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Morphological and Molecular Insights into the Biodiversity of Gastrointestinal Parasites from Canadian Grizzly (Ursus arctos horribilis) and Black Bears (Ursus americanu

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Morphological and Molecular Insights into the Biodiversity of Gastrointestinal Parasites from Canadian Grizzly (*Ursus arctos horribilis*) and Black Bears (*Ursus americanus*)

by

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Abstract

Classical and molecular parasitology are powerful tools for clinical diagnostics, for disease transmission surveys, and for designing strategies to control infections and outbreaks. Parasite communities have been demonstrated to strongly affect host population dynamics and viability. The absence of baseline data, and the potential detrimental effects on host health, supported the investigation of the gastrointestinal parasite fauna of free-living grizzlies (Ursus arctos horribilis) and black bears (Ursus americanus) from Alberta and British Columbia, Canada.

The study provided new insights into parasite biodiversity and infection patterns in Canadian bears. For the first time, the cestode species Diphyllobothrium dendtriticum, Diphyllobothrium nihonkaiense, and Taenia arctos have been unequivocally identified in North American bears. The present research also elucidated the systematics of the ursine hookworm species Uncinaria rauschi and Uncinaria yukonensis, determining their place within the family Ancylostomatidae.

Preface

Chapter Four is a modified version of the manuscript accepted for publication in the peer reviewed scientific journal Parasitology International. Text, associated tables and references have been formatted to follow thesis guidelines. As lead author, Stefano Catalano conducted the research with guidance from his committee, wrote the manuscript, and integrated comments and critique from coauthors and reviewers. The complete citation for this article is:

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To my Mom and Dad

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€And though there€s a new life line
I won€t forget the one I left behind
Jim James

CHAPTER ONE: INTRODUCTION

1.1 Bears of North America: History, Biology, and Ecology

Ursus americanus (American black bear), *Ursus arctos horribilis* (grizzly bear), and *Ursus maritimus* (polar bear) are mammals of the family Ursidae, order Carnivora. The grizzly bear, together with the Kodiak bear, *Ursus arctos middendorffi* of the Kodiak Islands (Alaska, USA), are North American subspecies of the brown bear *Ursus arctos* (Rausch 1963). Polar bears are considered marine mammals because of their high degree of specialization for life on the sea ice surface rather than on the adjacent land masses. This species inhabits Canada at its northern latitudes (Alberta, Newfoundland, Labrador, Nunavut, Northwest Territories, Quebec, Yukon, Ontario), United States (Alaska), Russia, Greenland/Denmark, Norway, Svalbard and Jan Mayen (Stirling and Derocher 1993; Amstrup et al. 2000).

Bears are slow-growing, large-bodied mammals with species-specific foraging behaviour. The black bear is the least carnivorous and the most vegetarian of the North American carnivores (Pelton 2003). In contrast, the polar bear is primarily carnivorous and predatory, with seals as their main prey (Amstrup 2003). The diet of the grizzly bear falls in between black and polar bears, being more carnivorous where ungulates (especially in the Arctic) or spawning salmons (coastal areas) are abundant (Sacco and Van Valkenburgh 2004; Mowat and Heard 2006).

Within the order Carnivora, the evolution of the family Ursidae started > 25 mya (million years ago) around the Oligocene-Miocene boundary. In this epoch, ursids first diverged from early canids, then from mustelids and pinnipeds (Davis et al. 2004; Hunt and Flynn 2005; Higdon et al. 2007). Members of the genus *Ursus* became a monophyletic group, believed to descend from the common ancestor *Ursus minimus*, a small forest-dwelling bear of the Pliocene (Kurtén and Anderson 1980). In Eurasia, approximately 2.8 mya *U. minimus* gave rise to *Ursus etruscus*, precursor of two distinct evolutionary lineages: the brown bear *U. arctos*, and the extinct cave bear *Ursus spelaeus* (Krause et al. 2008). The ancestors of the modern North American bears entered the Nearctic from Eurasia about 70-100 ka (kiloannum) by crossing the Bering Land Bridge (Kurtén and Anderson 1980). Kurtén and Anderson (1980) suggested that two independent migrations may have potentially occurred, allowing the speciation of *U. a. horribilis* and *U. a. middendorffi*. In contrast, black bears are evolutionarily distant from brown bears; genetic studies revealed that they split from a common ancestor 5.05 mya (Krause et al. 2008). The divergence of the black

bear precursors branched from the primitive *Ursus minimus*, or from its close relative *Ursus abstrusus*, approximately 4.6 mya. Later, approximately 4.1 mya the American and the Asiatic black bear (*Ursus thibetanus*) speciated, branching out from a common ancestor (Shields and Kocher 1991; Krause et al. 2008). Fossil evidence indicates that black bears have been present in North America for at least 2.4 mya (Kurtén and Anderson 1980). The youngest North American species of the family Ursidae is the polar bear, branching off the brown bear lineage. Surprisingly, recent studies suggested a much more ancient origin than the one estimated by palaeontologists. Although the oldest known polar bear fossils date to less than 130 ka (Kurtén and Anderson 1980; Davison et al. 2011), genetic data placed the speciation of polar bears at approximately 600–338 ka (Krause et al. 2008; Hailer et al. 2012).

1.1.1 American Black Bear

The American black bear is, by far, the most abundant, harvested, and studied bear (Garshelis and Hristienko 2006). Description of the species, and details about its biology, ecology, and physiology are reviewed by Pelton (2003). Black bears live at elevations ranging from the sea level to 3500m, inhabiting areas as diverse as dry Mexican scrub forests, Louisiana swamps, Alaskan rainforests, and Labrador tundra. Despite the loss of approximately 48% of its historical range (Pelton and Van Manen 1994), *Ursus americanus* still ranges through all the Canadian provinces and territories (extirpated only from Prince Edward Island in 1937), 41 states of the United States (with occasional sightings in at least three others), and through the Sierra Madre Occidental and the Sierra Madre Oriental of northern Mexico (Garsheliset al. 2008). Its status and density are variable along its range of distribution. The species is present with stable and abundant populations in northwestern United States and some Canadian provinces (British Columbia, Alberta, and Ontario). Conversely, the southeastern states of the USA are inhabited by small and isolated populations such as the Louisiana black bear, *Ursus americanus luteolus*, with concern about their viability and conservation (Pelton 2003). The American black bear lives in sympatry with the grizzly bear through much of its geographical distribution. However, the overlap between the two species is limited due to competitive exclusion by grizzly bears and to ecological and behavioural differences (Mowat et al. 2005; Belant et al. 2006).

The black bear is listed in the Appendix II, of the Convention on the International Trade of Endangered Species, but its main threats come from habitat destruction and human induced mortality caused by hunting, poaching, and road kills (Beckmann and Berger 2003; Pelton 2003). The species is classified as least concern, by the International Union for

Conservation of Nature (IUCN 2008), and as Not at risk, by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 1999).

1.1.2 Grizzly Bear

The grizzly bear is considered an iconic species in all the ecosystems it inhabits, including the Central Rockies Ecosystem (Simberloff 1999; Garshelis et al. 2005). The description of the species, and details about its biology, ecology, and physiology were reviewed by Schwartz and colleagues (2003). In the past century grizzly bears underwent a substantial decline throughout much of North America. In Canada, the prairie populations were designated extirpated in 1991, whereas currently there are small isolated populations in the southern portion of Alberta and British Columbia. In the rest of North America, grizzly bears now occupy only 2% of their historical range (McLellan 1998; McLellan and Banci 1999; Mattson and Merril 2002). Nowadays, the grizzly bear is present in Canada (Yukon, Northwest Territories, Nunavut, Alberta, and British Columbia) and northwestern USA (Alaska, Washington, Idaho, Montana, Wyoming). Its current range is described as one continuous metapopulation, with the exception of some isolated groups in the southern portion of British Columbia (Craighead and Vyse 1996; Proctor et al. 2004). The distribution range of the grizzly bear overlaps with that of the American black bear, and partially also with the polar bear (Burek et al. 2008).

The grizzly bear is listed in the Appendix II, of the Convention on the International Trade of Endangered Species. It is also listed as Special concern, under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2012), and, in Alberta, may be at risk, according to the Canadian Endangered Species Conservation Council (2006), although the status of Threatened, has been suggested (Festa-Bianchet 2010). Grizzly bear management and harvesting are under the federal legislation via the Canada Wildlife Act (1985) and the Canada National Parks Act (2000). In the United States (except Alaska), since 1975 the grizzly bear has been declared Threatened,, coming under the protection of the Endangered Species Act (US Fish and Wildlife Service 2009). Approximately 90% of all recorded grizzly bear mortalities are attributed to hunting, management removal, and road/rail kills (Schwartz et al. 2003). The lack of resilience towards humans is shown both by mortality rates significantly linked to contact with humans (Beckmann and Berger 2003; Nielsen et al. 2006), and by reproductive rates and body condition scores at the lowest extreme in individuals from the Canadian Rocky Mountain Parks (complex composed by the contiguous

national parks of Banff, Jasper, Kootenay, and Yoho), probably the most developed landscape where the grizzly still survives (Garshelis et al. 2005; Bertch and Gibeau 2010).

1.2 Parasites and Bears in North America

Limited research has been conducted on the helminth fauna of grizzly bears, whereas there are several parasitological studies on black bears. In the past, Rogers and Rogers (1976) summarized the available information on parasitic infection in ursids. This study reviews any published surveys on the gastrointestinal parasites of North American wild bears since 1977 (Table 1.1).

