodium regulation problems will arise. Wetlands at PG region are characterized by the highest nutrient level (i.e. phosphorus and nitrogen), salinity and pH, which might explain why this region has different species composition from the other two regions. In the reduced NHF environmental RDA model, two of the water quality variables (phosphorus and salinity) were indeed identified as important in explaining the variance. Of course, this does not mean that other water quality variables, like pH and nitrogen, are not influencing chironomid assemblages. Water quality variables are often highly correlated with each other. For example, in our study, pH is positively correlated with both phosphorus and salinity. Once a variable was

selected through the forward selection procedure, another correlated variable will not likely enter to the model unless it could still account for some of the residue variance after the variance explained by the first variable has been removed.

Maximum water depth and elevation were also found to correlate with chironomid assemblage structure. This might explain the unique chironomid composition at RF region as this region has the highest elevation with the deepest wetlands. The influence of water depth on chironomid composition has long been recognized in many lake studies (Walker et al. 1991, Quinlan et al. 1998, Larocque et al. 2001, Porinchu et al. 2002). Walker et al. (1991) suggested that the influence of lake depth on chironomid fauna is largely due to its cooling influence on surface water temperatures. In my study, I only found a weak negative correlation between the water depth and surface temperature (Spearman $r = -0.26$, $p < 0.001$). Larocque et al. (2001) argued that morphometric regulation of habitat availability (e.g., proportion and volume of the littoral and profundal zones) with increasing maximum depth might be the underlying mechanism regulating the chironomid assemblages. In my studied wetlands, the depth is relatively shallow (most wetlands < 2 meters deep), so the mechanism might be different from lakes. In wetlands, fish are more likely to occur in deeper ones (Baber et al. 2002) because deeper wetlands are less prone to extreme event such as freezing solid to the bottom in winter. And it has been found the presence or absence of fish was a very important factor in explaining invertebrate abundance and composition (e.g., Zimmer et al. 2001, Tangen et al. 2003, Tarr et al. 2005). In addition, Zimmer et al. (2000) suggested that increasing depth of wetlands could limit the light availability thus decreasing macrophytes abundance, which ultimately will affect the invertebrate composition. In addition, fish are

more likely to occur in deeper wetland. Elevation has been suggested to influence chironomid community indirectly; such as through water temperature, air temperature, lake productivity and water chemistry (Porinchu et al. 2002). My results showed that elevation was highly correlated with mean annual air temperature and mean annual precipitation. Thus, elevation might affect the distribution of chironomids through the local climatic conditions.

Open water area (i.e. wetland size) was also identified as an important variable in explaining the distribution of chironomids in our wetlands. A few explanations have been proposed to explain the effect of water body size on macroinvertebrates. First, larger water bodies tend to have higher habitat complexity (Allen et al. 1999, Heino 2000, Tarr et al. 2005); Second, larger water bodies are more likely to support larger population size that lower the extinction risk (Allen et al. 1999, Tarr et al. 2005). Third, larger water bodies might have higher immigration rates as many flying adults find water bodies through visual and olfactory cues that are more attracted to larger ones (Tarr et al. 2005, Baber et al. 2004). Fourth, larger water bodies are more resistant to extreme drought event.

In the reduced HF RDA model, agriculture and human created water bodies (e.g. canals, dugouts) were retained as significant in explaining the chironomid assemblage structure. Water quality in wetlands could be easily impacted by the surrounding land use (Grue et al. 1986, Euliss and Mushet 1996). Wetlands at CB and RF have good rooted surrounding vegetation (i.e., Larch, Black Spruce, Pine, and Mixedwood), while those at PG region were mainly associated with grass/shrub lands and agricultural activities. On one hand, increasing agriculture activities often cause contaminated run-offs with

increased nutrient input through fertilizer application, manure from livestock operation. On the other hand, the nutrient input in wetlands could be reduced by the nutrient uptake of the surrounding vegetation. For example, nitrate concentration in deforested watershed can be 50 times higher than in forested control watershed over several years (Falkenmark and Chapman 1989). Besides nutrients, salt levels can also be altered by land use through clearing and improper irrigation through changing the natural interactions between saline ground water and surface water (i.e. secondary salinity) (Jolly et al. 2008). When deep rooting trees are replaced with shallow crops, or excess water was applied to crops, more water would pass through root zone to groundwater, raising the water table and bringing salt to the surface.

Human-created water bodies include a diversity of structures such as dugouts and irrigation and drainage canals. They could easily alter the local hydrology (i.e. the pattern of water flow in an area) of the wetland (e.g., Rehage and Trexler 2006, Blann et al. 2009). For example, canals could change the speed and natural amount of the water that moves into and out of the wetland. The alteration of hydrology will then change the soil property and nutrient input, which could directly affect the local plant and animal community. Rehage and Trexler (2006) identified a strong correlation between increased aquatic animal density and phosphorus enrichment caused by canals. Besides, reservoirs may provide refuge habitats and increase connectivity to other aquatic habitat, which could also influence the local biological community (Rehage and Trexler 2006, Blann et al. 2009).

Because agricultural activities and human-created water bodies have poorer water quality and occur more often in the PG region, their effect on assemblages was shared

with NHF environmental variables (i.e. water quality) and space, which explained 3% of the variance, thus leaving near zero pure human-footprint effect after the variance partitioning.

3.4.2.2 Regional extent

At smaller spatial scale, dispersal limitation is less important and species distributions would be more linked to environmental conditions (both biotic and abiotic factors) (Diniz-Filho et al. 2012, Landeiro et al. 2012, Alahuhta and Heino 2013, Rezende et al. 2014). Within both PG and CB region, as expected there was an increase of the variance that is solely attributed to environmental effect (both NHF and HF effect) in comparison to the province analysis. However, there was a decrease of pure spatial effect only in the PG region, while CB region stayed the same as the provincial scale. But the pure spatial effect in the regional scale should be less linked to dispersal limitation than the provincial scale. Therefore, a larger portion of the pure spatial effect in the regional scale should be due to some unmeasured yet spatially structured environmental conditions.

For the CB (Canadian Shield-Boreal) region, salinity, frost-free period (FFP), phosphorus, maximum depth (Ddept) and open water area (wetland size) in the NHF RDA analysis were found to be statistically significant in explaining chironomid assemblages. All the variables except FFP were identified in the provincial analysis. FFP is closely related to the growing season (Kunkel et al. 2004), whose duration significantly affects the distribution and abundance of local vegetation including macrophytes, thus resulting in different habitat diversity (Sweetman and Rühland 2010), which could have a

large impact on chironomid assemblages. In the HF RDA analysis, the hard linear feature variable (e.g. gravel road) was selected as the best variable in explaining chironomid assemblages. In this region, hard linear features were strongly correlated to human created water bodies in CB region (Spearman $r = 0.74$, $p < 0.0001$). Although the information on what types of human created water bodies is not available, hard linear features, mainly roads, are often associated with drainage ditch alongside roadways. Run off from the road surface could transport more sediments and chemicals into the nearby wetland. Similarly, blown road dust from the road could also bring more sediments and chemicals to the wetland.

At PG (Parkland-Grassland) region, three variables (elevation, dissolved oxygen and maximum depth) were found to be significant in the NHF RDA analysis. However, the HF effect was not observed in the HF RDA analysis. The PG region has the most intensive human activities with good gradients and thus I had expected them to be important in affecting the chironomid community. The non-significant results might be due to a few reasons. First, the surrounding land of the wetlands in PG region might have been entirely altered in history by human activities and its effect on the local wetlands are still present. So the use of the current landscape information in our analysis might not explain the biological community very well. Second, we characterized the land use within 250 meter of each wetland, but larger landscape characteristics could play a more important role. For example, an assumed intact wetland (i.e. small human footprint within 250 m buffer zone) in our study could be a heavily affected site if the outer land (i.e. outside of the 250 m zone) is used for agricultural activities.

For the RF region, neither environmental nor spatial effect was observed. This region has the smallest spatial extent so it is not very surprising that spatial effect was not found. However, in the environmental model, it was expected that at least maximum depth and elevation could explain some of the variance in the chironomid assemblages within this region. Maximum depth and elevation had been identified as major factors to cause the distinct chironomid composition at RF region in the provincial analysis. More importantly, both variables had the largest gradient in RF region among all three regions. One possible explanation as to why no significance was found is that different chironomid taxa have different tolerances to the environment gradient. Most sites at RF region have elevations from 1000 m to 2000 m, while sites at PG region typically have elevations from 500 m to 1000 m. It is possible those taxa that mainly live above 1000 m are more tolerant to further elevation change so that an effect of elevation could not be observed at RF region.

The total variance that could be explained in both provincial and regional analysis was low (all less than 13%). There are several potential reasons. First, some important variables were not measured in our study. Many studies have found the presence or absence was the most important factor in explaining invertebrate abundance and composition (e.g., Zimmer et al. 2001, Tangen et al. 2003, Tarr et al. 2005). In addition, I only characterized the macrophytes by their density and height; however, macrophytes composition or other measurement vegetation complexity might play a more important role. So inclusion of those variables would improve the percentage of variance explained. Second, taxonomic resolution at genus level may have resulted in the loss of important ecological information. Species within the same genus of chironomids can exhibit a wide

range of environmental tolerances (Wrubleski 1987, Hudson et al. 1990, Armitage et al. 1995, Epler 1996). For example, King and Richardson (2003) failed to detect a stressor-response relationship for *Tanytarsus* spp. (6 species), yet four of its six species were found to be strongly associated with either disturbed wetlands or non-disturbed wetlands. Third, stochastic processes of recruitment, dispersal, local extinction etc. may play a more important role in controlling the macroinvertebrate distribution in wetlands than in rivers and streams (Davis et al. 2006, Batzer 2013). It's getting more established that macroinvertebrates in wetlands are more tolerant to environmental variations compared to stream macroinvertebrates, as they have adapted to cope with the highly variable nature of wetland environments, such as periods of flooding and drought, changing water quality conditions daily, seasonally and yearly (Batzer 2013).

3.5 Conclusion and future study

In this study, I looked at how environmental variables (including human footprint) and spatial factors are correlated with assemblage structure of wetland chironomids in Alberta. At different scales, the relative contributions of environment and space to the variance in chironomid assemblages were different. Environment filtering tends to become more important with decreasing spatial extent. We also identified the most important of the measured variables affecting the chironomid community at both province and regional extent.

In an effort to evaluate whether chironomid community could be used to reflect human footprint, I found that human footprint seemingly does affect chironomid composition, most likely by affecting the water quality. However, the use of chironomid

genus-level assemblages as indicators of human footprint seems to be of minor value. The common practice in biomonitoring is that an array of reference (no human footprint) sites is used to define the expected range of biological communities in the absence of human activities (Reece and Richardson 1999, Barbour et al. 1999). Potentially impacted sites are then compared to reference conditions to determine the extent of impairment. A critical aspect in determining reference conditions for test sites is to classify reference sites into groups to partition natural variability and then assign test sites to proper groups for comparison. It ensures that test sites only compare to reference sites within the same group so that the divergence between test sites and reference sites is only due to human disturbances. Based on our result, the total percentage of variance that could be explained by all our measured variables is very low, which will make it hard to define the reference condition.

For future research, more environmental variables, especially those that have been identified as important in affecting chironomid community from other studies (e.g., presence/absence of fish, types of macrophytes and sediments etc.), should be added, which should increase explanatory power. Furthermore, the response of richness, diversity index and individual taxa to human disturbance should be examined in detail to see whether any of them could be used to reflect human footprint.

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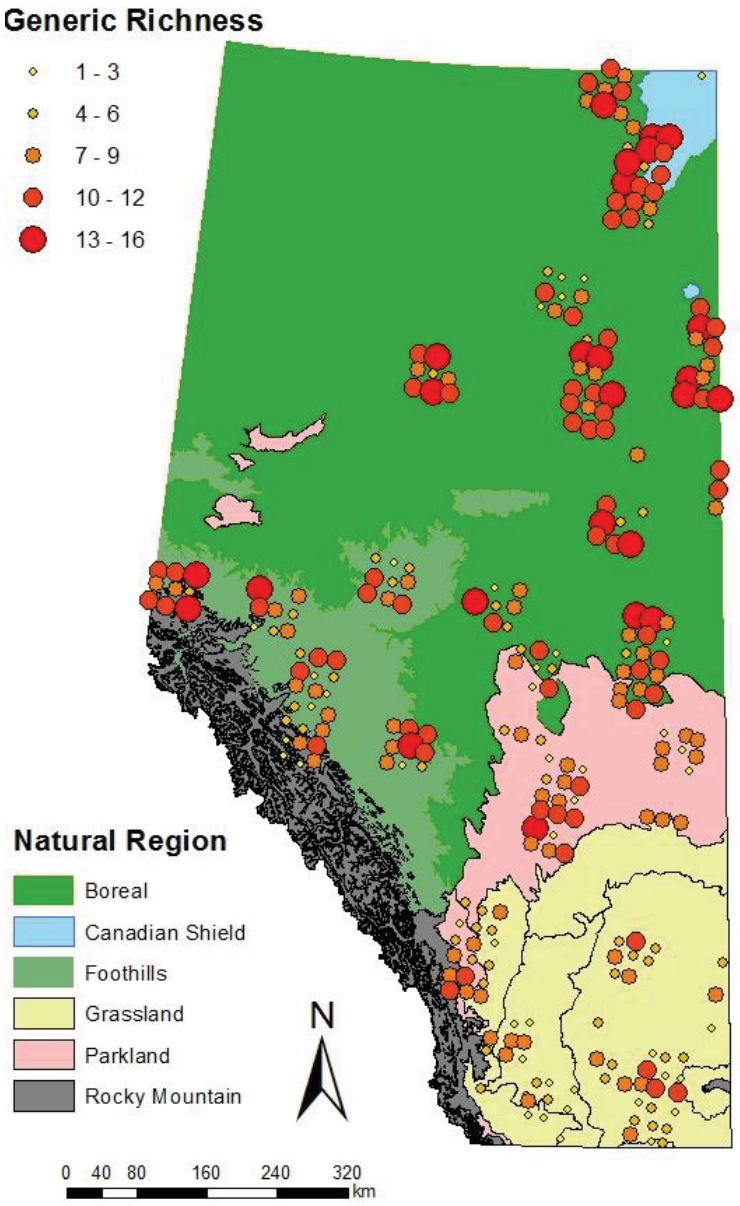

Figure 3.1. Locations of wetlands from which chironomids used in this study were sampled in Alberta, Canada. Size and color of the dots represent different generic richness of chironomid detected at each wetland.