In summary, data on the gastrointestinal parasite fauna of wild American black bears were provided for populations from Alberta (Hair and Mahrt 1970; Dies 1979), New Brunswick (Duffy et al. 1994), Ontario (Sprent 1950, 1951; Addison et al. 1978), Quebec (Frechette and Rau 1977; Juniper 1978), Yukon (Wolfgang 1956), Northwest Territories (Kutz et al. 2003a, 2003b, 2004a), Alaska (Rausch 1961; Olsen 1968; Rausch and Hilliard 1970), Minnesota (Vergeer 1938; Rogers 1975), Montana (Jonkel and Cowan 1971; Worley et al. 1976), southeastern USA (Crum et al. 1978; Conti et al. 1983; Foster et al. 2004), Washington (Poelker and Hartwell 1973), Wisconsin (Manville 1978), and Wyoming (Skinker 1931; Rush 1932; Scott 1932). Regarding North American brown bears, *U. a. horribilis* and *U. a. middendorffi*, information was reported for bears from Yukon (Choquette et al. 1969), Northwest Territories (Choquette et al. 1969; Gau et al. 1999), Alaska (Rausch 1954; Olsen 1968; Rausch and Hilliard 1970), and Montana (Worley et al. 1976; Seesee and Worley 1986). If baseline data on helminths of grizzlies and Kodiak bears are scarce, even less information is available on the parasites harboured by polar bears. With the exception of *Trichinella* sp. (Rausch 1970; Dick and Belosevic 1978; Larsen and Kapel 1983; Kapel 2000; Forbes 2000; Appleyard and Gajadhar 2000) and rarely detected microparasites (Taylor et al. 1991; Garner et al. 2000; Tryland et al. 2001), polar bear populations seem free from any macroparasite. Polar bears, due to their life-style, should be exposed to pathogens from both the terrestrial and the marine environment. Moreover, climate warming seems to be reducing the time polar bears can spend on the pack ice; it had forced them to alter their foraging behaviour, obliging them to scavenge or to eat the digestive tract and other tissues of the prey, making them more prone to encounter pathogens. As the climate warms it is possible that new pathogens may expand their ranges northward, with potential infection of naive populations of polar bears (Harvell et al. 2002; Derocher et al. 2004).

Table 1.1: Recent surveys on macroparasites of the digestive tract of wild bears in North America€•

Bear species	Parasite species	Location	Reference
Black bear	U. yukonensis(3), B. transfuga(3), D. ursi (3), T. hydatigena(3), T. krabbei(3)	Quebec, Canada	Frechette and Rau (1977)
Black bear	P. rara (2), A. americana(3), B. transfuga(3), T. krabbei(3)	Central Ontario, Canada	Addisonet al. (1978)
Black bear	G. pulchrum(1), C. putorii (2), Gnathostomasp. (2), A. caninum(3), B. transfuga(3), M. ingens(3), M. barbatus(3), P. lotoris (3), Strongyloidessp. (3)	Southeastern USA	Crum et al. (1978)
Black bear	D. ursi (3), ascarids (3), ancylostomatids (3)	Quebec, Canada	Juniper (1978)
Black bear	B. transfuga(3)	Northern Wisconsin, USA	Manville (1978)
Black bear	B. transfuga(3), T. hydatigena(3), T. krabbei(3)	Northwestern Alberta, Canada	Dies (1979)
Black bear	G. pulchrum(1), C. putorii (2), Gnathostomasp. (2), Physalopterasp. (2),L. sprenti(3), M. ingens(3), M. barbatus(3), P. procyonis(3), P. lotoris (3), Strongyloidessp. (3),T. leonina(3)	Florida, USA	Conti et al. (1983)
Black bear	G. pulchrum(1), C. putorii (2), Gnathostomasp. (2), Physalopterasp. (2),H. americana(3), L. sprenti(3), M. ingens(3), M. barbatus(3), P. procyonis(3), P. lotoris (3), Strongyloidessp. (3),T. leonina(3)	Florida, USA	Forrester (1992)
Black bear	B. transfuga(3), T. krabbei(3)	New Brunswick, Canada	Duffy et al. (1994)
Black bear	B. transfuga(3), U. rauschi(3), cestodes (3), trematodes (3)	Northwest Territories, Canada	Kutz (2003a, 2003b, 2004a)
Black bear	G. pulchrum(1), C. putorii (2), A. marcianae(3), A. caninum(3), A. tubaeforme(3), B. transfuga(3), B. virginianum(3), M. ingens(3), M. barbatus(3), Strongyloidessp. (3)	Florida, USA	Foster et al. (2004)
Grizzly bear	T. krabbei(3)	Montana, USA	Seesee and Worley (1986)
Grizzly bear	Diphyllobothriumsp. (3),Baylisascarissp. (3), Nematodirussp. (3),Uncinaria sp. (3)	Northwest Territories, Canada	Gau et al. (1999)
Polar bear	-	-	-

€ Numbers in parentheses indicate the niche within the host: (1) oesophagus; (2) stomach; (3) intestine.

• Complete names of the parasite species reported: Ancylostoma americana, Alaria marcianae, Ancylostoma caninum, Ancylostoma tubaeforme, Baylisascaris transfuga, Brachylaima virginianum, Capillaria putorii, Diphyllobothrium ursi, Gongylonema pulchrum, Heterobilharzia americana, Lagochilascaris sprenti, Macracanthorhynchus ingens, Molineus barbatus, Pharyngostomoides procyonis, Physaloptera rara, Placoconus lotoris (Arctocephalus lotoris), Taenia hydatigena, Taenia krabbei, Toxascaris leonina, Uncinaria rauschi, Uncinaria yukonensis

* Study conducted on faecal examinations.

Features and significance of the most common ursine parasites are discussed below.

ðSAscarids or roundworms (Nematoda: Ascaridiidae) are parasites of great medical and veterinary significance (O,Lorcain and Holland 2000). Baylisascarisspp., because of their zoonotic potential, are implicated in serious cases of human larva migrans (Gavin et al. 2005). B. transfugais the most common roundworm of bears, reported to cause larva migrans syndromes in several mammal species (Papini et al. 1993, 1996; Sato et al. 2004, 2005). In bears, the effects of B. tranfugahave not been studied in

depth, but a massive infection was described as the cause of death of a captive bear, probably because of intestinal obstruction (Mozgovoi 1953).

- Hookworms (Nematoda: Ancylostomatidae) are blood-sucking nematodes of humans, wild and domestic mammals. Species of the genus *Uncinaria*, as members of the family, develop as adults in the small intestine of the host, where they fasten causing anaemia, haemorrhagic enteritis, and hypoproteinemia (Hotez et al. 2004; Lyons et al. 2005). Infection patterns are definitely related to host-parasite species involved. In humans, the transmission route is primarily percutaneous; the age-intensity profile of hookworm infections is considered to increase with the age, with either a peak or a plateau in adulthood (Hotez et al. 2004). In canids, a large proportion of infections are acquired after birth via milk-borne larvae transmitted from the mother to the offspring during nursing, with peracute/acute disease from blood loss (Prociv 1998; Anderson 2000). Transmammary transmission is the main route in otariid pinnipeds, such as northern fur seals (*Callorhinus ursinus*) and New Zealand sea lions (*Phocarctos hookeri*). Adult hookworms persist in the intestine of the pup for maximum four months after birth (Lyons et al. 2003; Castinel et al. 2007a; Lyons et al. 2011a) causing mortality rates up to 61% for northern fur seals (Lucas 1899), and 13% for New Zealand sea lions (Castinel et al. 2007b). Death usually results from acute anaemia and occasionally from penetration of the intestinal wall and consequent peritonitis (Spraker et al. 2004, 2007; Lyons et al. 2011b). In bear species our knowledge of hookworms is limited to the taxonomic description of *U. yukonensis* (Wolfgang 1956), discovered in black bears from Yukon, and *U. krauschi* (Olsen 1968), from Alaskan black and grizzly bears.
- Cyclophyllidean tapeworms of the genus *Taenia* (Cestoda: Taeniidae) are parasites of terrestrial mammals, characteristically occurring as adult stages in carnivore definitive hosts, and as cystic metacestode stages (cysticercus, coenurus) in their prey. Ursids act as definitive hosts for several species of *Taenia*, with *T. krabbei* as the most reported one. However, studies conducted in Finland and Alaska (Haukisalmi et al. 2011; Lavikainen et al. 2011) have raised doubts about the potential misidentification of taeniid species in bears after the description of *T. arctos* from brown bears (definitive hosts) and moose/elk (intermediate hosts). In general, the low prevalence of *Taenia* spp. infections might reflect the feeding habits of bears, as well as the presence of additional potential definitive hosts (Jones and Pybus 2001).

ðS Pseudophyllidean tapeworms of the genus Diphyllobothrium (Cestoda: Diphyllobotriidae) reside as adults in the small intestine of mammals and birds feeding upon fish. Species of Diphyllobothrium use copepod crustaceans as first intermediate hosts, and marine and freshwater fish as second intermediate hosts (Rausch 1954; Scholz et al. 2009). The occurrence of Diphyllobothrium spp. in bears has been reported several times. Rausch (1955) described a fatal case after the experimental infection of a young black bear with an unidentified Diphyllobothrium species, probably D. ursi. Adult and intermediate stages of the provisionally named D. ursi were first described respectively from Kodiak bears and sockeye salmon from the Gulf of Alaska (Rausch 1954). Recently, Yamasaki et al. (2012) confirmed D. ursi as a valid, distinct species based on morphological traits and molecular markers.

1.3 Rationale of the Study

The research team composed by members of the Faculty of Veterinary Medicine, University of Calgary, and the Canadian Cooperative Wildlife Health Centre, Alberta Node, isolated unknown hookworm morphotypes parasitizing both grizzly and black bears from Banff National Park, Alberta. This preliminary finding raised questions about the identity and the significance of hookworms and other parasites in ursid hosts. The limited research conducted on parasites of bears in western Canada supported the need to further assess parasitological status of Ursus spp. in Alberta and British Columbia, and to better understand parasitic infections in ursid hosts. Uncinaria spp. infections are a significant cause of neonatal mortality for several species of the order Carnivora, but might go undetected in bears because of their denning behaviour, essential to cub early survival and maturation. The aetiology of natural mortality among cubs is still largely undetermined, and there is a general lack of insight on the role of parasitic infections in bears. Individual body condition scores and reproductive rates of Albertan bears are at the lowest for the species probably because of poor habitat quality and anthropogenic disturbance (Garshelis et al. 2005). The individual condition is of concern because body fat strongly affects reproduction in both black and grizzly bears. Females may forgo mating seasons because of the energetic stress, with persistent deferrals leading to a declining population trend (Hilderbrand et al. 1999). In such a scenario, parasitism may be determinant for host population dynamics. Parasites may exacerbate malnutrition and stress trade-offs in resource allocation with consequent negative effects on individual reproductive physiology and population viability (Combes 1996). Moreover, the progressive human-induced fragmentation of the Central Rockies Ecosystem

may have increased niche overlap and trophic relationships between grizzlies and black bears, with positive density-dependent effects on pathogen transmission. The outcome of higher prevalence and intensity of parasitic infections can be dramatic for species extremely vulnerable when constrained in degraded habitats. As a comparative example, in the last decade habitat loss and fragmentation led to disease emergence and mortality in giant pandas (Ailuropoda melanoleuca) parasitized by the intestinal nematode Baylisascaris schroederi (Zhang et al. 2008). In summary, despite much research on cub mortality rates and on habitat degradation in the regulation of bear populations (e.g., McLellan et al. 1999; Swenson et al. 2001; Apps et al. 2004; Garshelis et a. 2005; Nielsen et al. 2006; Dixon et al. 2007), parasites and diseases are too often forgotten as potential causes (Rogers 1993).

A better understanding of parasite diversity and life-history traits in ursid hosts can be gained by combining classical and molecular parasitology. The joint morphological and DNA-based analyses represent powerful tools for clinical diagnostics and disease transmission surveys. Nowadays, the genetic identification of taxa is by far the most applied molecular technique in parasitology (McKeand 1998; Criscione et al. 2005). The advances made in the field of molecular helminthology have improved our understanding of parasite biodiversity and species delimitation; molecular markers became essential tools not only to study the systematics of parasites, but also to shed light on their ecological and evolutionary processes (Brooks and Hoberg 2000; Nadler and Pérez-Ponce de León 2011). Species complexes of parasites can be revealed by genetic data where it was once thought there was single species (e.g., Leung et al. 2009; Ramovidil et al. 2010; Lavikainen et al. 2010). In contrast, two morphologically separate species have been found to be genetically identical, leading to synonymy and revision of their taxonomic classification (e.g., Stevenson et al. 1996; Dallas et al. 2000; Bell and Sommerville 2002). Although DNA-based approaches gained reliability and consensus through the years, traditional parasitology based on morphology is still considered as the principal method for parasite identification and the initial "prospecting" of any parasite species (McManus and Bowles 1996).

1.4 Objectives of the Study

The objectives of the study were to examine the parasite biodiversity in the gastrointestinal tract of grizzly and black bears from western Canada, and parasite infection patterns within these hosts. Classical and molecular approaches were combined to shed light on the parasite community infecting bears from Alberta and British Columbia. Historical parasitological studies on North American bears are few and dated. The recent findings of

previously undetected parasite taxa in ursids suggested the need to reassess their helminth fauna. Furthermore, this thesis focused on the discovery of novel hookworm morphovariants in Canadian bears. This finding raised the following questions:

- What does the morphological variability of *Uncinaria* spp. from black and grizzly bears mean? Are the isolated anatomical variants consistent with distinctive evolutionary lineages not yet described? Or, in contrast, are they just phenotypic attributes of the same parasite species?
- Are the ursine hookworms phylogenetically related to *Uncinaria* spp. of otariid pinnipeds? If so, are infections of bear cubs characterized by extremely high prevalence and intensity?

The substantial morphological diversity among the collected adult hookworm specimens and the relatively recent evolutionary divergence of ursids and otariids suggest the hypotheses of:

- Three distinct species of *Uncinaria* in North American bears.
- Close evolutionary relatedness between hookworm parasites of Ursidae and Otariidae.

To answer these questions and to test these assumptions, it was necessary to:

- Elucidate and describe the morphological trait variability among *Uncinaria* specimens collected from both grizzly and black bear hosts.
- Use informative molecular markers on selected parasite individuals already examined on morphology to disclose lineage exclusivity and identity.
- Assess the evolutionary linkage between the ursine *Uncinaria* spp. and other hookworm species from different carnivore hosts.
- Correlate levels of intestinal parasitic infection to host species and age class.

This thesis documents the parasite community harboured in the gastrointestinal tract of the opportunistically sampled grizzly and black bears from Alberta and British Columbia (Chapter 2). Chapter 3 determines whether the observed polymorphism for *Uncinaria* isolates corresponds to new/undiscovered taxa and explores the phylogenetic relatedness of the ursine hookworms with other species of the genera *Uncinaria* and *Ancylostoma*. Chapter 4 investigates taeniid tapeworm biodiversity in bears and reports the infection of Canadian grizzlies and black bears with *T. arctos*, a newly characterized tapeworm species. This study provides relevant baseline data on the gastrointestinal parasite fauna of bears from western Canada, and furthers our understanding of hookworm phylogeny. The significance and scope of the project are addressed in Chapter 5.

CHAPTER TWO: PATTERNS OF INFECTION FOR GASTROINTESTINAL PARASITES OF GRIZZLY AND BLACK BEARS FROM WESTERN CANADA

2.1 Introduction

Bears are highly iconic species in any ecosystem they inhabit (Simberloff 1999). In North America, black bears are the most abundant species of the family Ursidae. Black bears are classified as "Least concern", by IUCN (2008), and as "Not at risk", by COSEWIC (1999). However, some small and isolated populations are present in the southeastern states of USA (Pelton 2003). Grizzly bears, by contrast, are considered threatened throughout most of their habitat after the elimination from about the 98% of their historical range (US Fish and Wildlife Service 2009; Festa-Bianchet 2010; COSEWIC 2012). Their current distribution is restricted to Canada (Alberta, British Columbia, Northwest Territories, Nunavut, and Yukon) and the northwestern part of USA (Alaska, Idaho, Montana, Washington, and Wyoming). The range of the grizzly bear is described as one continuous metapopulation through western North America, with the exception of some insular populations in the southern portion of British Columbia (Craighead and Vyse 1996; Schwartz et al. 2003; Proctor et al. 2004).

To date, research on grizzlies and black bears has focused on several aspects of habitat quality, population dynamics, and anthropogenic influence (e.g., McLellan et al. 1999; Beckmann and Berger 2003; Apps et al. 2004; Garshelis et al. 2005; Mowat et al. 2005; Nielsen et al. 2006; Hristienko and McDonald 2007; Sawaya et al. 2012). However, the role of parasites and diseases is still largely undetermined. Many studies on populations of wild mammals and their pathogens demonstrated that parasites exacerbate malnutrition and stress trade-offs in resource allocation with consequent negative effects on individual reproductive physiology and population viability (e.g., Gulland 1992; Hudson et al. 1992, 1998; Catchpole et al. 2000; Martin et al. 2002; Stien et al. 2002; Luikart et al. 2008). Parasitism may influence the population dynamics of various hosts, but the interaction between wild hosts and their parasites is poorly documented. High parasitic infection levels may be responsible for poor body fat content (Hochberg et al. 1992), with a domino effect on female bear reproductive potential and rates of population growth: females may forgo mating seasons because of the energetic stress, with persistent deferrals leading to a declining population trend. Furthermore, malnourishment and weakness can make bear cubs and yearlings more vulnerable to predators and to a variety of pathogens (Ferguson 1987, 1993; Hilderbrand et al. 1999).

Despite the role of parasites as drivers of biodiversity within ecosystems, in the last 50 years limited research has been conducted on the helminth fauna of grizzlies and black bears,

particularly from populations of northwestern USA (Rausch and Hilliard 1970; Jonkel and Cowan 1971; Poelker and Hartwell 1973; Worley et al. 1976; Seesee and Worley 1986) and southwestern Canada (Hair and Mahrt 1970; Dies 1979). The acquired knowledge on the parasites of bears is largely ascribable to the parasitological surveys carried on black bear populations from the eastern part of North America (see Table 1.1, Chapter 1). These studies demonstrated that the latitudinal gradient has a strong influence on the parasite biodiversity of ursid hosts (Pence et al. 1983; Lindenfors et al. 2007). Surveys from the southeastern states of the USA recorded up to 12 different species of parasites infecting the digestive tract of black bears. In contrast, only three nematode species (*B. transfuga*, *P. rara*, *U. yukonensis*), three cestode species (*D. ursi*, *T. hydatigena*, *T. krabbei*) and one trematode species (*A. americana*) were isolated in black bears from eastern Canada (Rogers and Rogers 1976; Frechette and Rau 1977; Addison et al. 1978; Juniper 1978; Duffy et al. 1994).

The few and dated parasitological surveys on grizzly and black bears from western Canada suggested the need to reassess the parasite fauna of ursid hosts in this region. The aim of the present study was to provide data on gastrointestinal parasites of grizzly and black bears from Alberta and British Columbia. Our objectives were to define the parasite biodiversity in the sampled hosts, and to assess prevalence, intensity, and abundance of parasitic infections in correlation with host species and age class.

2.2 Materials and Methods

2.2.1 Specimen collection

From July 2011 to June 2013, the intestinal tracts of 40 black bears and 13 grizzlies from western Canada were collected and stored frozen at -20°C. Specimens from Alberta (22 black bears, 7 grizzlies) were gathered after post-mortem examination of bears found dead within the Alberta Parks and the Canadian Rocky Mountain Parks. For the bears from Alberta, the stomach was collected together with the intestine. Specimens from British Columbia (18 black bears, 6 grizzlies) were acquired from animals killed by vehicle, or by legal hunting within science-based wildlife management programs independent from the present work. Specimens from both Alberta and British Columbia were partitioned based on the seasons when they were collected: spring (12 black bears, 3 grizzlies), summer (16 black bears, 2 grizzlies), fall (8 black bears, 5 grizzlies), and winter (3 black bears). The information available for 4 of the sampled bears (1 black bear, 3 grizzlies) did not contain the date of death. The complete gastrointestinal tract of five black bears and four grizzly bears from

British Columbia was submitted to our laboratory; for the remaining bears (13 black bears, 2 grizzlies) the stomach was not provided. Information about age class, gender, date of death, and retrieval site of the carcass was collated for each individual. Age classification was calculated either based on the actual birth date of each individual when monitored since emergence from the den, or based on morphological traits and dentition with an assumed birth date at the beginning of February (Pelton 2003; Schwartz et al. 2003). Cubs were of 1 year old, juveniles were > 1 but < 4 years old, and adults were 4 or more years old.