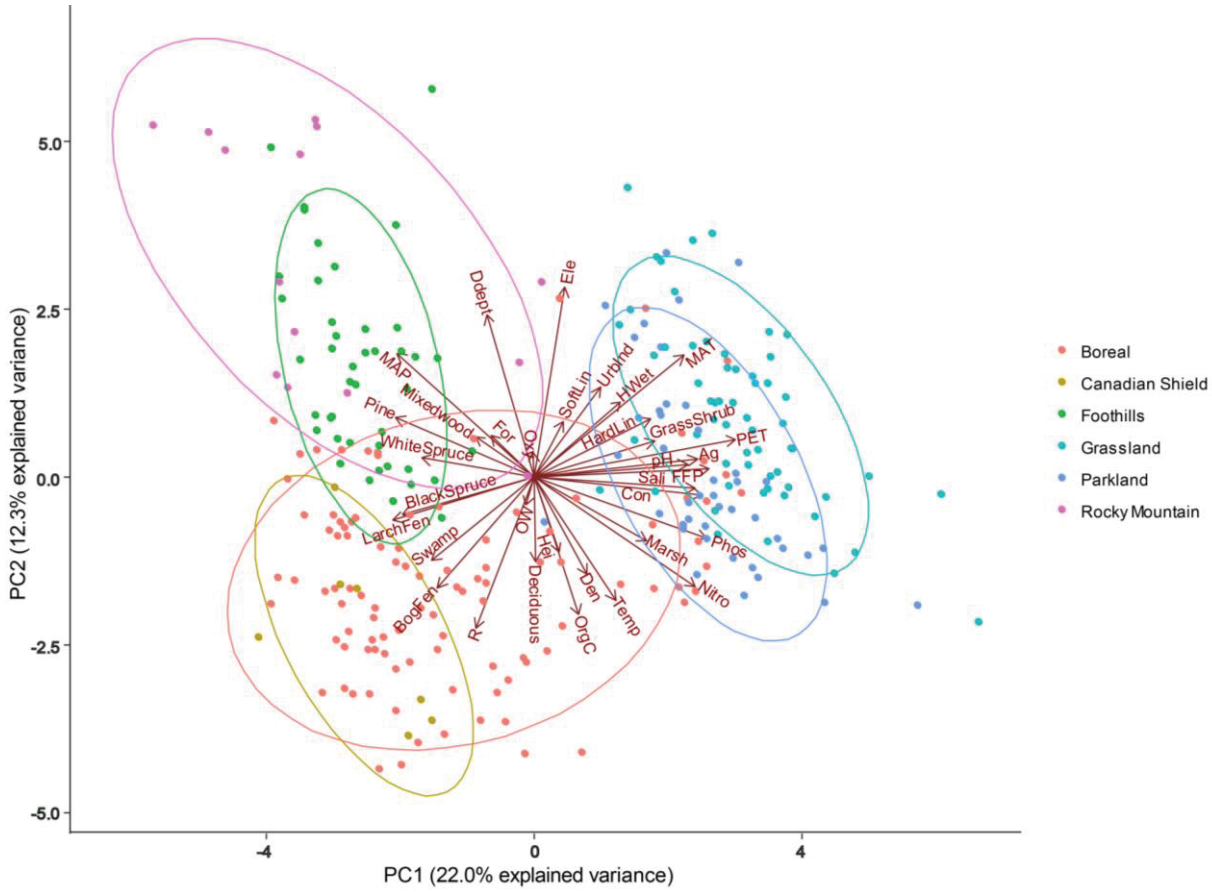

Figure 3.2. Groupings of study wetlands from six natural regions with respect to chemical and physical patterns at each site using principle components analysis (Axes 1 and 2). See Table 3.3 for abbreviations.

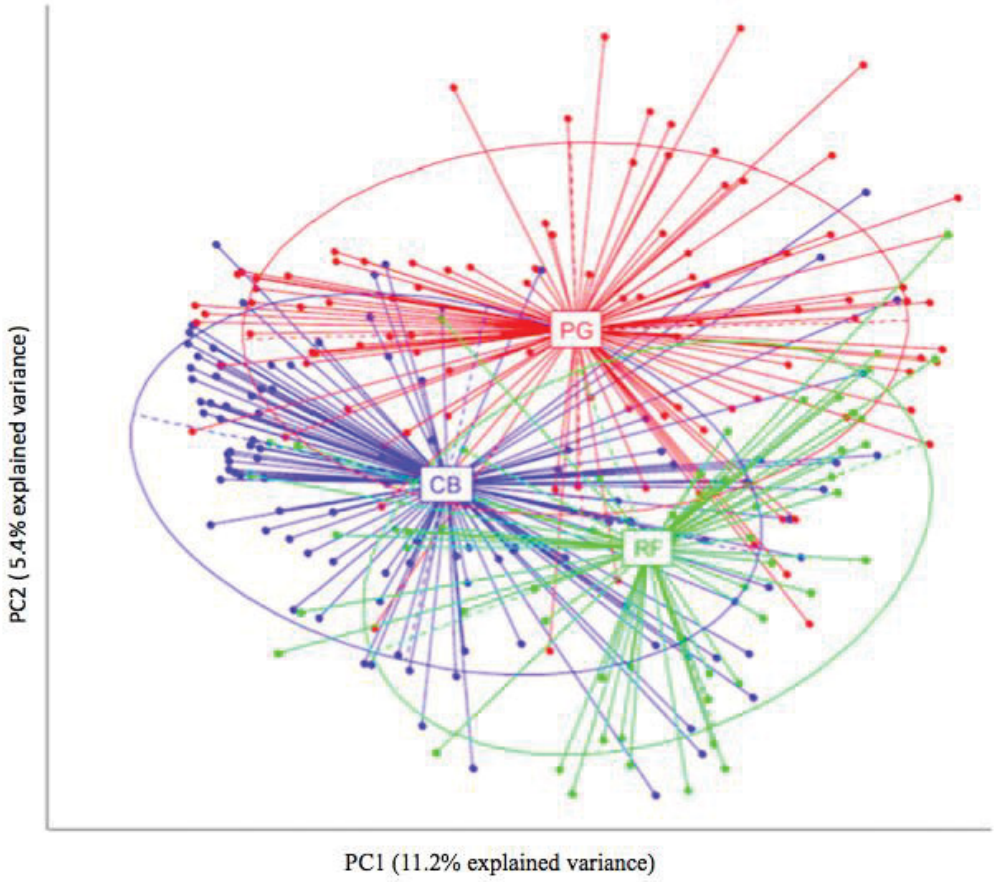

Figure 3.3. Groupings of study wetlands from three pooled regions with respect to chironomid assemblages at each site using principal coordinates analysis (Axes 1 and 2). Dots represent wetland sites and lines represent Bray-Curtis dissimilarities between each site and the group centroid. CB, Canadian Shield and Boreal region; PG, Parkland and Grassland region; RF, Rocky Mountain and Foothill region.

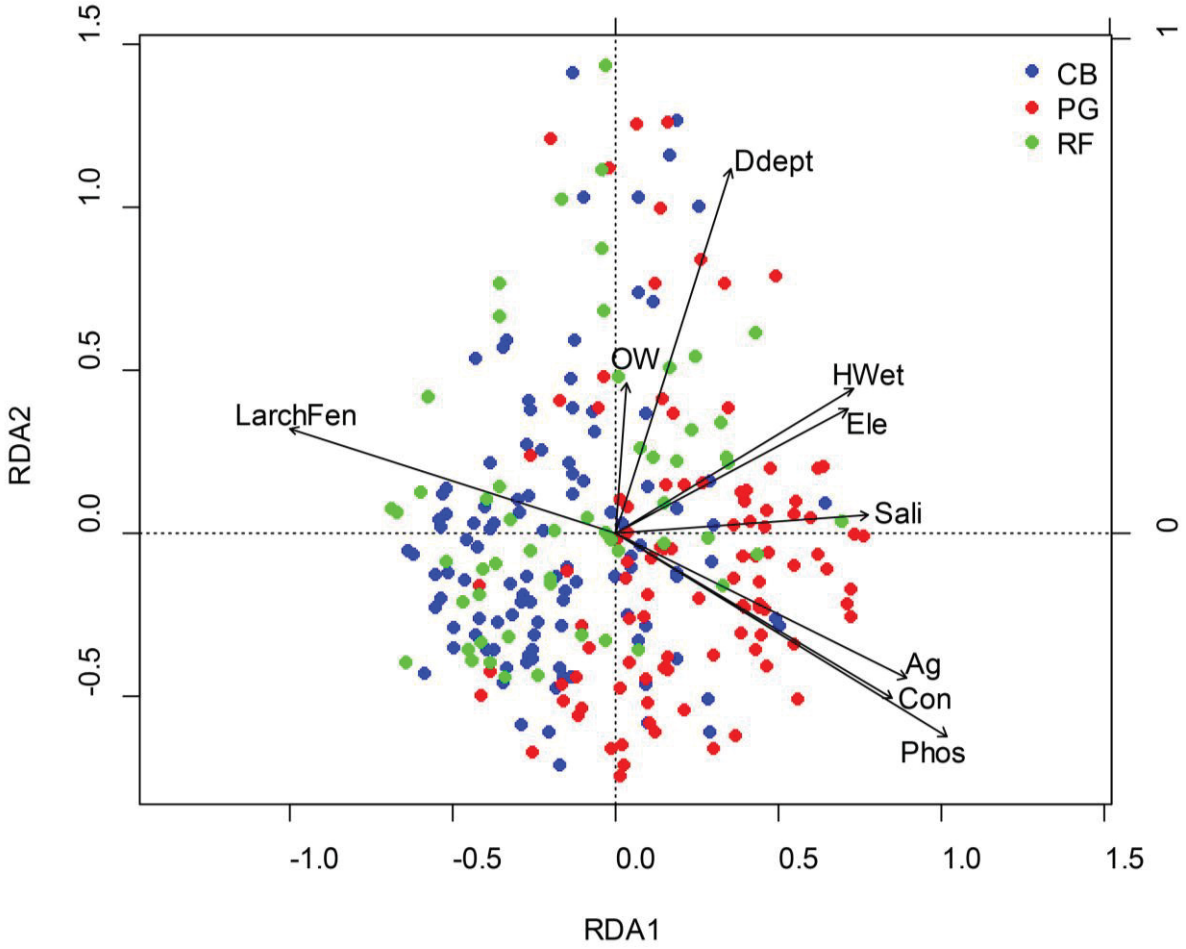

Figure 3.4. Redundancy analysis of chironomid assemblages and measured environment variables in the 270 wetland sites. Environmental variables on the graph were those selected as important in explaining chironomid assemblages and vectors of those environmental variables correspond to sites with higher values of that variable. CB, Canadian Shield and Boreal region; PG, Parkland and Grassland region; RF, Rocky Mountain and Foothill region. See Table 3.3 for abbreviations.

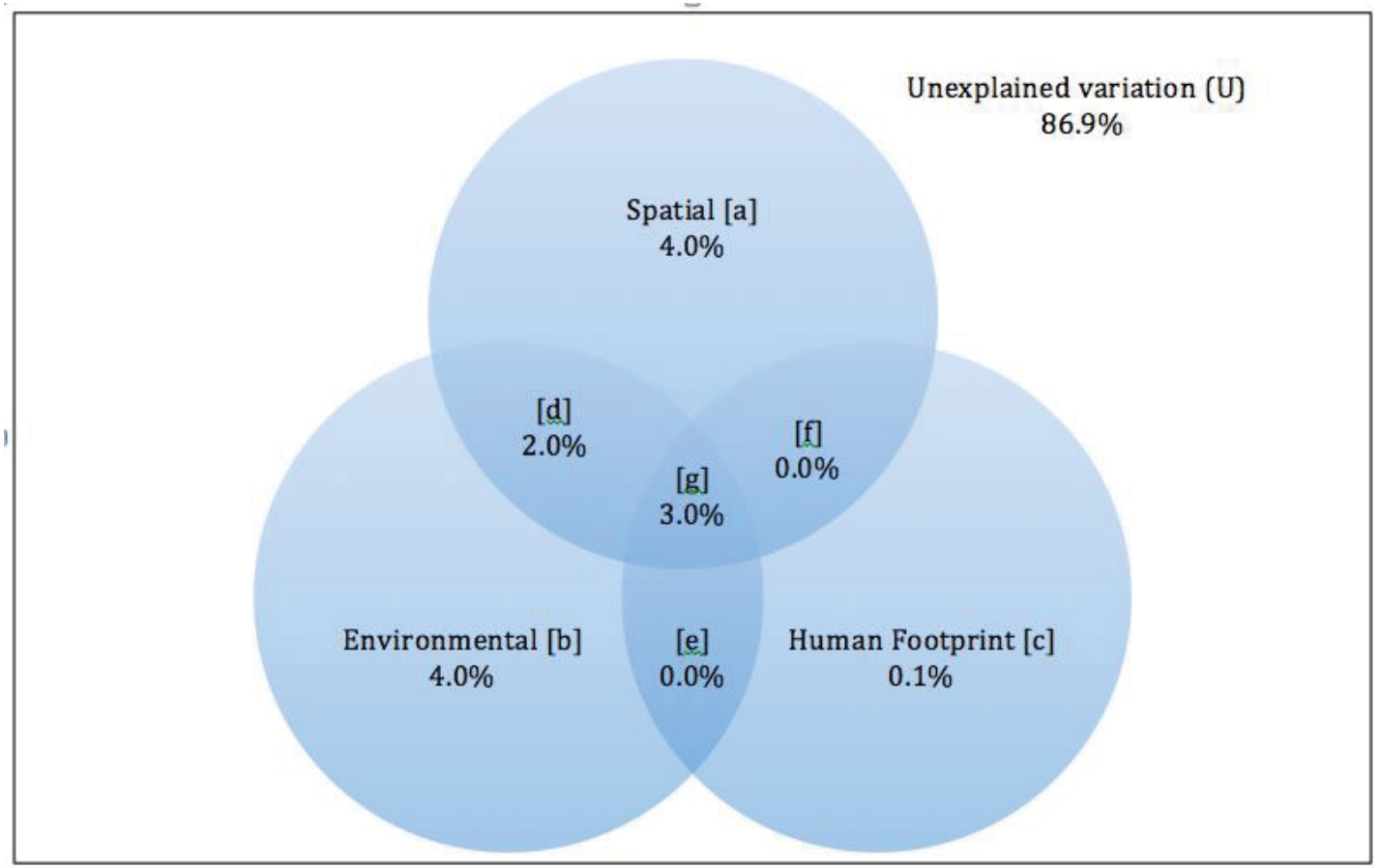

Figure 3.5.Variation partitioning results for chironomid assemblages at provincial scale based on partial redundancy analysis. Explainable variance was partitioned into: pure spatial effect [a], pure non-human-footprint (NHF) environmental effect [b], pure human footprint effect [c], interaction between space and NHF environment [d], interaction between space and HF [f], interaction between NHF environment and HF [e], interaction among all three [g].

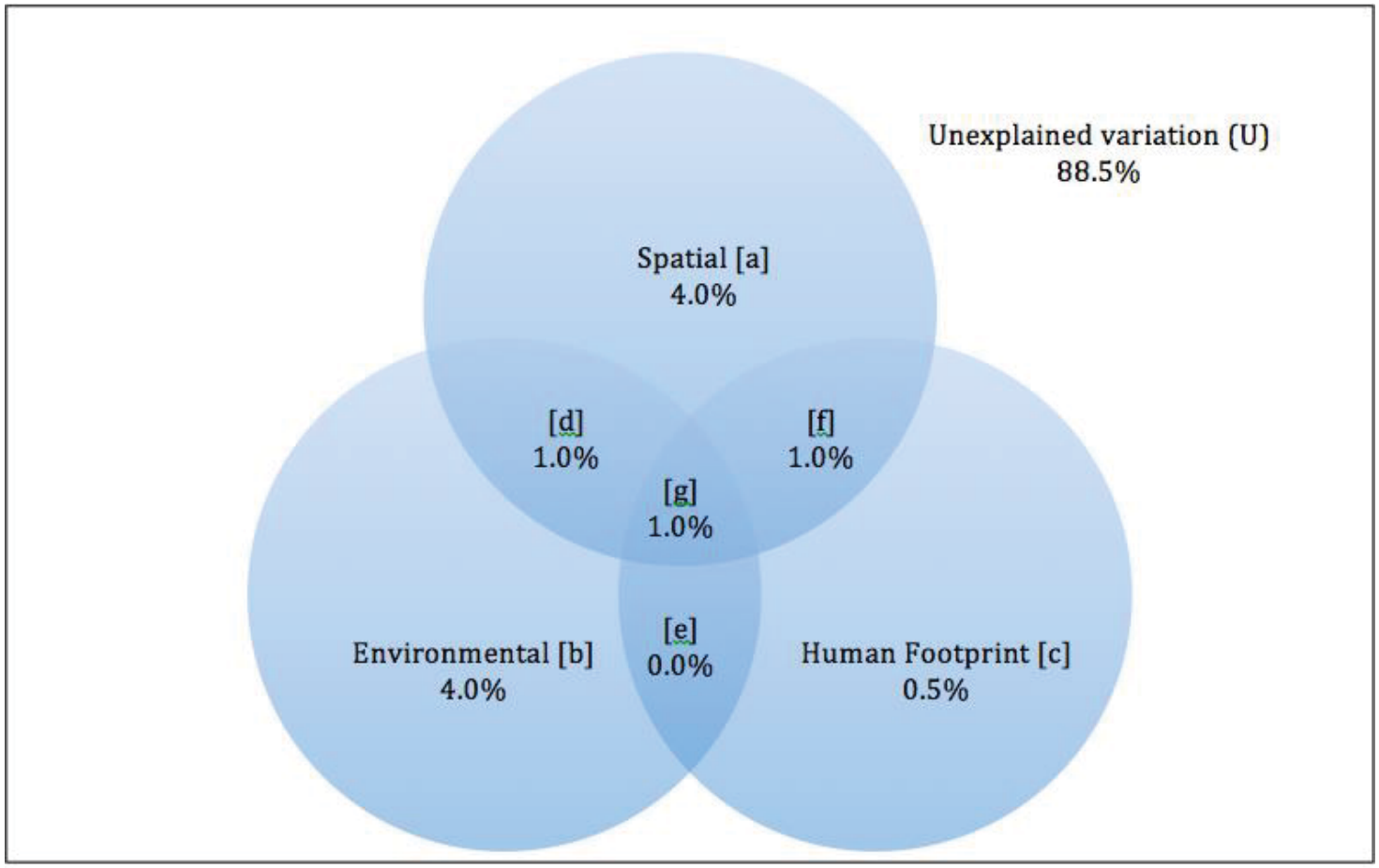

Figure 3.6.Variation partitioning results for chironomid assemblages at Canadian Shield and Boreal ecoregion (CB) based on partial redundancy analysis. Explainable variance was partitioned into: pure spatial effect [a], pure non-human-footprint (NHF) environmental effect [b], pure human footprint effect [c], interaction between space and NHF environment [d], interaction between space and HF [f], interaction between NHF environment and HF [e], interaction among all three [g].

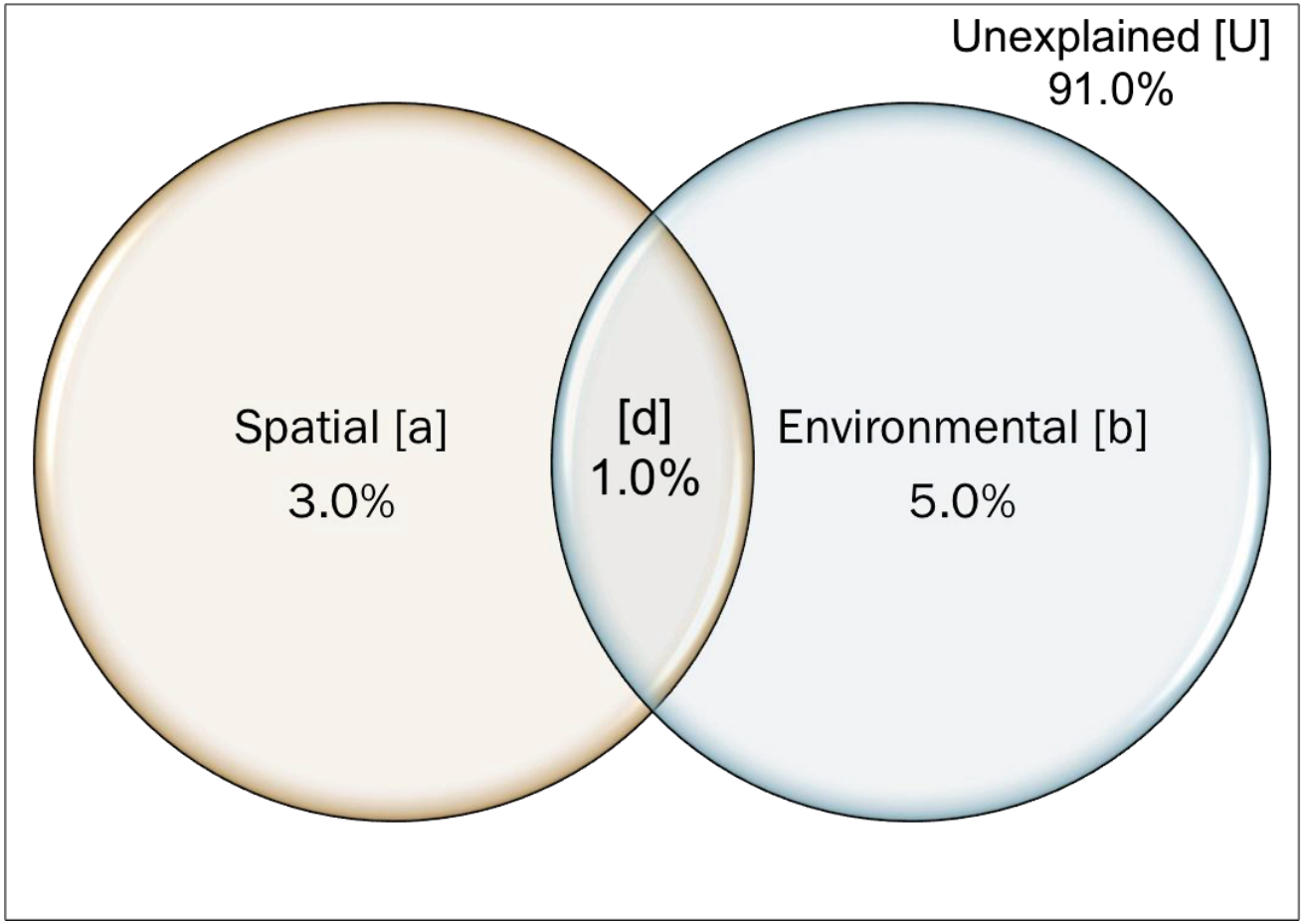

Figure 3.7.Variation partitioning results for chironomid assemblages at Parkland and Grassland ecoregions (PG) based on partial redundancy analysis. Explainable variance was partitioned into: pure spatial effect [a], pure non-human-footprint (NHF) environmental effect [b] and interaction between space and NHF environment [d].

Table 3.1. Comparison of the dispersion (mean+/- SD) of spatial extent, environment and chironomid assemblage composition among the three regions in the analysis of homogeneity of multivariate dispersions. Significant differences are indicated by superscript lower-case letters. Regions with the same letters were not significantly different according to Tukey's *post-hoc* tests. CB, Canadian Shield and Boreal region; PG, Parkland and Grassland region; RF, Rocky Mountain and Foothill region.

	CB	PG	RF	F-value	p-value
Spatial extent (Euclidean distance)	2.138 ± 0.97^{a}	1.809 ± 0.56^{b}	1.438 ± 1.01^{c}	$F_{2,264}=13.103$	0.001
Environmental heterogeneity (Euclidean distance)	4.89 ± 1.06^{a}	4.74 ± 1.23^{a}	5.38 ± 1.71^{b}	$F_{2,264}=4.6195$	0.009
Beta-diversity of chironomid assemblages (Bray-Curtis dissimilarity)	0.4655 ± 0.15^{a}	0.4942 ± 0.11^{ab}	0.5255 ± 0.12^{b}	$F_{2,264}=4.0039$	0.017

Table 3.2. Redundancy analyses (RDA) to evaluate the relationship between the chironomid assemblages and explanatory variables. RDA was used separately for different explanatory variable groups: spatial variables, human footprint (HF) variables and non-human-footprint (NHF) environmental variables. In the case where the global model was statistically significant ($p < 0.05$ from 9999 Monte Carlo permutations), a forward selection procedure was performed to retain the most important variables in explaining the chironomid assemblages. Numbers for spatial variables indicate the spatial scale with smaller numbers representing broad spatial scale. The spatial, non-human-footprint (NHF) environmental and human footprint (HF) variables were shown in the order of importance. See Table 3.3 for abbreviations.