2.2.2 Laboratory analysis

Once thawed, the stomach was dissected, contents noted, and the lining examined for parasites. The entire intestine was longitudinally opened and washed into a bucket. The intestinal contents were rinsed through a sieve (500 µm pores), then diluted and stored in a graduated beaker and examined for parasites in a tray against a black background. Isolated parasites were counted and preserved in 70% ethanol at -20°C for both morphological and molecular analyses. Tapeworms were counted based on the number of isolated scolices. The statistical significance of infection prevalence, intensity, and abundance [see Bush et al. (1997) and Rózsa et al. (2000) for definitions] in the different bear species and age classes was assessed with non-parametric methods: the Wilcoxon rank sum test with continuity correction and the Kruskal-Wallis rank sum test, respectively applied to compare two and more than two groups ($P < 0.05$ for statistical significance). The non-normal distribution of the data, assessed by the Shapiro-Wilk W-test, justified the application of non-parametric tests. The Wilcoxon test was used to compare infection intensity and abundance between species and genders. The Kruskal-Wallis test was used to evaluate infection values among the three age classes. Statistical analyses were run in the R software environment (R Development Core Team 2012).

2.2.3 Parasite identification

After rehydration in tap water, isolated nematode species were morphologically identified according to keys (Wolfgang 1956; Olsen 1968; Sprent 1968; Soulsby 1986). Cestode parasites were characterized to the genus level based on the morphology of the scolex when found, and on the anatomy of mature proglottids when specimens were in an adequate state of preservation for staining. Two segments from one tapeworm specimen were stained with Semichon's acetic carmine, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. Cestode species were molecularly identified based on the analysis of the

mitochondrial DNA (mtDNA) cytochrome c oxidase subunit 1 (cox1) gene. After rehydration in nuclease-free water, genomic DNA from each isolate was extracted using Epicentre® MasterPure™ Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI). DNA elution was made up in 20µl TE buffer, stored at -20°C until Polymerase Chain Reaction (PCR). Specimens were amplified for a fragment of the cox1 gene using primers no. 2575 (forward, 5'-TTTTTTGGGCATCCTGAGGTTTAT) and 3021 (reverse, 5'-TAAAGAAAGAACATA ATGAAAATG) by Bowles et al. (1992) for Taenia isolates; primers BW3 (forward, 5'-TTTTTTGGCCACCCCGAAGTATAT) and BW4.5 (reverse, 5'-TAGTGACATTACATAGTGGAAGTG) by Wicht et al. (2007) for Diphyllobothrium isolates. For Taenia sp., enzymatic amplification and thermocycling protocol were performed as described by Lavikainen et al. (2011). For Diphyllobothrium sp., 2µl DNA template were mixed in a 25µl reaction containing 2.5µl 10X PCR buffer, 3mM $MgCl_2$, 200µM deoxynucleoside triphosphates, 0.5µM of each primer, 1µg bovine serum albumin (New England Biolabs, Ipswich, MA), and 0.3µl Taq DNA polymerase (5000units/ml). Cycling parameters consisted of an initial nucleic acid denaturation at 94°C for 5 min, followed by 33 cycles of 94°C for 45 sec, 50°C for 45 sec, and 72°C for 1 min at 72°C, with a final 5 min extension at 72°C.

2.2.4 Sequence analysis

The PCR products were purified with E.Z.N.A.™ MicroElute Cycle-Pure Kit (Omega Bio-Tek, Norcross, GA), then sequenced using an Applied Biosystems 3730xl DNA Analyzer with BigDye Terminator™ chemistry (Perkin-Elmer, Waltham, MA). The sequences were double-stranded. Contig assembly and editing were performed with CodonCode Aligner (CodonCode Corporation, Centerville, MA). Invariant flanking regions corresponding to the PCR primers were removed from each contig before alignment. Obtained sequences were compared with previously published DNA data available on the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) by alignment using the software Geneious 5.5 created by Biomatters (www.geneious.com).

2.3 Results

The nematodes *B. transfuga* and *Uncinaria* sp., and the cestodes *D. dendriticum*, *D. nihonkaiense*, and *T. arctos* were recovered from the digestive tract of 53 Canadian grizzlies (n=13) and black bears (n=40). The gastric contents were analysed for 38 bears (27 black bears, 11 grizzlies) since the stomach of 13 black bears and 2 grizzlies from British Columbia

was not provided. Only two grizzly bears from Owikeno Lake, British Columbia (51° 42‡ 30ˆ N, -126° 54‡ 52ˆ W), harboured *B. transfuga* juveniles in their stomach. Parasitic infections of the intestinal tract were found in 49/53 bears (92.5%): 38/40 black bears (95%) and 11/13 grizzlies (84.6%). *Uncinaria* sp. was isolated from 29 black bears (72.5%) and 7 grizzlies (53.8%); infection by *B. transfuga* was found in 24 black bears (60%) and 7 grizzlies (53.8%); *T. arctos* infected 4 black bears (10%) and 1 grizzly (7.7%). Two grizzly bears, one from Owikeno Lake and one from Kitwancool Lake, British Columbia (55° 24‡ 32ˆ N, -128° 8‡ 51ˆ W), respectively hosted *D. dendriticum* and *D. nihonkaiense*. Out of 49 parasitized bears, multiple infections by *B. transfuga*, *Uncinaria* sp., and cestodes were only found in 4 bears (8.2%). Infections by both *B. transfuga* and *Uncinaria* sp. were found in 20 bears (40.8%), whereas 25/49 infected hosts (51%) harboured a single helminth species.

The finding of unknown *Uncinaria* sp. morphotypes parasitizing both grizzly and black bears necessitated a more detailed study to shed light on hookworm biodiversity in North American bears (data presented in Chapter 3). Herein, the morphological identification of hookworm specimens is limited to the genus level. In contrast, the poor quality of the *Taenia* isolates did not allow their morphological characterization. Taeniid tapeworms were found in one black bear from Vancouver Island, British Columbia (49° 35ˆ N, -124° 31‡ 2ˆ W), and in one grizzly and three black bears from the general area of the Canadian Rockies within Alberta. The specimens were molecularly identified as *T. arctos* based on the similarity of the obtained *cox1* sequences (396 base pairs) with data previously reported (Lavikainen et al. 2011). Morphological and molecular analyses were not successful for the only *Taenia* specimen found in a black bear from Jasper National Park (52° 51‡ 35ˆ N, -118° 4‡ 35ˆ W); in this case, the infection with *T. arctos* was assumed since the black bear came from the same area of Alberta as the other *T. arctos*-positive bears. Similarly to *T. arctos*, the pseudophyllidean cestodes *D. dendriticum* and *D. nihonkaiense* were characterized based on the obtained molecular amplicons (368–397 base pairs) of the mtDNA *cox1* gene (Table 2.1). Before the specific diagnosis based on molecular markers, two segments from two adult tapeworms later identified as *D. nihonkaiense* were stained for morphological characterization (Figure 2.1). Indisputable species identification of *Diphyllobothrium* taxa is extremely difficult on morphology; in this case no species-specific trait was observed.

Table 2.1: Pairwise similarity scores (%) for the mitochondrial DNA cytochrome c oxidase subunit 1 (cox1) of *Diphyllobothrium* spp.; sequences from the present study were aligned and compared with data deposited in GenBank.

Parasite species	*D. nihonkaiense* ‰	*D. dendriticum* ‰	AB015755[a]	AM412738[b]	AY972071[c]	AB605762[d]
D. nihonkaiense ‰	-	94.5%	99.2-100%	93.2-93.7%	92.4-92.9%	95-95.5%
D. dendriticum ‰	94.5%	-	94.5%	98%	92.7%	95%
AB015755[a]	99.2-100%	94.5%	-	93.7%	92.9%	95.5%
AM412738[b]	93.2-93.7%	98%	93.7%	-	92.4%	94.2%
AY972071[c]	92.4-92.9%	92.7%	92.9%	92.4%	-	91.4%
AB605762[d]	95-95.5%	95%	95.5%	94.2%	91.4%	-

‰ Present study

[a] *D. nihonkaiense* (Miyadera et al. 2001).

[b] *D. dendriticum* (Wicht et al. 2008).

[c] *D. latum* (Yera et al. 2006).

[d] *D. ursi* (Yamasaki et al. 2012).

Figure 2.1: Scolex (A), eggs (B), and gravid proglottids of *D. nihonkaiense*. The segments were stained using Semichon's acetic carmine, and photographed at 2X (C) and 5X (D) magnification.

The results of the parasitological analysis are summarized in Tables 2.2 and 2.3. The prevalence, intensity, and abundance of parasitic infections were grouped by host species and age class. The 40 sampled black bears included 7 cubs (17.5%), 19 juveniles (47.5%), and 14 adults (35%); 8 of them were females (20%), and 32 males (80%). The 13 sampled grizzly bears included 1 cub (7.7%), 5 juveniles (38.5%), and 7 adults (53.8%); 7 of them were females (53.8%), and 6 males (46.2%). The intensity of *Uncinaria* sp. and *B. transfuga* in the

infected hosts are displayed in Figure 2.2 and Figure 2.3, respectively. No statistical comparison between host species, genders, and age classes using either the Wilcoxon rank sum test or the Kruskal-Wallis rank sum test resulted in significant ($P > 0.05$).

Figure 2.2: Intensity of *Uncinaria* sp. infection for the different age classes of the sampled grizzly and black bears. For the distribution of the data within each group (adults, cubs, juveniles) the thicker line indicates its median value, the box shows the distance between first and third quartile, the whiskers represent the lowest and the highest datum respectively within 1.5 interquartile range of the lower and upper quartile. Outliers are indicated by dots.