	Significance of full model (p-value)			Variables identified as important factors		
Dataset	Spatial	NHF environmental	HF	Spatial	NHF Environmental	HF
Province	0.005	0.005	0.005	2 + 1 + 3 + 8 + 7 + 15 + 4 + 6 + 11 + 57 + 14	Phos + Ddept + Ele + LarchFen + Con + Sali + OW	Ag+Hwet
CB	0.01	0.005	0.01	3 + 6 + 5 + 9	Sali + FFP + Phos + Ddept + OW	HardLin
PG	0.026	0.005	0.14	3 + 6 + 4	Ele + Oxy + Ddept	-
RF	0.62	0.16	0.69	-	-	-

Table 3.3. Abbreviations of the environmental variables used in the study.

For	Forestry
Ag	Agriculture
UrbInd	Urban and Industrial developments
SoftLin	Soft Linear Features
HardLin	Hard Linear Features
HWet	Human-Created Water bodies
Temp	Temperature
pH	pH
Oxy	Dissolved Oxygen
Con	Conductivity
Sali	Salinity
Nitro	Total Nitrogen
Phos	Total Phosphorus
OrgC	Dissolved Organic Carbon
Ddept	Maximum water depth
Ele	Elevation
PET	Potential evapotranspiration
FFP	Frost-free period
MAP	Mean annual precipitation
MAT	Mean annual temperature
Hei	Vegetation height
Den	Vegetation density
R	Riparian area
OW	Open water area
Deciduous	Deciduous
Mixedwood	Mixedwood
WhiteSpruce	White Spruce
Pine	Pine
BlackSpruce	Black Spruce

LarchFen	Larch Fen
BogFen	Bog and Fen
Marsh	Marsh
Swamp	Swamp
GrassShrub	Grass and Shrub

Table 3.4. Human footprint and Vegetation type descriptions.

Human Footprint types	
Agriculture	Percent area converted for crops and pasture
Forestry	Percent area with forest harvesting (clear-cut or partial retention timber extraction.
Urban/Industrial Development	Percent area converted for human use (e.g., residences) and industrial activity (e.g. mines)
Hard Linear Features	Percent area converted for linear features that are paved or gravel (e.g. highways and logging roads)
Soft Linear Features	Percent area converted for linear features that are grass or natural vegetation after disturbance (e.g., pipelines)
Human-Created water bodies	Percent area converted to reservoirs, dugouts, canals etc.
Vegetation types	
Deciduous	Upland, combined trembling aspen, balsam poplar and white birch comprise >80%
Mixedwood	Upland, deciduous >20% and combine conifer species >20%
White Spruce	Upland, combined white spruce and balsam fir > 80%
Black Spruce	Upland, black spruce is the leading species
Larch Fen	Wetland, larch is the leading species
Bog and Fen	Wetland, sphagnum moss and sedge are the leading species
Marsh	Wetland, non-woody aquatic plants.
Swamp	Wetland, open shrub or closed shrub.
Grass and Shrub	Non-forested land, non-woody plants and soil regime is dry or mesic

Chapter 4 : Responses of chironomid genus richness, chironomid diversity, total abundance and abundance of common chironomid genera to environmental factors in Albertan wetlands

4.1 Introduction

Wetlands are areas characterized by poorly drained soils and water-loving plants, being saturated with water, either permanently or seasonally. They are important as they not only support unique communities, but also influence the adjacent terrestrial ecosystem (Davis et al. 2006). They provide a wide range of services, including improving water quality through filtration, providing habitats for wildlife, and storing floodwaters (Gibbs 2000, Wray and Bayley 2006) . In Alberta, wetlands cover about 21% of the landscape of the province (Wray and Bayley 2006) . However, many wetlands in Alberta have been altered or even destroyed by human activities. The dominant cause of continued degradation is the change in land use practices due to industrial development and human settlement. An effective monitoring strategy is essential for proper wetland management. Development of a biological monitoring (biomonitoring) strategy requires two steps: evaluation of the effect of environmental factors on biological responses (e.g., species richness), and then using the biological responses to identify and monitor changes in the environment (Barbour et al. 1999, Reece and Richardson 1999) . However, freshwater biomonitoring has historically been focused on lotic habitats (i.e. rivers and streams) and large lentic systems (i.e. lakes); more research is needed to further evaluate monitoring strategies in small wetlands (Fennessy et al. 2004, Davis et al. 2006) .

Aquatic macroinvertebrates have been widely used as indicators of ecosystem health (Rosenberg and Resh 1993, Barbour et al. 1999) . The advantages and difficulties of using macroinvertebrates in biomonitoring were very well summarized (e.g.

Rosenberg and Resh 1993, Barbour et al. 1999). Those general conclusions are equally applicable to the family Chironomidae (commonly known as non-biting midges), as it is one of the most abundant and diverse groups of macroinvertebrates in freshwater environments (Pinder 1986, Ferrington 2008) . Compared to other macroinvertebrate families, chironomids provide an advantage in offering the widest possible spectrum of responses to environmental stressors (Rosenberg 1992). However, the diversity of this abundant and ecologically important family is often neglected in freshwater studies due to the extra effort required to identify them more finely than subfamily (Rosenberg 1992, Epler 2001) . As a result, there is a lack of information establishing responses of common chironomid taxa (e.g. genus or species) to different types of environmental stressors.

Many studies have examined how assemblages of macroinvertebrate in wetlands are influenced by environmental factors. Examined factors range from local influences within the wetlands such as vegetation (Krieger 1992, De Szalay and Resh 2000, Thomaz et al. 2008, Hinojosa-Garro et al. 2010) and water chemistry (De Szalay and Resh 2000, Heino 2000, Battle and Golladay 2001) , to landscape factors such as the land-use practices (Steinman et al. 2003, Hall et al. 2004, Meyer et al. 2015) . However, results from many of these studies are not consistent with each other indicating these results may be applicable only to the geographical regions of the study. For example, some studies (e.g., Nicolet et al. 2004, Thomaz et al. 2008, Hinojosa-Garro et al. 2010) found an increase in macroinvertebrate abundance and diversity with increased vegetation types and plant cover while the opposite pattern was observed in other studies (e.g., McLaughlin and Harris 1990, De Szalay and Resh 2000) . Similarly, land-use practice (e.g. agriculture) may strongly affect macroinvertebrate abundance and diversity (Euliss

and Mushet 1999, Steinman et al. 2003, Meyer et al. 2015) or have no significant impact on macroinvertebrates (Tangen et al. 2003). Those inconsistent results and their apparent regional specificity suggest that further studies are needed to establish relationships between biological responses and environmental factors at different geographic locations.

The main goal of this study was to assess the responses of chironomid genera richness, chironomid diversity (Shannon-Wiener index) and abundance of common chironomid genera to local habitat factors (e.g., water quality) and surrounding landscape factors (e.g., anthropogenic disturbance and surrounding vegetation) in Alberta, Canada. Identifying the important factors associated with different aspects of chironomids would help us to evaluate the utility of including chironomids in wetland biomonitoring in Alberta.

4.2 Method

4.2.1 Study Sites

The study was conducted at wetland sites in Alberta selected and sampled by the Alberta Biodiversity Monitoring Institute (ABMI) (http://www.abmi.ca/home.html) (Figure 2.1). The ABMI sampling program contains 1656 terrestrial sites evenly distributed throughout Alberta using the 20 km National Forest Inventory (NFI) grid (ABMI 2008). Near each NFI site, a wetland is chosen randomly from a pool of suitable wetlands that meet the following criteria: 1) be permanent; 2) have >1.0 ha of open water and still be >0.5 m deep in July; 3) have a well-developed zone of vegetation (ABMI 2013). However, the selection criteria did not exclude human created wetlands. Data and

specimens used in this study were collected at 270 wetlands across the province by ABMI from June 15 to July 31 between 2009 and 2011.

4.2.2 Potential Explanatory Variables

4.2.2.1 Water Quality

Physiochemistry for each sampled water body was measured by ABMI personnel at the deepest point of the wetland and at 2 additional points located at 25 m intervals moving toward the geometric center of the wetland (ABMI 2013). Water temperature, pH, dissolved oxygen, conductivity and salinity were determined at each point using a Hydrolab multi-probe meter in the field. A one litre water sample was collected at each of the three points and processed in the laboratory using standard protocols to determine total nitrogen (TN), total phosphorus (TP), and total dissolved organic carbon (DOC). Water samples and physiochemical data were taken between 1:00 and 2:00 pm to avoid variation caused by time of day.

4.2.2.2 Climate data

To characterize the local climatic conditions of each site, mean annual temperature (MAT), mean annual precipitation (MAP), frost-free period (FFP) and potential evapotranspiration (PET) were derived by ABMI personnel by overlaying the wetland location with the climate layer, which is based on interpolated climate data (Daly et al. 2002, Hijmans et al. 2005) from weather stations for the period 1961–1990. The western North American portion of these data is described by Wang et al. (2012).

4.2.2.3 Habitat Characteristics

Vegetation height above substrate and density were surveyed by ABMI personnel at three transects (10 x 2 m) in the open water at each wetland (ABMI 2013). First, the transects were categorized based on vegetation height as Non-Vegetated (floating or submerged plants <10% cover), Short Submerged (>50% vegetation extending into the water column 0.0-0.3 m above the substrate), Medium Submerged (>50% vegetation extending into the water column 0.3-1.3 m above the substrate), Tall Submerged (>50% vegetation extending into the water column >1.3 m above the substrate), and Floating (>50% vegetation with floating leaves on the water surface). Second, the transects were categorized based on vegetation density as Non-Vegetated (floating or submerged plants <10% cover), Sparse (aquatic vegetation covering 10 - 24% of the substrate, Moderate (aquatic vegetation covering 25-75% of the substrate), and Dense (aquatic vegetation covering >75% of the substrate). Then the categorical variables of vegetation height and density were coded as ordinal variables from 0-4 and 0-3, respectively. Wetland bathymetry was characterized using one primary and two secondary axes (ABMI 2013). Water depth measurements were taken at 12 points equally spaced along the first axes and at eight points equally spaced along the two additional axes.

Human footprint and surrounding vegetation types were characterized by ABMI personnel by manually interpreting 1:30,000 air photos and existing geographic information system (GIS) data layers (ABMI 2012). 'Human footprint' is defined by ABMI (2012) as "the geographic extent of areas under human use that have either lost their natural cover (e.g., roads, agricultural land) or whose natural cover is periodically or temporarily replaced by resource extraction activities (e.g., forestry, surface mining)."

Human footprints are categorized into five broad types: Agriculture, Forestry, Hard Linear Features (e.g., roads, rails), Soft Linear Features (e.g. pipelines, seismic lines), Human-Created Water Bodies and Urban Industrial Features. Surrounding vegetation was categorized by ABMI into 10 broad types: Deciduous, Mixedwood, White Spruce, Pine, Black Spruce, Larch Fen, Bog Fen, Marsh, Swamp and Grass Shrub. The percentage area of these human footprint and vegetation types were quantified within a 250 m wide buffer around the open water of the wetland using ArcGIS and ArcView software by ABMI. In addition, the area of the open water and riparian zone of each wetland, and the natural ecoregion and subecoregion in which each wetland is located, were also determined by ABMI personnel from existing GIS data layers.

4.2.2.4 Spatial Variables

Geographic coordinates (latitude, longitude) and elevations were determined for each wetland site by ABMI personnel.

4.2.3 Aquatic invertebrates

Aquatic invertebrates were sampled by ABMI personnel at ten locations in each wetland using a modified D-ring dip net with a mesh size of 500 μm (ABMI 2013). Samples were preserved using 10% buffered formalin in the field. Once back to the lab, the preservative was changed to 70% ethanol (ABMI 2011).

In the lab, all ten samples from the same wetland were first put through a 500 μm sieve and combined to create a composite sample (ABMI 2011). Then the composite sample was elutriated to go through 2.8 mm and 500 μm sieves. The elutriated material

retained by both the 2.8 mm and 500 μm sieves was rinsed into a Marchant box sub-sampler (Marchant 1989) to fill each of the 100 separate cells. The Marchant box was closed, inverted and gently swirled to evenly distribute the elutriated materials in the Marchant box cells. A random-numbers table was used to select a cell whose contents were transferred to a Petri dish. ABMI target taxa (Table 1.1) were sorted into separate vials filled with 70% ethanol using a 10-40x microscope with a fiber-optic light source. The goal was to collect at least 350 undamaged specimens of all target taxa combined. If this was not reached from the first cell, another cell was randomly selected and sorted until there were 350 target organisms or all 100 cells were sorted.

I then picked through the chironomid samples to identify the specimens more finely. If there were more than 100 specimens in a single sample, it was subsampled. For 2009 and 2010 samples, I randomly selected 100 chironomids for identification from each sample. To do this, I placed the chironomid larvae in a Petri dish overlying a grid of 100 cells and used a random number generator to randomly sample cells until a total of 100 chironomids were reached. To identify the chironomid specimens I first put them into lactic acid for 12-24 hours to clear before slide-mounting them in polyvinyl alcohol mounting medium (PVA, cat. #6371A from BioQuip, Rancho Dominguez, California). Slides were then left on slide warmers for 2-3 days at ~45°C before they were examined under a compound light microscope. I used various taxonomic keys (Oliver and Roussel 1983, Coffman and Ferrington 1996, Epler 2001) to identify the chironomids to the finest possible taxonomic level. Originally I tried to identify all chironomids to morphospecies, however, verification of the morphospecies identification by other chironomid taxonomy expert (Robert Hinchcliffe from Royal Alberta Museum) showed a

large number of chironomids couldn't be reliably identified at morphospecies. So in all my analysis, I only used the chironomid data at genus-level. For 2011 samples, chironomids were subsampled and identified by ABMI personnel. Their subsampling strategy was to randomly select no more than 100 chironomid specimens (instead of exact 100 as I used) for fine identification. To determine the number of chironomids that needs to be identified in a sample with more than 100 specimens, the number of chironomid larvae in the entire sample was divided by the number of Marchant box cells sorted, which was then multiplied by a whole number to get a number as close to 100 without going over.

Finally, the true abundance of each taxon in the original (un-subsampled) sample was estimated as (relative abundance of this taxon * the total number of chironomids sorted)/proportion of Marchant box cells sorted. For example, if 250 chironomids were sorted from a site from 50 Marchant box cells, and 40 out of the 100 identified chironomids were from the genus *Chironomus*, then the relative abundance of *Chironomus* is 40/100=0.4, the proportion of Marchant box cells sorted is 50/100=0.5, and the abundance of *Chironomus* in the complete sample is estimated as (0.4*250)/0.5=200.

4.2.4 Data Analysis

To assess the responses of chironomid genus richness, chironomid diversity (Shannon-Wiener index) and abundance of common chironomid genera to local habitat factors (e.g., water quality) and surrounding landscape factors (e.g., anthropogenic disturbance and surrounding vegetation), I used the generalized linear model (GLM) to

model their relationships. Common genera were those taxa that occurred in at least 20% of the surveyed sites. These genera are *Chironomus, Dicrotendipes, Endochironomus, Glyptotendipes, Parachironomus, Paratanytarsus, Tanytarsus, Acricotopus, Corynoneura, Cricotopus, Nanocladius, Psectrocladius, Ablabesmyia*. Shannon indices were log-transformed before the analysis and were modeled with a normal distribution, which is equivalent to a multiple linear regression. Chironomid genus richness and total chironomid abundance was modeled with negative binomial distribution to account for over-dispersion. Over-dispersion was measured using the regression-based test for mean variance equality (Cameron and Trivedi 1990) in the R Environment (R Development Core Team 2013). The abundance of individual taxa was modeled with zero-inflated negative binomial models to account for over-dispersion and high occurrences of zero count in many sites. I used the R package glmmADMB (Bolker et al. 2012) to fit the GLM and R package glmulti (Calcagno 2013) to select the best models among candidate models. Because there is a huge number of possible candidate models (i.e. 2^{30} = 1073741824 possible combinations of predictor variables), it's difficult to use the traditional exhaustive screening procedure to evaluate every single model. Instead, the genetic algorithm -a global optimization procedure that reduces the total number of models that must be assessed - was used to find the best models (Wasserman and Sudjianto 1994, Wallet et al. 1997) .

I used the variance inflation factors (VIF) to examine multicollinearity among environmental predictor variables. VIF measures how much of the variance of an estimated regression coefficient would be inflated as compared to when predictor variables are not linearly dependent (Dormann et al. 2013). A VIF of one shows no

multicollinearity and increasingly larger values suggest increasing multicollinearity. A VIF exceeding 10 is often considered a sign of serious multicollinearity (Dormann et al. 2013). I calculated the VIF for all my predictor variables and removed the predictor with the largest VIF, recalculated, and repeated until all VIFs dropped below 10.

I used the corrected Akaike information criterion (AIC_c) to evaluate the relative model fit. Models with $\Delta AIC_c < 2$ in comparison to the best model (i.e. the model with the smallest AIC_C) were selected as confidence models (Burnham and Anderson 2002) . Then I averaged coefficient estimates and the estimate of precision (i.e. standard errors) across all confidence models based on their relative likelihood (i.e., Akaike weights) to get one averaged model using the "model.avg" function of the R package MuMln (Barton 2014). This approach incorporates model selection uncertainty directly into the estimate of precision (i.e. standard errors) thus reduces the bias in parameter estimates. All the variables selected in the final averaged model were then listed in decreasing order based on their relative importance (determined by Akaike weights) for further evaluation. A clearly sharp drop in their relative importance was used as a cut-off point above which variables were considered as the most important variables in affecting the biological responses. After fitting the model, all residuals were checked for spatial autocorrelation through Moran's I index using the R package ape (Paradis et al. 2012). No significant residual spatial autocorrelation was detected, justifying the use of non-spatial regression methods for data analysis.

I used D^2 (deviance explained) to assess the absolute model fit of the final averaged model. D^2 is a measure of the variance reduction in models based on maximum-likelihood estimation (Guisan and Zimmermann 2000) . It's analogous to R^2 (coefficient

of determination) in an ordinary least square (OLS) regression. I could not calculate the D^2 for averaged models directly, but instead estimated these values by calculating D^2 for each of the confidence models ($\Delta AIC_c < 2$), then calculating a weighted average across those confidence models based on their AIC_c weights.

4.3 Results

I identified a total of 40 genera of chironomids from 2009 and 2010 samples and ABMI identified another 9 genera from 2011 samples, with 13 of those genera occurring in >20% of the surveyed wetlands (Table 4.1). See Chapter 2 for a detailed taxonomic review of the 40 genera I identified.

4.3.1 Relationship Between Diversity and Abundance Metrics and Environmental Variables

The Shannon diversity index model fitted the data relatively well, with 32.79% of the variation explained by the environmental variables (Table 4.2). The variables with the largest likelihood (i.e. most important variables) in influencing Shannon index contained two human footprint variables (agriculture and forestry), five surrounding vegetation variables (deciduous, larch fen, mixedwood, pine and swamp) and four other habitat variables (conductivity, vegetation density in the open water, elevation and potential evapotranspiration). Shannon diversity decreased with conductivity and elevation, but increased with all the other variables. For the average total richness model, the variation explained by environmental variables was only 7.9% (Table 4.2). One human footprint variable (human-created water bodies), six surrounding vegetation variables (grass shrub, larch fen, mixedwood, pine, white spruce and deciduous), and three other habitat

variables (conductivity, vegetation density in the open water and elevation) were most important in influencing the richness. Similar to the Shannon index, chironomid genus richness was negatively affected by conductivity and elevation. In addition, increases in human-created water bodies, grass shrub and white spruce were also associated with decreasing richness. For total abundance, the most important variables in influencing it were three surrounding vegetation variables (black spruce and marsh) and seven other habitat variables (deepest depth, vegetation density and height in the open water, elevation, organic compound, oxygen, phosphorus and potential evapotranspiration). Total abundance of chironomids was negatively correlated with deepest depth, elevation, vegetation height and oxygen level, but positively correlated with all other variables. In addition, surrounding pine, open water area and soft linear features were also relatively important, as they were included in many of the confidence models. However, their coefficient estimates had 95% confidence intervals that crossed zero, indicating large uncertainties on how the total abundance responded to those three variables. Overall, the environmental variables could explain only 2.85% of the variation (Table 4.2).

4.3.2 Relationship Between Individual Chironomid Genera and Environmental Variables

For individual genera, the most important variables identified in the final averaged models contained between 6 and 13 variables (Table 4.3). Both human footprint and surrounding vegetation variables were quite common in individual taxon models. Surrounding vegetation variables occur in every taxon model and human footprint variables were only absent from the *Psectrocladius* abundance model. The surrounding vegetation variables that occurred quite often in the final models were bog and fen, and

grass and shrub. For the human footprint variables, human-created water bodies were quite common (9 out of 13 models) in the final models. However, agriculture and forestry were only important in affecting one or two taxa. Besides surrounding landscape factors, other most important variables are elevation (included in 9 out of 13 taxa models), phosphorus (6 out of 13), aquatic vegetation (6 out of 13) and deepest depth (5 out of 13). The remaining variables (e.g., nitrogen, oxygen, temperature) were only important in affecting a few (fewer than five) chironomid genera. But the negative effect of oxygen on *Chironomus* and the negative effect of nitrogen on *Endonchironomus, Parachironomus, Paratanytarsus* are worth a mentioning here, because oxygen and nitrogen are much more influential in those models based on their beta standardized coefficients (Table 4.3) compared to other important environmental variables.

Although several environmental variables were identified as significant and independent predictors of chironomid abundance, the amount of explained taxonomic variation was relatively small (Table 4.3). *Chironomus, Parachironomus* and *Acricotopus* were the highest with about 7% of their variance explained by the environmental variables. All the other ten chironomid genera had no more than 5% of the variance explained by the environmental variables.

4.4 Discussion

4.4.1 Effect of human footprint and surrounding vegetation

I discovered that human footprint variables (Agriculture, Forestry, Hard Linear Features and Soft Linear Features) explained taxonomic variation across chironomid communities (13 out of 16 diversity/abundance metrics and genera, Table 4.2 and 4.3). Human-creation of water bodies was the most influential anthropogenic influence on

chironomids, though it comprised the smallest surface area of the total human footprint. Total genus-richness, *Dicrotendipes, Parachironomus, Chironomus, Paratanytarsus, Acricotopus, Corynoneura* and *Ablabesmyia* were negatively affected by human-created water bodies, while *Endochironomus, Tanytarsus,* and *Psectrocladius* were positively correlated with them. Human-created water bodies include a diversity of structures such as dugouts and irrigation and drainage canals. They could easily alter the local hydrology (i.e. the pattern of water flow in an area) of the wetland (e.g., Rehage and Trexler 2006, Blann et al. 2009) . For example, canals could change the speed and natural amount of the water that moves into and out of the wetland. The alteration of hydrology will then change the soil property and nutrient input, which could directly affect the local plant and animal community. Rehage and Trexler (2006) identified a strong correlation between increased aquatic animal density and phosphorus enrichment caused by canals. Besides, reservoirs may provide refuge habitats and increase connectivity to other aquatic habitat, which could also influence the local biological community (Rehage and Trexler 2006, Blann et al. 2009) .

Other human footprints also affected a few of the chironomid responses. For example, Shannon diversity index and *Tanytarsus* abundance were positively correlated with surrounding agriculture practice. Several studies (Grue et al. 1986, Euliss and Mushet 1996) have showed that surrounding land use change could lead to wetland degradation. For example, increased agriculture activities often cause increased sedimentation, which could affect biological community by reducing habitat heterogeneity, burying invertebrates and altering productivity of algae (Martin and Neely 2001, Gleason et al. 2003) . In addition, land use could also degrade the wetland through

habitat fragmentation (Kantrud 1993), discharge of chemicals into wetlands (Grue et al. 1986) and alteration of local hydrological cycle (Euliss and Mushet 1996) . Therefore, the positive correlation between Shannon diversity and agriculture land use was not expected. However, agriculture land use could also influence nutrient loadings and I did find a good positive correlation between phosphorus in a water body (Spearman $r=0.56$, $p<0.0001$) and adjacent agriculture land use. Increased input of phosphorous within a threshold could lead to increased productivity of primary producers, which could favor certain groups of aquatic invertebrates. The total abundance and eight of the thirteen genera abundance were found to be increasing with elevated phosphorus levels. This might explain why Shannon diversity was positively correlated with agriculture land use.

Some surrounding vegetation types were always identified as important variables in every chironomid response model (Table 4.2 and 4.3). For example, the Shannon index was positively correlated with upland deciduous, mixedwood, pine trees and lowland larch dominated fen and shrub dominated swamp (Table 4.2). The riparian vegetation could influence the type and abundance of aquatic invertebrates for many reasons. First, like discussed above, adjacent vegetation could reduce nutrient and sediment input to a water body (Batzer et al. 2000). Second, riparian trees may shade the aquatic habitat and light availability is important in structuring the aquatic plant community (Batzer et al. 2000, Kemp et al. 2004, Lacoul and Freedman 2006) . High light availability will favor vascular emergent plant while low light availability will support more non-vascular bryophytes and submerged vegetation (Lacoul and Freedman 2006) . Trees may also affect the type and abundance of aquatic invertebrate directly by providing organic matter through litter fall. Leaf litter from different trees has different nutritional quality for

invertebrates (Richardson et al. 2004). For example, deciduous leaves are much better food recourses than conifer leaves because they are relatively easier to decompose (Naiman et al. 2010).

4.4.2 Effect of other environmental factors

Elevation was identified as among the most important variables in many models. In a lake study by Nyman et al. (2005), chironomid richness was found to be highest at mid-elevation (400 m above sea level) and decreases towards both ends of the elevation gradient. The range of the elevation in their study was between 150 m and 1150 m, which is similar to my study (between 192 m and 2174 m but with only nine sites at above 1500 m). However, I found all chironomid diversity metrics (i.e., Shannon index, genera richness and total abundance) to be negatively correlated with elevation. The contradictory results are probably because elevation affects the distribution of chironomids indirectly. In the study by Nyman et al. (2005), the elevation gradient covaries with many environmental variables such as air temperature, precipitation and nutrient supply. Those environmental variables were probably more ecologically meaningful variables, but some of their effects are likely to be captured by elevation (Porinchu et al. 2002). In my study, elevation was positively correlated with mean annual air temperature (Spearmen $r= 0.625$, $p<0.0001$) and mean annual precipitation (Spearman $r=0.473$, $p<0.0001$). Thus, elevation might affect the distribution of chironomids through the local climatic conditions.