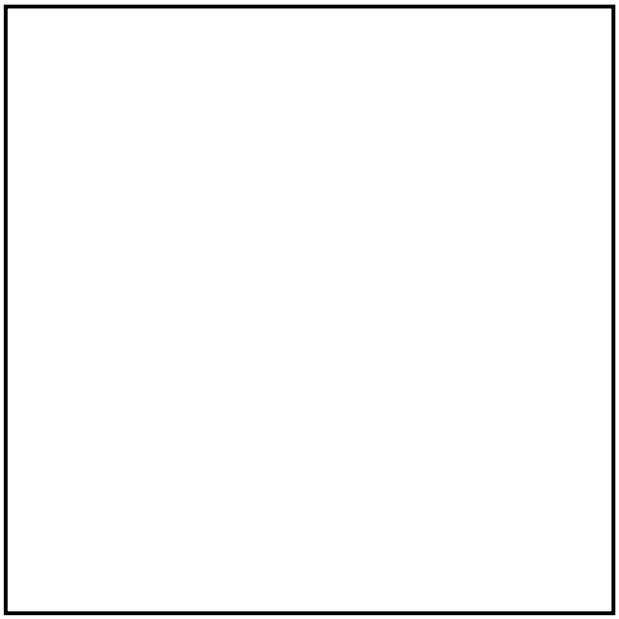
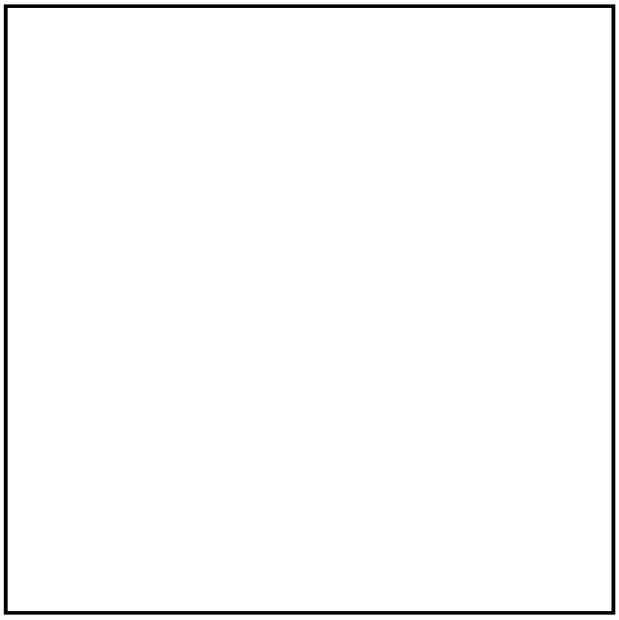


Figure 2.3: Intensity of *B. transfuga* infection for the different age classes of the sampled grizzly and black bears. For the distribution of the data within each group (adults, cubs, juveniles) the thicker line indicates its median value, the box shows the distance between first and third quartile, the whiskers represent the lowest and the highest datum respectively within 1. interquartile range of the lower and upper quartile. Outliers are indicated by dots.



Voucher specimens were archived in the U.S. National Parasite Collection (USNPC), U.S. Department of Agriculture (USDA), under the accession numbers USNPC 106982, 106983, 106985, 107885, 107886 for Uncinaria sp., USNPC 106984 and 106986 for T. arctos, USNPC 106987 and 106988 for B. transfuga, and USNPC 107884 for D. nihonkaiense. The quality of D. dendriticum specimens was too low for submission to the archival collection. The mtDNA cox1 sequence data were deposited in the NCBI GenBank database (accession numbers KF356386, KF356387, KJ026488, and KJ026489 for T. arctos; KJ026490 for D. dendriticum; KJ026491–KJ026494 for D. nihonkaiense).

Table 2.2: Infection prevalence, median intensity (range in parentheses), and mean abundance for the intestinal parasites of black bears (BB). The number of analysed intestinal tracts for each group is reported in parentheses.

Parasite species	BB cubs (n=7)		BB juveniles (n=19)		BB adults (n=14)		BB total (n=40)		
	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity	Abundance
Nematoda									
B. transfuga	42.9% (3/7)	3 (1-6)	73.7% (14/19)	8 (1-78)	50% (7/14)	11 (4-40)	60% (24/40)	7.5 (1-78)	9.30 ± 16.79
Uncinaria sp.	71.4% (5/7)	21 (5-740)	57.9% (11/19)	24 (2-739)	92.9% (13/14)	158 (2-506)	72.5% (29/40)	118 (2-740)	133.05 ± 201.38
Cestoda									
T. arctos	-	-	5.3% (1/19)	N/A (1)	21.4% (3/14)	2 (1-3)	10% (4/40)	1.5 (1-3)	0.17 ± 0.59

Table 2.3: Infection prevalence, median intensity (range in parentheses), and mean abundance for the intestinal parasites of grizzly bea (GB). The number of analysed intestinal tracts for each group is reported in parentheses.

Parasite species	GB cubs (n=1)		GB juveniles (n=5)		GB adults (n=7)		GB total (n=13)		
	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity	Abundance
Nematoda									
B. transfuga	N/A (1/1)	N/A (6)	40% (2/5)	10.5 (4-17)	57.1% (4/7)	10.5 (4-26)	53.8% (7/13)	9 (4-26)	6.00 ± 8.11
Uncinaria sp.	N/A (1/1)	N/A (20)	40% (2/5)	96.5 (22-171)	57.1% (4/7)	62.5 (30-188)	53.8% (7/13)	46 (20-188)	42.77 ± 65.16
Cestoda									
D. dendriticum	-	-	-	-	14.3% (1/7)	N/A (3)	7.7% (1/13)	N/A (3)	0.23
D. nihonkaiense	N/A (1/1)	N/A (27)	-	-	-	-	7.7% (1/13)	N/A (27)	2.08
T. arctos	-	-	20% (1/5)	N/A (1)	-	-	7.7% (1/13)	N/A (1)	0.08

2.4 Discussion

This study describes the parasite community harboured in the gastrointestinal tract of grizzly and black bears from Alberta and British Columbia. Previous data from Alberta were provided by Hair and Mahrt (1970) on *Eimeria* spp. (Apicomplexa: Eimeriidae) in 52 faecal samples from black bears, and by Dies (1979) who examined 91 gastrointestinal tracts of black bears from the Peace River region, northwestern Alberta (see Table 1.1, Chapter 1). In contrast, bear populations from British Columbia have never been investigated for parasites in the past. Further records on parasites of bears from western Canada come from Yukon and Northwest Territories (Choquette et al. 1969; Gau et al. 1999; Kutz 2003a, 2003b, 2004a). Choquette et al. (1969) reported the infection of grizzly bears with adults of *B. transfuga*, *U. yukonensis*, and *Diphyllobothrium* sp. (presumably *D. ursi*). More recent studies added the presence of *Nematodirus* sp. eggs from faecal samples of grizzly bears (Gau et al. 1999), and of *U. rauschi* from the intestinal tract of black bears (Kutz 2003a).

The current survey confirms the common occurrence of *B. transfuga* and *Uncinaria* sp. in North American bears, indicating these taxa as core species (i.e., regionally common and locally abundant parasite taxa) of the parasite community of bears at northern latitudes. Furthermore, this study is the first report of adult *T. arctos* tapeworms in grizzly and black bears. Previously, *T. arctos* has been described only in brown bears from Finland as definitive hosts (Lavikainen et al. 2011). The application of molecular tools also allowed the identification of adult *D. nihonkaiense* and *D. dendriticum* in the small intestine of two grizzly bears from British Columbia. The species *D. nihonkaiense* was already reported in Russian brown bears as definitive host (Arizono et al. 2009). In contrast, the diagnosis of *D. dendriticum* in ursid hosts was never confirmed before by molecular methods.

The ascarid *B. transfuga* is a cosmopolitan nematode of *Ursus* species (e.g., Dies 1979; Foster et al. 2004; Sato et al. 2004; De Ambrogi et al. 2011). This parasite is ubiquitous through North America, occurring in northern regions as at southern latitudes. However, its lower prevalence in black bears of the southeastern USA may ensue from limited egg survival as a consequence of a warmer climate (Pence et al. 1983; Foster et al. 2004), and/or from reduced transmission potential induced by a lower host population density (Anderson and May 1978; Arneberg et al. 1998; Hristienko and McDonald 2007). Environmental limitations and inadequate host-parasite densities explained the similar decrease of *B. procyonis* prevalence in raccoon (*Procyon lotor*) populations from northern to southern USA (Kazacos and Boyce 1989; Kazacos 2001). Accordingly, *B. transfuga* is highly prevalent in northern USA and western Canada, as documented by the current and historical studies (e.g., Worley et

al. 1976; Manville 1978; Dies 1979). These data support the role of *B. transfuga* as a core parasite species of bears at northern latitudes. Nevertheless, specific studies on the pathology of *Baylisascaris* spp. in ursids are absent. Hundreds of adult *B. transfuga* can cause the death of bears by obstructing the lumen of the digestive tract (Mozgovoi 1953; Kazacos 2001; Testini et al. 2011). Similar mechanisms are described for *B. schroederi* infections, a major cause of death in giant pandas (Xue 1987; Zhang et al. 2008, 2011). Heavy burdens of *B. schroederi* in pandas, fostered by higher host densities in the degraded, patchy habitat pandas inhabit, were reported to clog the pylorus (i.e., the junction of the stomach to the beginning of the small intestine) of their hosts, causing their death (Xue 1987). The findings on *B. schroederi* in giant pandas highlight the need for further study on *B. transfuga* and its pathogenicity in bears.

Hookworm nematodes of the genus *Uncinaria* were identified based on the pattern of the bursal rays and on a keyhole-shaped buccal capsule with two cutting plates and subventral lancets (Lyons and DeLong 2005). The morphological characterization of *Uncinaria* spp. stopped at the genus level because hookworm isolates never described before were found. The hypothesis of multiple species/polymorphism for *Uncinaria* spp. in bears was tested in Chapter 3. This survey is the first report of hookworm infection in bears from Alberta and British Columbia. To date, our knowledge on ursine hookworms is limited to the description of *U. yukonensis* by Wolfgang (1956) and *U. rauschi* by Olsen (1968). Further findings from North American populations of bears supported the taxonomic classification of their hookworms and their distribution in northern ecosystems. These studies isolated *U. yukonensis* in black bears (Rausch 1961; Jonkel and Cowan 1971; Frechette and Rau 1977) and grizzlies (Choquette et al. 1969), *rauschi* in black bears (Kutz 2003a), and unidentified strongylid specimens in both bear species (Wyont et al. 1976; Manville 1978; Gau et al. 1999), always reporting a low prevalence of infection (< 17%). Only Choquette et al. (1969) in northwestern Canada found a higher prevalence of *U. yukonensis*, with 10/21 (47.6%) positive grizzly bears. Similarly to Choquette et al. (1969), the current study reports a total 67.9% prevalence for *Uncinaria* sp. among the sampled hosts (72.5% in black bears, 53.8% in grizzlies). Adult bears were found to be the heaviest infected age class by *Uncinaria* sp., with a prevalence of 92.9% in black bears and 57.1% in grizzlies. These higher hookworm infection levels in adult bears resemble the infection patterns in humans, where hookworm transmission is mainly percutaneous and infection intensity peaks during adulthood (Hotez et al. 2004). Data on hookworm-infected bears contrast with information on *Uncinaria* spp. transmitted primarily through a transmammary route. This alternative-life-cycle was described for

Uncinaria spp. of otariid pinnipeds such as northern fur seals and New Zealand sea lions. It causes elevated parasite burdens in the intestine of pups, which have been found to harbour hundreds of adult hookworms. The parasites persist in the intestinal tract of the pinniped pups for a maximum of four months after birth, after which they are expelled if the animal survives (Lyons et al. 2003; Castinel et al. 2007a; Lyons et al. 2011b).

Pseudophyllidean cestodes of the genus Diphyllobothrium were already described in bears as definitive hosts, but this study provides the first molecularly confirmed report of Diphyllobothrium spp. in Canadian bears. A segment of the mtDNA cox1 gene allowed the diagnosis of D. dendriticum in a grizzly bear from Owikeno Lake, and of D. nihonkaiense in a grizzly bear from Kitwancool Lake. The species D. dendriticum has been reported from a broad range of definitive hosts in North America, including bears (Andersen et al. 1987). However, previous investigations on parasites of Canadian bears mainly isolated D. ursi. Diphyllobothrium ursi was first characterized by Rausch (1954) from Alaskan Kodiak bears. Further reports come from black bears of Quebec (Frechette and Rau 1977; Juniper 1978), and grizzly bears of northwestern Canada (Choquette et al. 1969) and Montana (Worley et al. 1976). In most cases the identity as D. ursi was just assumed. The morphological reexamination of putative D. ursi from bears of British Columbia pointed out the potential misidentification of D. dendriticum with D. ursi, and synonymized D. ursi with D. dendriticum (Andersen et al. 1987). Eventually, a recent study recognized D. ursi as a valid species with support of both morphological and molecular data (Yamasaki et al. 2012). This example, as many others (e.g., Rausch 1954; Post 1971; deVos et al. 1990), state the extreme difficulty of a definitive morphological identification of Diphyllobothrium species. In this study, morphological criteria were inadequate to characterize the specimens collected from the two grizzly bears of Owikeno and Kitwancool Lakes. The application of molecular tools provided the first indisputable record of D. dendriticum adult stages in the intestinal tract of a bear, unambiguously expanding the host spectrum of the parasite to ursids as potential definitive hosts. The second isolated species, D. nihonkaiense, was never characterized before from grizzlies and black bears. However, this cestode species was been previously reported in Russian brown bears from Lake Azabache, Kamchatka Peninsula, Russia (Arizono et al. 2009). The current study is the first record of D. nihonkaiense in North American bears. This parasite is transmitted to definitive hosts by salmonids of the genus Oncorhynchus (Ando et al. 2001), anadromous fish (i.e., which migrate from marine waters upstream) whose role as intermediate hosts for D. nihonkaiense has been recently proved in Canada (Yera et al. 2006; Wicht et al. 2007).

This survey is also the first report in North America for the cyclophyllidean cestode *T. arctos* for grizzly and black bears as definitive hosts. The only previous isolation of this parasite on the continent was the detection of its larval forms in Alaskan moose (*Alces americanus*) and muskoxen (*Ovibos moschatus wardi*) from west Greenland using molecular techniques (Lavikainen et al. 2011; Raundrup et al. 2012). Bears are reported as definitive hosts for several species of *Taenia*, with a prevalence never higher than 11% (e.g., Frechette and Rau 1977; Choquette et al. 1969; Dies 1979). Only two studies on nine (Rogers 1975) and 12 black bears (Duffy et al. 1994) found a higher prevalence (28.6% and 25%, respectively), but the small sample size and the collection of the carcasses from a relatively limited area may have affected the prevalence value. This study reports a low total prevalence (9.4%) and intensity (range 1-3) for *T. arctos* infections in the analysed grizzly and black bears. One *T. arctos*-positive black bear was from Vancouver Island; three *T. arctos*-positive black bears (4/40, 10% prevalence) and the only *T. arctos*-positive grizzly bear (1/13, 7.7% prevalence) were retrieved from the general area of the Canadian Rocky Mountains of Alberta: the northernmost black bear was a yearling female from Jasper National Park (52° 51′ 35″ N, -118° 4′ 35″ W), the southernmost was an adult male from the Crowsnest Pass area (49° 34′ N, -114° 10′ 53″ W); the grizzly bear was a subadult male from Kananaskis Country, Alberta (51° 2′ 39″ N, -114° 41′ 59″ W). These results further support the specificity of *T. arctos* for ursids as definitive hosts, and potentially expand the geographic distribution of the parasite to ecosystems of North America where bears and moose are sympatric. The isolation of *T. arctos* and its importance are further explored in Chapter 4.

In agreement with preceding surveys, the results of this study showed the low parasite diversity in bear populations at northern latitudes. In the southeastern USA, where bears are exploited by a broader range of parasite species (e.g., Conti et al. 1983; Foster et al. 2004), *B. transfuga* was the only parasite with marked specificity for ursid hosts. Further parasites isolated from black bears at southern latitudes mainly occur in other carnivore hosts phylogenetically related to the family Ursidae, raccoons in particular. The contrasting parasite biodiversity in North American bears at different latitudes may result from the considerably lower density of raccoons as reservoir hosts, and from environmental factors limiting the distribution of parasitic infective stages in Canada and in the northern states of USA (Pence et al. 1983). Despite the finding of few helminth species, this research expands parasite biodiversity in North American grizzlies and black bears with the first report of *D. dendriticum*, *D. nihonkaiense*, and *T. arctos*. Despite the new information gained, the role of parasites in ursids is still poorly understood. Their integration into wildlife management and

conservation programmes is limited (Cleaveland et al. 2002). The outcome of higher parasitic infectionscan be dramatic for ursid species, in particular if constrained in degraded, crowded habitats. In the last decade habitat loss and fragmentation led to disease emergence and mortality in giant pandas parasitized by the intestinal nematode *Baylisascaris schroederi* (Zhang et al. 2008, 2011). The importance of habitat quality has been discussed many times for the natural regulation of bear populations (e.g., Apps et al. 2004; Mowat et al. 2005; Nielsen et al. 2006) but the need to start including parasites and diseases as crucial given the potentially detrimental effects of infectious agents on bear health and viability.

CHAPTER THREE: MORPHOLOGICAL VARIABILITY AND MOLECULAR IDENTITY OF UNCINARIA SPP. FROM GRIZZLY AND BLACK BEARS: NEW SPECIES OR PHENOTYPIC PLASTICITY?

3.1 Introduction

Hookworms are haematophagous nematodes responsible for significant pathology in humans, wild and domestic mammals. They develop to the adult stage in the small intestine of their hosts, where they attach to the mucosa and submucosa with their armed buccal capsule. The most important infection route for hookworms is the presence of larvae in the soil, which enter a suitable host by penetrating the skin and/or by oral ingestion (Anderson 2000; Traversa 2012). A third transmission strategy is the transmammary passage of infective larvae from dam to offspring during nursing (Olsen and Lyons 1965; Castinel et al. 2007a).

Transmammary transmission is a main infection route for hookworms of otariid pinnipeds. It has been convincingly documented for Uncinaria spp. of California sea lions (Lyons et al. 2000, 2005; Spraker et al. 2007), northern fur seals (Lyons 1994; Lyons et al. 2011a), and New Zealand sea lions (Castinel et al. 2007a), but it has been inferred also for Uncinaria spp. of Juan Fernández fur seals, Arctocephalus philippii (Sepúlveda and Alcaíno 1993; Sepúlveda 1998), and South American sea lions (Beveridge et al. 2004). Nursing as a relevant infection source of hookworm larvae for pups has been demonstrated also for Ancylostoma caninum, a zoonotic species with canids as primary hosts (Stone and Girardeau 1968; Stone and Peckham 1970; Prociv 1998; Bowman et al. 2010), and it has been postulated for Ancylostoma duodenale, a hookworm found principally in humans (Nwosu 1981; Schad 1991; Yu et al. 1995; Bethony et al. 2006). In contrast, milk-borne larvae have not been documented for U. stenocephala and other Ancylostoma spp. of canids and felids (Walker and Jacobs 1985; Traversa 2012; Traub 2013). The pathogenesis of hookworm disease is primarily a consequence of blood consumption, intestinal ulceration, and haemorrhage. Death may result from haemorrhagic enteritis and acute anaemia, but perforation of the intestinal wall by adult hookworms with subsequent peritonitis may also occur (Spraker et al. 2004b; Traub 2013). Symptoms can be severe and life-threatening particularly for newborns, whereas immune resistance increases with the age of the host and the exposure to hookworms (Anderson 2000; Castinel et al. 2007a, 2007b; Traversa 2012).