Aquatic vegetation was also identified important in influencing several chironomid responses. It has been suggested that an increase of macroinvertebrates abundance and

diversity is often associated with increased aquatic vegetation complexity (Nicolet et al. 2004, Thomaz et al. 2008, Hinojosa-Garro et al. 2010) , though some studies (e.g., McLaughlin and Harris 1990, De Szalay and Resh 2000) found different results. In my study, vegetation density influences 9 chironomid responses that all show a positive correlation with it. These responses are Shannon index, total richness, total abundance, *Chironomus, Paratanytarsus, Tanytarsus, Cricotopus*, *Psectrocladius* and *Ablabesmyia*. In contrast, vegetation height negatively influences 6 responses: total abundance, *Chironomus, Tanytarsus, Cricotopus, Psectrocladius* and *Ablabesmyia.* Vegetation could affect invertebrates by influencing food availability and microhabitats. Similar to the riparian vegetation, aquatic vegetation varies in nutritional values. For example, bryophytes contain more refractory matter (e.g., fibrous materials), which is a poor food source for many invertebrates (Suren and Winterbourn 1991, Thomaz and Cunha 2010) . On the other hand, macrophytes increase habitat complexity and heterogeneity thus providing more microhabitat types (Thomaz and Cunha 2010) . Higher vegetation density tends to increase habitat complexity and provide more food sources. However, tall emergent plants may decrease the habitat complexity below water because there are mainly stems. Beside, the tall emergent plants could shade the water and low the productivity of algae deeper in the water.

Water quality is another important determinant of chironomid assemblages (Pinder 1986, Armitage et al. 1995, Quinlan et al. 1998, Porinchu et al. 2002) . I found total abundance, *Endochironomus, Glyptotendipes, Parachrionomus, Paratanytarsus, Corynoneura, Cricotopus, Nanocladius* were all positively affected by increased phosphorous. Chironomids have long been used to characterize the trophic status of lakes

(Saether 1979, Brooks et al. 2001, Porinchu et al. 2002) . Increased input of phosphorous and nitrogen often lead to increased productivity of primary producers, which could favor certain groups of aquatic invertebrates, although extreme eutrophication can cause massive algal blooms, and subsequent death and decomposition of the algae can lower dissolved oxygen concentrations to lethal levels. In addition, I found that total midge abundance and *Chironomus* abundance were negatively associated with dissolved oxygen. This was unexpected because many aquatic organisms depend on sufficient dissolved oxygen for respiration. Most of the studied wetlands had dissolved oxygen concentration above 5mg/L so the oxygen is not likely to be a limiting factor for chironomids. Other unmeasured factors or processes yet correlated to dissolved oxygen could have caused the negative relationship.

Maximum water depth was also found to affect many chironomid responses negatively including total abundance, *Chironomus, Dicrotendipes, Parachironomus, Tanytarsus, Acricotopus*. The influence of water depth on chironomids has long been recognized in many lake studies (Walker et al. 1991, Quinlan et al. 1998, Larocque et al. 2001, Porinchu et al. 2002). Walker et al. (1991) suggested that the influence of lake depth on chironomid fauna is largely due to its cooling influence on surface water temperatures. In my study, I only found a weak negative correlation between the water depth and surface temperature (Spearman $r = -0.26$, $p < 0.001$). Larocque et al. (2001) argued that morphometric regulation of habitat availability (e.g., proportion and volume of the littoral and profundal zones) with increasing maximum depth might be the underlying mechanism regulating the chironomid assemblages. In my studied wetlands, the depth is relatively shallow (most wetlands < 2 meters deep), so the mechanism might

be different from lakes. In wetlands, fish are more likely to occur in deeper ones (Baber et al. 2002) because deeper wetlands are less prone to extreme event such as freezing solid to the bottom in winter. And it has been found the presence or absence of fish was a very important factor in explaining invertebrate abundance and composition (e.g., Zimmer et al. 2001, Tangen et al. 2003, Tarr et al. 2005). In addition, Zimmer et al. (2000) suggested that increasing depth of wetlands could limit the light availability thus decreasing macrophytes abundance, which ultimately will affect the invertebrate composition. In addition, fish are more likely to occur in deeper wetland

4.4.3 Weak correlation with environmental factors

Despite all those environmental factors identified as important in explaining various chironomid responses, I found that all the chironomid responses, except for the Shannon index, were poorly explained by the predictor variables. Of all the 13 taxa tested, none of them had >8% of the variation explained.

There are several potential reasons. First, some important variables were not measured in my study. It has been found the presence or absence of fish was the most important factor in explaining invertebrate abundance and composition (e.g., Zimmer et al. 2001, Tangen et al. 2003, Tarr et al. 2005) . Also many studies have found that an increase of macroinvertebrates abundance and diversity is often associated with increased aquatic vegetation complexity (Nicolet et al. 2004, Thomaz et al. 2008, Hinojosa-Garro et al. 2010) . Although I characterized the macrophytes by their density and height, this might not be a good way to reflect the vegetation complexity. So in future studies, inclusion of other variables, especially those that have been identified as important in

affecting macroinvertebrates or chironomids would improve the percentage of variance explained. Inclusion of those variables would improve the percentage of variance explained. Second, although using above-species-level taxa when biomonitoring with freshwater invertebrates is often a very effective approach (Lenat and Resh 2001) , identifying only to the genus level may have resulted in the loss of important ecological information. Species within the same genus of chironomids can exhibit a wide range of environmental tolerances (Wrubleski 1987, Hudson et al. 1990, Armitage et al. 1995, Epler 1996). For example, King and Richardson (2002) failed to detect a stressor-response relationship for the genus *Tanytarsus*, yet four of the six species they analyzed were found to be strongly associated with the degree of wetland disturbance. Third, stochastic processes of recruitment, dispersal, local extinction etc. may play a more important role in controlling the macroinvertebrates in wetlands than in rivers and streams (Davis et al. 2006, Batzer 2013) . There is growing evidence that macroinvertebrates in wetlands are more tolerant to environmental variation compared to stream macroinvertebrates, as they have been selected to cope with the highly variable nature of wetland environments, such as periods of flooding and drought, changing water quality conditions daily, seasonally and yearly (Batzer 2013).

4.5 Conclusion

In this study, I assessed the statistical responses of chironomid richness, chironomid diversity (Shannon index) and 13 common chironomid genera to local habitat factors (e.g., water quality) and surrounding landscape factors (e.g., human footprint and surrounding vegetation) at 270 Albertan wetlands. The results show that local habitat

factors and surrounding landscape factors likely influence the richness, abundance and composition of chironomids. So those factors need to be taken into consideration for management and conservation of wetlands. Conversely, the amount of variation of each chironomid response that could be explained by the environmental variables was relatively small. All the response variables, except Shannon index, had less than 8% of their variance explained by the environmental variables. This low explanatory power makes it hard to develop chironomid-based index to reflect human disturbances. The weak correlation between chironomids and environmental variables could be due to the lacking of other important environmental variables and insufficient taxonomic resolution, as well as chironomids being generalists (i.e., insensitive/tolerant to environmental variation) in wetlands.

For future research, more environmental variables, especially those that have been identified as important in affecting invertebrates and chironomids from other studies (e.g., presence/absence of fish, types of macrophytes and sediments etc.) should be added, which should increase the explanatory power. Further developing regional keys to identify chironomids to species is needed to improve the sensitivity of chironomids to environmental signals. In addition, my study involves a broad range of wetlands using descriptive approach (i.e. nothing is manipulated). Too many variables are examined at the same time and those variables could be very dynamic and interactive, which makes it difficult to draw conclusions. In future studies, manipulated field experiments (e.g., changing water levels, Wrubleski 2005) could also be performed to better understand the role different environmental factors play in influencing chironomids and other invertebrates in Albertan wetlands.

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Table 4.1. Percent occurrences of the 13 most common chironomid genera (i.e. occurring in >20% of the surveyed wetlands) collected from 270 wetlands across Alberta by Alberta Biodiversity Monitoring Institute (ABMI) from 2009 to 2011.

Taxa	% Occurrence
Subfamily Chironominae	
Chironomus	30
Dicrotendipes	43
Endochironomus	42
Glyptotendipes	43
Parachironomus	38
Paratanytarsus	63
Tanytarsus	53
Subfamily Orthocladiinae	
Acricotopus	25
Corynoneura	50
Cricotopus	80
Nanocladius	24
Psectrocladius	71
Subfamily Tanypodinae	
Ablabesmyia	57

Table 4.2. Final averaged models across all confidence models ($\Delta AIC_c < 2$) to examine the relationship between chironomid biodiversity metrics (Shannon index, genus richness and total abundance) and environmental factors. Bolded text indicated the most important variables based on their importance weight. Refer to Table 3.3 for abbreviations.

	Environmental Factors	Relative Importance	Coefficient Estimate	95% CI		D^2
				Lower	Upper	
Shannon Index						0.328
	Ag	1	0.038	0.008	0.069	
	Con	1	-0.070	-0.099	-0.041	
	Deciduous	1	0.053	0.024	0.082	
	Den	1	0.055	0.025	0.084	
	Ele	1	-0.070	-0.100	-0.041	
	For	1	0.031	0.002	0.060	
	LarchFen	1	0.058	0.028	0.088	
	Mixedwood	1	0.035	0.007	0.064	
	PET	1	0.035	0.006	0.065	
	Pine	1	0.058	0.029	0.088	
	Swamp	1	0.032	0.003	0.061	
	HWet	0.71	-0.018	-0.054	0.004	
	Ddept	0.44	-0.010	-0.051	0.007	
	pH	0.43	-0.008	-0.049	0.009	
	BlackSpruce	0.2	0.004	-0.008	0.050	
	FFP	0.14	-0.003	-0.048	0.010	
	Nitro	0.11	-0.002	-0.046	0.012	
	R	0.08	0.001	-0.012	0.046	
	WhiteSpruce	0.04	-0.001	-0.045	0.012	
	BogFen	0.03	0.000	-0.017	0.040	
	GrassShrub	0.03	0.000	-0.038	0.020	
	Temp	0.03	0.000	-0.020	0.037	
Genus Richness						0.079
	Con	1	-0.141	-0.220	-0.061	
	Den	1	0.114	0.060	0.168	
	Ele	1	-0.133	-0.191	-0.075	
	GrassShrub	1	-0.065	-0.128	-0.002	
	HWet	1	-0.102	-0.169	-0.035	
	LarchFen	1	0.062	0.009	0.116	
	Mixedwood	1	0.065	0.017	0.113	
	Pine	1	0.070	0.013	0.126	
	WhiteSpruce	0.98	-0.054	-0.112	0.002	
	Deciduous	0.96	0.048	0.001	0.100	
	HardLin	0.23	-0.009	-0.098	0.020	
	Marsh	0.2	-0.008	-0.098	0.014	

	For	0.12	0.004	-0.017	0.076	
	R	0.12	0.004	-0.020	0.082	
	OW	0.11	-0.004	-0.095	0.020	
	Swamp	0.1	0.003	-0.015	0.084	
	BogFen	0.07	-0.002	-0.076	0.030	
	Ddept	0.07	-0.003	-0.102	0.022	
	BlackSpruce	0.06	0.002	-0.019	0.080	
	Oxy	0.04	0.001	-0.030	0.060	
	FFP	0.03	-0.001	-0.091	0.031	
	Hei	0.02	0.000	-0.043	0.088	
	OrgC	0.02	0.001	-0.036	0.086	
	pH	0.02	-0.001	-0.084	0.035	
	Phos	0.02	0.000	-0.069	0.043	
	Temp	0.02	0.000	-0.044	0.080	
	UrbInd	0.02	0.000	-0.036	0.074	
Total Abundance						0.029
	BlackSpruce	1	0.210	0.005	0.414	
	Ddept	1	-0.334	-0.558	-0.110	
	Den	1	0.667	0.386	0.948	
	Ele	1	-0.729	-0.967	-0.492	
	Hei	1	-0.527	-0.795	-0.260	
	Marsh	1	0.261	0.019	0.503	
	OrgC	1	-0.316	-0.522	-0.110	
	Oxy	1	-0.303	-0.445	-0.162	
	PET	1	0.331	0.035	0.626	
	Phos	1	0.393	0.175	0.611	
	Pine	0.95	0.236	-0.002	0.498	
	OW	0.83	-0.125	-0.298	-0.005	
	SoftLin	0.78	-0.141	-0.380	0.020	
	HWet	0.1	0.011	-0.105	0.332	
	pH	0.1	-0.012	-0.354	0.116	
	R	0.09	0.008	-0.119	0.308	
	FFP	0.07	-0.015	-0.557	0.128	
	Ag	0.06	-0.008	-0.381	0.112	
	Nitro	0.05	-0.005	-0.313	0.089	
	Sali	0.05	-0.007	-0.435	0.147	
	Con	0.04	-0.006	-0.427	0.174	
	Swamp	0.04	0.003	-0.183	0.334	
	WhiteSpruce	0.04	-0.004	-0.341	0.157	

Table 4.3. Final averaged models across all confidence models ($\Delta AIC_c < 2$) to examine the relationship between chironomid genera abundance and environmental factors. Bolded text indicated the most important variables based on their importance weight. Refer to Table 3.3 for abbrevations.