In bear species, our knowledge of hookworm biodiversity is limited to the taxonomic descriptions of U. yukonensis discovered in black bears from the Yukon Territory, Canada (Wolfgang 1956), and U. rauschi from black and grizzly bears from Alaska, USA (Olsen

1968). Further reports of *U. yukonensis* are moderately common for both bear species in North America (Rausch 1961; Choquette et al. 1969; Jonkel and Cowan 1971; Frechette and Rau 1977), and for brown bears from the Russian Far East (Rausch et al. 1979). By contrast, additional reports of *U. rauschi* have been restricted to black bears from Fort Providence, Northwest Territories, Canada (Kutz 2003a). The few morphological studies on the two hookworm species of ursids (Wolfgang 1956; Olsen 1968; Rausch et al. 1979) provided several discriminatory traits of taxonomic value:

- For adults of both genders, *U. yukonensis* is considerably larger than *U. rauschi*
- Male spicule length of 1.6–1.8mm for *U. yukonensis*, and 0.82–0.95mm for *U. rauschi*.
- Females of *U. yukonensis* characterized by prominent pre-vulvar flap, whereas *U. rauschi* female specimens lack a pronounced flap.

During a survey on causes of mortality and diseases in grizzly and black bears found dead within Banff National Park, Alberta, Canada (51° 11′ 5″ N, -115° 45′ 3″ W), unknown hookworm morphotypes parasitizing the intestinal tract of both bear species were isolated. Specifically, female specimens with either one or two knobs defining the vulvar opening were found to infect the examined bears. Are these anatomical variants consistent with distinctive evolutionary lineages not yet described? Alternatively, are they phenotypic attributes of a polymorphic parasite species? Hookworm species are well studied in many mammalian hosts such as humans, canids, and primates. By contrast, there is a dearth of information on *Uncinaria* spp. in ursid hosts. Further reports are rare, and the species status of both *U. yukonensis* and *U. rauschi* was based solely on the morphological examination of few specimens, with no support from genetic data. The present study determined whether the observed polymorphism represents a new/undiscovered taxon by combining classical and molecular techniques in parasite systematics for the analysis of *Uncinaria* specimens.

3.2 Materials and Methods

3.2.1 Specimen collection

During 2011 and 2012, the intestinal tracts of three grizzlies and four black bears from Alberta were gathered during the post-mortem examination of bears found dead within the Alberta Parks and the Canadian Rocky Mountain Parks. Specimens from one grizzly and five black bears of British Columbia, Canada, were collected from animals killed by legal hunting, within science-based wildlife management programs independent from the present study (Figure 3.1). The collected intestines were stored at -20°C. Once thawed and dissected, the

intestinal contents were diluted and examined for the presence of parasites. Isolated hookworms and other helminths were preserved in 70% ethanol at -20°C for both morphological and molecular analyses.

Figure 3.1: Distribution map of the sites where grizzly (triangle) and black bear (circle) carcasses were retrieved in Alberta and British Columbia. Map developed using the software ArcGIS 10.1 ESRI®.

3.2.2 Hookworm morphology

After rehydration in tap water and clarification with a few drops of lactophenol, adult hookworms were examined using an Olympus BX53 microscope and its software (http://microscope.olympus-global.com/en/ga/product/bx53/sf04.cfm). Measurements were made for 269 adult female and 166 adult male hookworms: 150 (99 females and 51 males) from the four grizzlies and 285 (170 females and 115 males) from the nine black bears examined. Following previous studies on Uncinaria morphometrics (Nadler et al. 2000a; Castinel et al. 2006), measurements for the males included body length and width, buccal capsule depth, oesophageal length and width at the base, distance of the nerve ring from the anterior end of the oesophagus, distance of the excretory pore from the anterior end of the body, copulatory bursa length and width, spicule length, gubernaculum length and width, and

dorsal ray length and width. The first seven of these measurements were also made for the females, along with distance of vulva from the posterior end, tail length and width, and egg length and width. After the morphological analysis, each specimen was cleaned from the lactophenol using 70% ethanol before preservation at 90% ethanol in numbered vials.

Morphometric data for the collected hookworms were analysed using the statistical software R (R Development Core Team 2012). The normal distribution of the data was assessed with the Shapiro-Wilk W-test. The Wilcoxon rank sum test with continuity correction was used to determine significant biometric differences among the analysed specimens ($P < 0.05$ for statistical significance).

3.2.3 Polymerase Chain Reaction (PCR) amplification

The identity of individual hookworms previously examined on morphology was verified using the internal transcribed spacers (ITS-1 and ITS-2) of the nuclear ribosomal DNA (rDNA). The specimens were rehydrated in TE buffer. Genomic DNA was extracted from the excised mid-body using the Epicentre® MasterPure™ Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI). DNA was eluted in 20µl TE buffer and stored at -20°C until PCR. Samples from each specimen were amplified for the ITS-1 and ITS-2 genes using primers no. 93 (forward, 5'-TTGAACCGGGTAAAAGTCG) and 264 (reverse, 5'-CGTTTTTCATCGATACGCG) for ITS-1; primers no. 623 (forward, 5'-ACGTCTGGTTCAGGGTTGTT) and 94 (reverse, 5'-TAGTTTCTTTTCCTCCGCT) for ITS-2 (Nadler et al. 2000a, 2000b). Amplifications were performed in a 25µl reaction mixture containing 2.5µl 10X PCR buffer, 3mM $MgCl_2$, 200µM deoxynucleoside triphosphates (dNTPs), 0.5µM of each primer, 1.25µl of bovine serum albumin (New England Biolabs, Ipswich, MA), 0.2µl Taq DNA polymerase (5000units/ml), and 2µl DNA template. Cycling parameters consisted of an initial nucleic acid denaturation at 95°C for 5 min, followed by 32 cycles of 95°C for 1 min, 54°C for 1 min, and 1 min at 72°C, with a final 5 min extension at 72°C.

3.2.4 Sequence and phylogenetic analyses

After either enzymatic treatment with exonuclease I and shrimp alkaline phosphatase (GE Healthcare Biosciences, Piscataway, NJ), or purification with E.Z.N.A.™ MicroElute Cycle-Pure Kit (Omega Bio-Tek, Norcross, GA), the PCR products were sequenced using an Applied Biosystems 3730xl DNA Analyzer with BigDye Terminator™ chemistry (Perkin Elmer, Waltham, MA). The sequences were double-stranded using the amplification primers.

Contig assembly and editing were performed with CodonCode Aligner (CodonCode Corporation, Centerville, MA). The invariant flanking regions, corresponding to PCR primers and poor quality 5′ and 3′ ends, were removed from each contig before sequence alignment and analysis. The ITS-1 and ITS-2 sequences from individual parasites were aligned separately using Clustal W 2.0 (Larkin et al. 2007). The ITS rDNA data from the collected hookworms were also aligned with ITS sequences available for other hookworm species in the NCBI GenBank database. The analysis included the ITS regions of *U. stenocephala* from the arctic fox, *Alopex lagopus* (GenBank accession number AF194145); *U. stenocephala* from the island fox, *Urocyon littoralis* (HQ262052); *U. lucasi* from the Steller sea lion, *Eumetopias jubatus* (HQ262132); *U. hamiltoni* from the South American sea lion, *Otaria flavescens* (HQ262104); *Uncinaria* sp. from the New Zealand sea lion (HQ262089). The ITS-1 and ITS-2 regions were concatenated for a combined analysis. The species *A. duodenale* (EU344797) and *A. caninum* (JQ812694) were used as outgroups to construct the phylogenetic tree (Table 3.1). Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses were conducted using PAUP* 4.0b10 (Swofford 1998). The heuristic search option was selected for a bootstrap MP search using 2,000 replicates. For the ML analysis, tree construction was estimated with the model HKY+G. This model was selected using the Akaike Information Criterion (AIC) as implemented in MrModeltest version 2.3 (Nylander 2004). It assumes unequal base frequencies, different substitution rates for transversions and transitions, and gamma distribution of rates at variable sites. Nodal support in the ML analysis was estimated by a bootstrap search using 2,000 replicates and the heuristic search option.

Table 3.1: Internal transcribed spacer sequences, nuclear ribosomal DNA, of the hookworm taxa included in the study. Host species, sampling area, GenBank accession number, and reference study are provided.

Taxon	Host species	Location	GenBank accession no.	Reference
U. stenocephala	Island fox	California, USA	HQ262052	Nadler et al. (2013)
U. stenocephala	Arctic fox	Alaska, USA	AF194145	Nadler et al. (2000b)
U. hamiltoni	South American sea lion	Uruguay	HQ262104	Nadler et al. (2013)
U. lucasi	Steller sea lion	Alaska, USA	HQ262132	Nadler et al. (2013)
Uncinaria sp.	New Zealand sea lion	Enderby Island, New Zealand	HQ262089	Nadler et al. (2013)
A. caninum	Domestic dog	Michigan, USA	JQ812694	Lucio-Forster et al. (2012)
A. duodenale	Human	Fujian, China	EU344797	Wang et al. (unpublished)

3.3 Results

3.3.1 Hookworm identity

The examined hookworms were collected from the intestinal tract of four grizzly and nine black bears from Alberta and British Columbia. All the analysed specimens (269 female and 166 male adult hookworms) presented a keyhole-shaped buccal capsule with two cutting plates and subventral lancets (Figure 3.2) distinctive of *Uncinaria* spp. (Lyons and DeLong 2005). They also presented well-defined cervical papillae, filariform oesophagus constricted by a nerve ring at its mid-posterior region, and excretory pore slightly behind the nerve ring. The male bursa was characterized by one small dorsal and two ventral lobes, and by one dorsal ray divided in two tridigitate branches, as described also for *U. stenocephala* of canids and felids (Gibbs 1961). The two spicules were slender and of approximate equal size. The gubernaculum had ellipsoidal shape, widest at its posterior. Male hookworms were classified as either *U. rauschi* or *U. yukonensis* based on the identification keys (Wolfgang 1956; Olsen 1968). The spicule and gubernaculum mean length respectively measured 913.1 ± 46.8µm and 112.9 ± 6.1µm for the former, 1641.4 ± 86.2µm and 185.0 ± 3.8µm for the latter (Figure 3.3). All the bears harboured males of *U. rauschi*. Only one adult female grizzly from Banff National Park was co-infected with *U. yukonensis*. Along with males of *U. rauschi*, single-parasitized bears hosted female hookworms featuring body length of 10.77-17.77mm, and either one or two knob-like vulvar appendages respectively posterior to the vulvar opening and delimiting it; in few cases the isolates lacked any vulvar knob (Figure 3.4). These isolates were provisionally identified as three morphovariants of the same species, *U. rauschi*. The only host infected with both *U. rauschi* and *U. yukonensis* males harboured several female hookworms that exhibited additional distinct morphological features. These isolates had body length of 12.89-16.89mm, and were characterized by a linguiform-vulvar flap, or by obvious bi-lobed knob-like appendages remarkably similar to the structures described for the female hookworms found within hosts exclusively *U. rauschi*-positive (Figure 3.5). In addition, they had typical ovejectors, clearly visible in almost all of them. These specimens were provisionally identified as *U. yukonensis*, but unequivocal characterization was needed. The high anatomical variability of the vulvar lobes among the isolated hookworm female specimens, their largely overlapping body size, and the incongruence of our findings with historical descriptions made necessary a molecular approach for the confirmation of the diagnosis.