	Environmental Factors	Relative Importance	Beta Coefficient	95% CI		D^2
				Lower	Upper	
Chironomus						0.077
	Con	1	-0.602	-1.183	-0.021	
	Ddept	1	-1.295	-1.712	-0.878	
	Den	1	0.666	0.161	1.171	
	GrassShrub	1	-0.555	-0.958	-0.152	
	Hei	1	-0.753	-1.197	-0.309	
	HWet	1	-2.039	-3.448	-0.631	
	Oxy	1	-2.999	-3.932	-2.065	
	Pine	1	1.033	0.589	1.477	
	Sali	1	0.670	0.000	1.339	
	LarchFen	0.96	-0.430	-0.814	-0.084	
	WhiteSpruce	0.96	-0.467	-0.865	-0.113	
	pH	0.87	0.295	0.010	0.670	
	R	0.74	0.223	-0.019	0.617	
	Ele	0.3	-0.088	-0.670	0.081	
	Temp	0.08	0.013	-0.105	0.449	
	OrgC	0.07	0.014	-0.206	0.605	
	OW	0.06	-0.010	-0.508	0.179	
	UrbInd	0.06	-0.017	-0.906	0.312	
	Deciduous	0.05	0.006	-0.195	0.431	
	Swamp	0.05	0.005	-0.201	0.398	
	BlackSpruce	0.04	-0.013	-0.652	0.054	
	PET	0.04	-0.006	-0.622	0.346	
	Phos	0.04	0.004	-0.204	0.373	
Dicrotendipes						0.034
	BogFen	1	-0.501	-0.859	-0.143	
	Ddept	1	-0.689	-1.113	-0.266	
	Ele	1	-1.147	-1.610	-0.685	
	GrassShrub	1	-0.594	-1.061	-0.126	
	HardLin	1	-0.395	-0.721	-0.069	
	HWet	1	-0.736	-1.220	-0.252	
	Sali	1	-0.688	-1.371	-0.006	
	For	0.95	-0.658	-1.393	0.008	
	BlackSpruce	0.79	-0.243	-0.646	0.028	
	Deciduous	0.17	0.045	-0.167	0.694	
	Oxy	0.14	-0.054	-0.744	-0.040	

	OrgC	0.08	-0.024	-0.800	0.242	
	Con	0.07	-0.046	-1.547	0.301	
	Mixedwood	0.05	-0.015	-0.755	0.189	
	Nitro	0.05	-0.050	-3.569	1.687	
	Pine	0.05	0.011	-0.180	0.591	
	LarchFen	0.04	-0.008	-0.726	0.317	
	Marsh	0.04	0.005	-0.382	0.677	
	PET	0.04	0.009	-0.314	0.761	
	Phos	0.04	0.006	-0.256	0.535	
	R	0.04	0.004	-0.206	0.425	
	Swamp	0.04	0.008	-0.344	0.737	
Endochironomus						0.020
	Ele	1	-0.820	-1.240	-0.399	
	HWet	1	0.346	0.013	0.679	
	Nitro	1	-14.560	-24.044	-5.075	
	Pine	1	0.485	0.060	0.911	
	Phos	0.93	0.212	-0.032	0.487	
	GrassShrub	0.83	0.500	-0.001	1.207	
	Marsh	0.66	-0.260	-0.749	-0.045	
	SoftLin	0.65	-0.274	-0.812	-0.027	
	Deciduous	0.13	0.025	-0.092	0.476	
	FFP	0.11	-0.026	-0.608	0.113	
	Mixedwood	0.1	0.034	-0.187	0.873	
	HardLin	0.06	-0.018	-0.723	0.093	
	WhiteSpruce	0.06	-0.013	-0.788	0.339	
	Sali	0.05	-0.003	-0.306	0.179	
	Swamp	0.05	-0.006	-0.526	0.297	
Glyptotendipes						0.048
	BogFen	1	-0.650	-1.079	-0.220	
	Ele	1	-1.531	-2.168	-0.893	
	LarchFen	1	-0.440	-0.715	-0.166	
	Mixedwood	1	0.762	0.036	1.489	
	PET	1	0.686	0.221	1.150	
	Phos	1	0.619	0.110	1.128	
	Pine	0.96	-0.447	-0.969	0.034	
	SoftLin	0.88	-0.350	-0.819	0.025	
	Swamp	0.17	-0.058	-0.717	0.050	
	Hei	0.15	0.035	-0.112	0.599	
	Den	0.11	0.027	-0.127	0.598	
	Oxy	0.09	-0.021	-0.522	0.038	
	HWet	0.06	-0.012	-0.558	0.175	
	R	0.06	0.015	-0.272	0.740	
	UrbInd	0.04	-0.008	-0.666	0.222	
	Ag	0.03	0.003	-0.288	0.538	
	BlackSpruce	0.03	-0.003	-0.337	0.155	

	Ddept	0.03	-0.004	-0.609	0.297	
	Deciduous	0.03	0.004	-0.252	0.518	
	FFP	0.03	0.006	-0.522	0.934	
	GrassShrub	0.03	0.003	-0.249	0.484	
	HardLin	0.03	0.004	-0.333	0.640	
	Nitro	0.03	-0.018	-3.861	2.748	
	OrgC	0.03	-0.007	-0.942	0.476	
	OW	0.03	-0.004	-0.428	0.175	
	WhiteSpruce	0.03	-0.007	-0.636	0.234	
Parachironomus						0.075
	BogFen	1	-0.670	-1.016	-0.325	
	Ele	1	-0.954	-1.365	-0.543	
	GrassShrub	1	0.403	0.035	0.771	
	HWet	1	-0.981	-1.428	-0.533	
	LarchFen	1	-0.566	-1.007	-0.125	
	Nitro	1	-16.846	-29.989	-3.704	
	Phos	1	0.479	0.111	0.848	
	R	1	0.403	0.062	0.743	
	SoftLin	1	-0.773	-1.213	-0.333	
	WhiteSpruce	1	-0.576	-0.993	-0.159	
	Ddept	0.93	-0.271	-0.540	-0.041	
	OW	0.92	-0.418	-0.865	-0.045	
	BlackSpruce	0.12	0.030	-0.161	0.654	
	Deciduous	0.09	-0.015	-0.532	0.177	
	Den	0.09	0.015	-0.187	0.526	
	Con	0.08	-0.020	-0.758	0.291	
	Mixedwood	0.08	0.016	-0.294	0.692	
	Swamp	0.08	-0.015	-0.661	0.281	
	Sali	0.07	0.006	-0.229	0.400	
	Temp	0.07	-0.004	-0.301	0.169	
Paratanytarsus						0.043
	Den	1	0.557	0.227	0.886	
	Ele	1	-1.264	-1.613	-0.914	
	HardLin	1	-0.407	-0.788	-0.026	
	HWet	1	-0.713	-1.080	-0.347	
	Marsh	1	0.518	0.076	0.960	
	Nitro	1	-8.930	-16.271	-1.589	
	PET	1	0.662	0.109	1.214	
	Phos	1	0.119	0.007	0.231	
	Temp	0.95	-0.163	-0.340	-0.003	
	BlackSpruce	0.65	0.122	-0.060	0.434	
	SoftLin	0.21	-0.040	-0.427	0.050	
	FFP	0.12	-0.035	-0.780	0.199	
	Ddept	0.09	-0.011	-0.390	0.147	
	Con	0.08	0.004	-0.122	0.237	

	Hei	0.06	-0.011	-0.521	0.136	
	LarchFen	0.05	-0.008	-0.550	0.202	
	Oxy	0.05	-0.004	-0.166	0.032	
	Sali	0.05	0.004	-0.114	0.273	
	Mixedwood	0.04	0.005	-0.228	0.470	
	OW	0.04	-0.008	-0.579	0.204	
	pH	0.04	-0.001	-0.208	0.125	
Tanytarsus						0.048
	BogFen	1	-0.483	-0.781	-0.184	
	Ddept	1	-0.875	-1.225	-0.524	
	Den	1	0.957	0.444	1.471	
	Ele	1	-1.298	-1.803	-0.792	
	HardLin	1	-0.541	-0.845	-0.237	
	Marsh	1	-0.765	-1.072	-0.459	
	Oxy	1	-0.778	-1.644	0.087	
	Pine	1	0.850	0.360	1.339	
	SoftLin	1	-0.321	-0.588	-0.053	
	Ag	0.96	0.340	-0.085	0.791	
	Hei	0.86	-0.310	-0.735	0.016	
	Sali	0.55	-0.224	-0.865	0.046	
	GrassShrub	0.19	-0.060	-0.692	0.056	
	pH	0.18	0.058	-0.128	0.767	
	Deciduous	0.11	0.018	-0.131	0.468	
	FFP	0.1	0.029	-0.207	0.781	
	Con	0.05	-0.016	-0.815	0.159	
	PET	0.05	0.012	-0.284	0.785	
	HWet	0.04	-0.006	-0.486	0.198	
	R	0.04	-0.003	-0.368	0.208	
	Temp	0.04	-0.006	-0.599	0.291	
	UrbInd	0.04	0.003	-0.196	0.358	
Acricotopus						0.073
	Ddept	1	-2.156	-3.396	-0.916	
	LarchFen	1	-0.436	-0.795	-0.076	
	pH	1	0.565	-0.026	1.156	
	Pine	1	-3.115	-5.280	-0.949	
	R	1	-0.614	-1.344	0.116	
	UrbInd	1	0.498	-0.247	1.243	
	GrassShrub	0.97	0.384	-0.050	0.840	
	SoftLin	0.97	-0.456	-0.926	-0.018	
	HWet	0.95	-0.582	-1.225	0.000	
	Con	0.93	-0.828	-1.826	0.038	
	For	0.43	-0.613	-7.491	4.619	
	Temp	0.1	0.020	-0.198	0.599	
	Deciduous	0.09	0.016	-0.174	0.512	
	Ele	0.08	-0.013	-0.534	0.195	

	OrgC	0.08	-0.034	-1.147	0.345	
	WhiteSpruce	0.07	-0.022	-1.207	0.539	
	Sali	0.06	-0.015	-1.164	0.628	
	Swamp	0.06	-0.011	-0.738	0.376	
	Hei	0.04	-0.006	-0.470	0.167	
	HardLin	0.03	0.015	-0.116	1.176	
	Marsh	0.03	-0.003	-0.479	0.260	
	Oxy	0.03	-0.006	-0.854	0.408	
	PET	0.03	0.005	-0.286	0.581	
Corynoneura						0.031
	BogFen	1	-0.401	-0.655	-0.147	
	Con	1	-1.106	-2.049	-0.163	
	Ele	1	-0.976	-1.342	-0.610	
	For	1	0.362	0.057	0.666	
	GrassShrub	1	0.498	0.093	0.902	
	HWet	1	-0.558	-0.922	-0.194	
	LarchFen	1	-0.589	-0.899	-0.279	
	Phos	1	0.497	0.105	0.890	
	Pine	1	-0.431	-0.769	-0.094	
	OrgC	0.35	0.164	-0.134	1.076	
	SoftLin	0.15	-0.028	-0.467	0.087	
	Ddept	0.13	-0.025	-0.502	0.113	
	Nitro	0.11	-0.146	-4.640	1.979	
	Hei	0.09	-0.019	-0.585	0.159	
	Ag	0.08	0.015	-0.318	0.674	
	Oxy	0.06	-0.021	-0.814	0.130	
	R	0.05	0.005	-0.188	0.427	
	Swamp	0.05	-0.011	-0.604	0.188	
	Den	0.04	0.011	-0.156	0.722	
	Marsh	0.04	0.005	-0.229	0.480	
	WhiteSpruce	0.04	-0.006	-0.509	0.216	
Cricotopus						0.031
	BlackSpruce	1	0.275	0.027	0.523	
	Den	1	0.903	0.525	1.280	
	Ele	1	-0.634	-0.921	-0.347	
	Hei	1	-0.461	-0.830	-0.093	
	PET	1	0.609	0.284	0.933	
	Phos	1	0.401	0.016	0.786	
	SoftLin	1	-0.386	-0.589	-0.184	
	OrgC	0.96	-0.267	-0.530	-0.029	
	HardLin	0.93	0.303	-0.047	0.696	
	Mixedwood	0.92	-0.300	-0.662	0.012	
	Pine	0.1	0.025	-0.101	0.607	
	Oxy	0.07	-0.012	-0.392	0.042	
	BogFen	0.06	-0.009	-0.403	0.108	

	FFP	0.06	-0.014	-0.722	0.231	
	GrassShrub	0.06	-0.008	-0.441	0.139	
	Deciduous	0.05	0.006	-0.170	0.406	
	R	0.05	0.004	-0.139	0.323	
	Con	0.04	-0.004	-0.449	0.250	
	Ddept	0.04	0.004	-0.295	0.497	
	Marsh	0.04	0.003	-0.253	0.423	
	OW	0.04	-0.002	-0.219	0.109	
	Sali	0.04	-0.004	-0.447	0.234	
	Swamp	0.04	-0.003	-0.424	0.251	
	UrbInd	0.04	0.003	-0.207	0.352	
Nanocladius						0.028
	BlackSpruce	1	0.513	-0.015	1.041	
	Deciduous	1	0.970	0.256	1.684	
	Phos	1	0.731	0.103	1.360	
	FFP	0.98	-0.837	-1.646	-0.063	
	Marsh	0.93	-0.704	-1.451	-0.057	
	BogFen	0.84	-0.564	-1.377	0.029	
	Con	0.59	-0.574	-2.269	0.331	
	HWet	0.17	0.086	-0.339	1.323	
	R	0.1	0.041	-0.206	1.063	
	WhiteSpruce	0.07	-0.031	-1.242	0.349	
	HardLin	0.05	-0.017	-1.123	0.381	
	Hei	0.05	-0.016	-0.941	0.255	
	GrassShrub	0.04	0.018	-0.483	1.275	
	OW	0.04	0.005	-0.241	0.492	
	PET	0.04	0.017	-0.468	1.229	
	pH	0.04	0.010	-0.381	0.862	
	Swamp	0.04	-0.007	-0.688	0.295	
	UrbInd	0.04	-0.016	-1.482	0.616	
	Mixedwood	0.03	-0.008	-0.975	0.516	
	Ag	0.02	0.005	-0.608	1.177	
	LarchFen	0.02	-0.004	-0.828	0.406	
	OrgC	0.02	0.010	-0.728	1.600	
	Oxy	0.02	-0.007	-0.940	0.234	
	Pine	0.02	-0.002	-0.514	0.311	
	Sali	0.02	0.009	-0.955	1.932	
Psectrocladius						0.026
	Den	1	0.834	0.417	1.252	
	Ele	1	-0.734	-1.060	-0.408	
	OW	1	-0.913	-1.329	-0.497	
	Oxy	1	-0.393	-0.635	-0.152	
	Hei	0.97	-0.380	-0.774	-0.012	
	HWet	0.89	0.250	-0.041	0.603	

	Marsh	0.87	0.232	-0.060	0.593	
	pH	0.64	-0.198	-0.617	-0.002	
	Sali	0.59	0.188	-0.066	0.704	
	WhiteSpruce	0.19	-0.045	-0.546	0.062	
	Nitro	0.11	-0.032	-0.598	-0.006	
	Ddept	0.1	-0.024	-0.582	0.109	
	BogFen	0.07	-0.006	-0.348	0.170	
	LarchFen	0.07	0.010	-0.171	0.459	
	BlackSpruce	0.05	0.008	-0.144	0.460	
	For	0.03	0.003	-0.184	0.350	
	PET	0.03	0.003	-0.293	0.476	
Ablabesmyia						0.040
	BlackSpruce	1	0.419	0.052	0.787	
	Den	1	1.180	0.686	1.674	
	Ele	1	-0.413	-0.765	-0.061	
	FFP	1	0.662	0.228	1.096	
	GrassShrub	1	-0.529	-0.896	-0.161	
	Hei	1	-0.525	-0.939	-0.112	
	HWet	1	-0.472	-0.773	-0.172	
	Marsh	1	0.516	0.136	0.896	
	OW	1	-0.393	-0.685	-0.100	
	Ddept	0.91	-0.132	-0.324	0.034	
	Nitro	0.91	-4.290	-10.843	1.376	
	pH	0.78	-0.131	-0.441	0.106	
	Deciduous	0.12	0.020	-0.133	0.464	
	Oxy	0.11	-0.010	-0.228	0.032	
	PET	0.1	0.035	-0.295	1.020	
	Temp	0.08	-0.005	-0.220	0.092	
	Phos	0.06	0.005	-0.081	0.246	
	R	0.05	-0.008	-0.509	0.175	
	Ag	0.04	-0.008	-0.626	0.270	
	HardLin	0.04	0.005	-0.266	0.500	
	OrgC	0.04	0.001	-0.102	0.172	
	SoftLin	0.04	0.005	-0.208	0.452	
	UrbInd	0.04	-0.004	-0.382	0.197	

Chapter 5: Synthesis and General Discussion

5.1 Research Summary

The overall goal of this thesis was to explore the diversity of chironomids in Albertan wetlands, assess their responses to particular environmental factors, and evaluate their utility as biomonitoring tools. I recorded a total of 40 genera of chironomids in 4 subfamilies from 270 wetlands sampled by the Alberta Biodiversity Monitoring Institute. Thirteen of these genera occurred in >20% of the surveyed wetlands, with *Cricotopus* (80% occurrence) and *Psectrocladius* (71% occurrence) being the most ubiquitous taxa. In Chapter 2, I created a morphological atlas of all the chironomid genera identified, which includes a glossary describing critical features of chironomids and a description of morphological and ecological features of each genus. As far as I am aware, this is the first chironomid survey at the provincial scale in Alberta, so it will provide baseline information and a good taxonomic reference for future chironomid studies.

In Chapter 3, I studied how entire chironomid assemblages were correlated to environment conditions. At different spatial scales, the relative contributions of environment effect and spatial effect to the variance in chironomid assemblages were different. As expected, environmental factors become more important than space with decreasing spatial extent. However, the total variance in chironomid assemblage structure that could be explained in both provincial and regional analyses was low (all less than 13%). At the provincial scale, the significant environmental factors identified to explain chironomid assemblages were two human-footprint variables (agriculture and human

created water bodies), three water-quality variables (phosphorus, conductivity and salinity), one surrounding-vegetation variable (larch-dominated fen) and three other general habitat variables (elevation, maximum water depth and open water area). Within the Canadian Shield and Boreal ecoregion (CB), one human footprint variable (hard linear features), two water quality variables (salinity and phosphorus), one climate variable (frost-free period) and two other general habitat variables (elevation and maximum water depth) were identified as significant variables. Within the Parkland and Grassland ecoregion (PG), elevation, dissolved oxygen level and maximum water depth were selected as significant variables. In the Rocky Mountain and Foothill ecoregion (RF), no environmental variables were identified as significant in explaining chironomid assemblages.

In Chapter 4, I assessed the statistical responses of chironomid diversity (Shannon index), chironomid richness, total chironomid abundance and 13 common chironomid genera to environmental conditions. I found that all the chironomid responses, except for that of the Shannon index at 33%, were poorly explained (less than 8 % variation) by the measured environmental variables. Generally, each response variable was significantly associated with a combination of 6 to 13 environmental variables. The most universally significant environmental variables were those describing surrounding vegetation and human footprint. Surrounding vegetation variables occurred in every model and human footprint variables were only absent from the *Psectrocladius* abundance model. In addition, elevation (included in 12 out of 16 response models), aquatic vegetation (9 of 16), phosphorus (7 of 16), and maximum water depth (7 of 16) were also quite common

in final models. Other variables (e.g., nitrogen, oxygen, temperature) were significantly associated with only a few (fewer than five) responses each.

5.2 Relevance to Wetland Biomonitoring and Future Research

A good understanding of the effect of environmental factors on biological responses (e.g., species richness) is usually the first step in developing a proper biomonitoring strategy (Barbour et al. 1999, Reece and Richardson 1999). In this study, I assessed the relationship between various aspects of chironomid community and environmental factors. However, I was unable to develop useful chironomid-based indices for wetland biomonitoring in Alberta due to the relative weak correlations of chironomid taxa with the assessed environmental variables.

First, the weak correlation between chironomids and environmental variables could be due to the lacking of other important environmental variables. Several studies have found the presence or absence of fish to be very important in determining invertebrate abundance and composition (e.g., Zimmer et al. 2001, Tangen et al. 2003, Tarr et al. 2005). So biological interaction (e.g., predation) could play a more important role in controlling macroinvertebrate community than abiotic factors. Absence of fish information in the ABMI database prevents me from testing the effect of fish. Although other invertebrate groups were sampled by ABMI, I did not summarize them and include those potential predators in the analysis in this study. In addition, the 250 m buffer zone may not have been large enough to capture landscape effects on chironomids. The landscape scale to which an organism responds appears to depend on both disturbance types and organism types (Brazner et al. 2007). For example, agricultures often affect the

whole watershed while urbanization type disturbances appear to be more important in influencing an adjacent water body. Organisms that are large-bodied and/or good dispersers tend to respond to the environment across larger spatial scales. Although larval chironomids are restricted in aquatic environment, the adult chironomids are terrestrial fliers thus could disperse actively on the landscape (Delettre and Morvan 2000). The adult dispersal is mediated by the distance to other water bodies, landscape heterogeneity and landscape openness. So in future studies, the effect of predators, and the effect of surrounding vegetation and human footprint at different spatial scales could be tested. Besides, the connectivity (e.g., distance) of the wetland to other water bodies could also be added in the analysis.

Second, the way that ABMI sampled and characterized some of the environmental variables may have compromised the explanatory power in my analysis. For example, ABMI sampled the water quality, aquatic vegetation and macroinvertebrates at different locations in the wetland. However, a single water body could have many different microhabitats that differ in both environment conditions and biological community (Barbour et al. 1999, Liston et al. 2008). In addition, ABMI grouped the human footprint into six types, which might be too coarse to capture the variation in effect. For example, the effect of croplands seeded to canola could be very different from croplands seeded to clover. However, they were all categorized as agriculture type. So in future studies, improving the sampling design and using finer classification of human footprint would likely to improve the variation explained.

Third, identifying chironomids only to the genus level may have masked responses detectable at finer taxonomic levels. Different views exist on what level of taxonomic

resolution is sufficient for accurate biomonitoring (e.g., Bowman and Bailey 1997, Lenat and Resh 2001, Defeo and Lercari 2004, Huggins and Kriz 2005). In many stream biomonitoring programs (Barbour et al. 1999), keeping chironomids at above-species-level seemed to be a common practice and it has a minimum effect on the overall monitoring result. Rabeni and Wang (2001) even found the exclusion of chironomid data from the macroinvertebrate had no negative effect on the ability to detect stream impairment. This is probably because streams have many other sensitive groups of macroinvertebrates such as EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa. However, wetland monitoring could be quite different from stream monitoring as wetlands do not have many EPT taxa or other macroinvertebrate groups that have been documented as particularly responsive to human disturbances (King and Richardson 2002). Chironomids still hold a great potential for wetland monitoring if we could identify chironomids to the species. In a wetland study, King and Richardson (2003) failed to detect a stressor-response relationship for the *Tanytarsus* at the genus level, yet at the species level, four of the six species they analyzed were strongly associated with either the most disturbed sites or intact sites. In addition, many paleolimnology studies have shown that morphospecies-level chironomids respond to environment strongly thus robust models have been developed to reconstruct past environment using chironomid remains from lakes (Hofmann 1988, Porinchu et al. 2002, Barber et al. 2013). In my study, many of my identified chironomid genera are quite diverse. The most commonly occurring genus, *Cricotopus*, was represented by more than ten morphospecies in my samples. So identifying chironomids to species or morphospecies should improve the sensitivity of chironomids to environmental signals. But the lack of species descriptions

and species-level keys focused on larvae makes it difficult to identify chironomids collected as part of the usual macroinvertebrate sampling procedures to species/morphospecies. More taxonomic research on chironomids is needed to develop local species checklist and local keys to species based on larvae.

Fourth, stochastic processes of recruitment, dispersal, local extinction etc. may play a more important role in controlling the macroinvertebrate distribution in wetlands than in flowing waters (Davis et al. 2006). After reviewing wetland invertebrate studies from 14 areas of North America, Batzer (2013) concluded that wetland invertebrate assemblages are extremely hard to predict and generalizations are not evident. In my chironomid assemblage analysis for RF region (Rocky Mountain and Foothill ecoregion), neither environmental nor spatial effects were observed, which supports the idea that wetland invertebrates are more tolerant/resilient to environmental variations compared to stream invertebrates, so wetland invertebrates may only respond strongly to extreme environmental changes (e.g., dramatic shifts in vegetation, extreme flooding or drying).

My study involves a broad range of wetlands using descriptive approach (i.e. nothing is manipulated). One major drawback is that too many variables are examined at the same time and those variables could be very dynamic and interactive, which makes it difficult to draw conclusions. In future studies, manipulated field experiments (e.g., changing water levels, Wrubleski 2005) could also be performed to better understand the role different environmental factors play in influencing chironomids and other invertebrates in Albertan wetlands.

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Supplementary Thesis Materials

The following supporting materials have been deposited in the University of Alberta's institutional repository: Education and Research Archive (ERA), and are accessible through the link: https://era.library.ualberta.ca/files/1j92gb13w

Appendix 1: Spearman's rank correlation matrix for all measured environmental variables (separate electronic files in Excel format).

Appendix 2: Table of all identified chironomid genera with their true abundance (# of individuals at each site). The true abundance was estimated from the abundance of subsamples as described in Chapter 3 (separate electronic files in Excel format).

Appendix 3: Complete table of all measured environmental data for this study (separate electronic files in Excel format).

Appendix 4: Maps of chironomids in Alberta wetlands including the total generic richness, total abundance and abundance of the 13 common genera (separate electronic files in jpg format in a single folder).