Figure 3.2: Buccal capsule of a female *Uncinaria* sp. collected from a black bear; lateral (A) and dorsoventral view (B).

Figure 3.3: Spicules of male *U. yukonensis* (A) and *U. rauschi* (B) collected from a grizzly bear.

Figure 3.4: Female morphovariants of *U. rauschi* from black bear; lateral view of no (A), one (B), and two (C) vulvar knobs.

Figure 3.5: Female morphovariants of *U. yukonensis* from grizzly bear; lateral view of the pre-vulvar flap (A) and of the two knob-like vulvar appendages (B).

3.3.2 Sequence and phylogenetic analyses

A total of 16 ITS-1 and 56 ITS-2 sequences was obtained from 35 grizzly and 21 black bear hookworms (40 females, 16 males). Gene fragment size was 432 base pairs (bp) for ITS-1, and 274 bp for ITS-2. The sequence alignment revealed two distinct genotypes, polymorphic at five fixed sites for ITS-1, and seven for ITS-2. The two loci exhibited sets of fixed patterns, concordant in outlining the presence of two exclusive lineages, with no sequence variation related to host species, geographical location, or morphotype for female hookworms (Table 3.2). Voucher specimens representing intact males and each examined female morphotype for *U. rauschi* and *U. yukonensis* were deposited in the USNPC, USDA, under the accession numbers 106982 (*U. yukonensis* from Alberta grizzly bear), 106983 (*U. rauschi* from British Columbia black bear), 107885 (*U. rauschi* from British Columbia grizzly bear), 106985 (*U. rauschi* from Alberta grizzly bear), 107886 (*U. rauschi* from Alberta black bear). ITS-1 and ITS-2 rDNA data from individual hookworm specimens were deposited in the NCBI GenBank database under the accession numbers KJ626425-KJ626519. Anterior and posterior ends of the hookworm specimens used for the genetic analysis were archived as vouchers in 90% ethanol in the collection of the Canadian Cooperative Wildlife Health Centre, University of Calgary, Canada, and in the Nematode Collection, University of California Davis, USA (Table 3.3).

Table 3.2: Sites of polymorphism within the internal transcribed spacers (ITS-1 and ITS-2), nuclear ribosomal DNA, of *U. rauschi* and *U. yukonensis*. The analysis included specimens from both Alberta (AB) and British Columbia (BC). Five nucleotide differences were detected within the ITS-1, seven within the ITS-2.

Host species (location)	Hookworm species	ITS-1 sequence variation sites					ITS-2 sequence variation sites						
		126	177	208	218	271	21	51	54	120	158	175	209
Black bear (AB, Canada)	*U. rauschi*	C	A	C	G	G	C	T	C	A	G	A	C
Black bear (BC, Canada)	*U. rauschi*	C	A	C	G	G	C	T	C	A	G	A	C
Grizzly bear (AB, Canada)	*U. rauschi*	C	A	C	G	G	C	T	C	A	G	A	C
Grizzly bear (BC, Canada)	*U. rauschi*	C	A	C	G	G	C	T	C	A	G	A	C
Grizzly bear (AB, Canada)	*U. yukonensis*	T	G	T	T	A	T	C	T	C	A	G	T

Table 3.3: Hookworm species, specimen gender, female morphotype, host species, host sampling area in western Canada (AB for Alberta, BC for British Columbia), hookworm specimen identifier, and GenBank accession numbers for the internal transcribed spacers (ITS-1 and ITS-2) of the nuclear ribosomal DNA of *U. rauschi* and *U. yukonensis*

Hookworm species	Hookworm gender	Female morphotype¶	Host species	Host location	Hookworm identifiers	ITS-1 accession no.	ITS-2 accession no.
U. rauschi	Female	0 vk	Black bear	Banff National Park, AB	X7237	-	KJ026507
U. rauschi	Female	1 vk	Black bear	Lake Louise, AB	X71	-	KJ026516
U. rauschi	Female	2 vk	Black bear	Jasper National Park, AB	X7229	-	KJ026517
U. rauschi	Male	-	Black bear	Canmore, AB	X70	-	KJ026515
U. rauschi	Female	1 vk	Black bear	Fort Nelson, BC	X20	KJ026501	KJ026508
U. rauschi	Female	2 vk	Black bear	Cowichan Lake, BC	X7206	KJ026502	-
U. rauschi	Female	2 vk	Black bear	Yoho National Park, BC	X7223	-	KJ026509
U. rauschi	Male	-	Black bear	Quesnel, BC	X25	KJ026500	KJ026506
U. rauschi	Female	0 vk	Grizzly bear	Banff National Park, AB	X7217	-	KJ026511
U. rauschi	Female	1 vk	Grizzly bear	Banff National Park, AB	X7214	-	KJ026512
U. rauschi	Female	2 vk	Grizzly bear	Banff National Park, AB	X35	KJ026505	-
U. rauschi	Female	2 vk	Grizzly bear	Lake Louise, AB	X7212	-	KJ026514
U. rauschi	Male	-	Grizzly bear	Lake Louise, AB	X26	KJ026503	-
U. rauschi	Male	-	Grizzly bear	Banff National Park, AB	X16	-	KJ026510
U. rauschi	Female	1 vk	Grizzly bear	Kitwancool Lake, BC	X50	KJ026504	-
U. rauschi	Female	2 vk	Grizzly bear	Kitwancool Lake, BC	X49	-	KJ026518
U. rauschi	Male	-	Grizzly bear	Kitwancool Lake, BC	X51	-	KJ026519
U. yukonensis	Female	1 vf	Grizzly bear	Banff National Park, AB	X7341	KJ026495	KJ026498
U. yukonensis	Female	2 vk	Grizzly bear	Banff National Park, AB	X11	KJ026496	KJ026499
U. yukonensis	Male	-	Grizzly bear	Banff National Park, AB	X15	-	KJ026497

¶ Number of observed vulvar knobs (vk) or flaps (vf).

These isolates generated a sequence alignment of 429 bp and 269 bp for ITS-1 and ITS-2, respectively, when compared to the ITS data deposited for other defined species of Uncinaria. Pairwise comparison of the ITS sequences for U. rauschi and U. yukonensis showed similarity scores of 98.8% for ITS-1 and 97.3% for ITS-2. Between U. yukonensis and U. stenocephala (AF194145) identity was observed for 424/429 bp for ITS-1 (98.8% similarity score), and 264/269 bp for ITS-2 (98.1%). Between U. rauschi and U. stenocephala the identity was of 423/429 bp for ITS-1 (98.6%), and 261/269 bp for ITS-2 (97.0%). Similarity was lower when ursine hookworms were compared with Uncinaria spp. (HQ262104 and HQ262132) from pinniped hosts (Table 3.4).

Table 3.4: Pairwise similarity scores (%) for the internal transcribed spacers (ITS-1 and ITS-2), nuclear ribosomal DNA, of Uncinaria species. Sequence similarity is reported for both ITS-2 and ITS-1 (in parentheses).

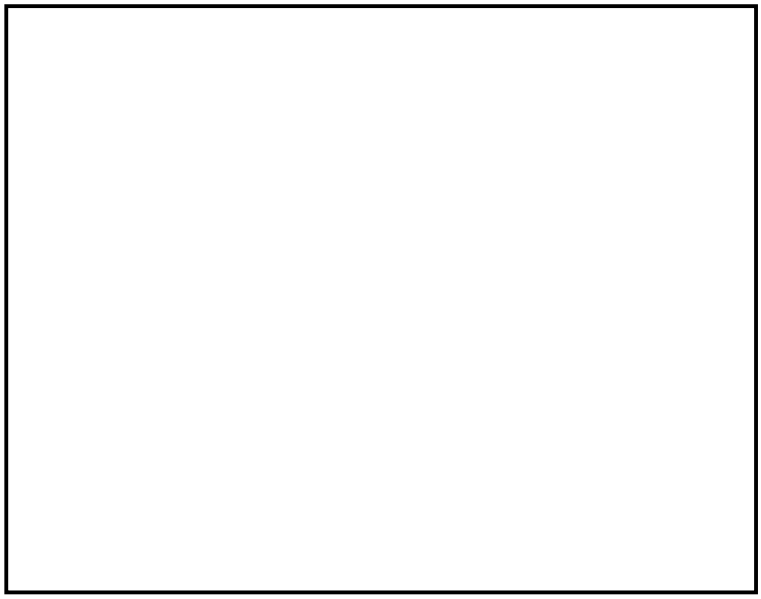
	U. yukonensis	U. rauschi	U. stenocephala[a]	U. stenocephala[b]	U. lucasi[c]	U. hamiltoni[d]
U. yukonensis	-	97.3% (98.8%)	98.1% (98.8%)	97.3% (98.8%)	90.5% (93.5%)	92.0% (93.3%)
U. rauschi	97.3% (98.8%)	-	97.0% (98.6%)	96.2% (98.6%)	89.8% (93.7%)	90.2% (92.8%)
U. stenocephala[a]	98.1% (98.8%)	97.0% (98.6%)	-	99.2 % (100%)	90.2% (93.7%)	90.9% (93.3%)
U. stenocephala[b]	97.3% (98.8%)	96.2% (98.6%)	99.2 % (100%)	-	89.4% (93.7%)	90.2% (93.3%)
U. lucasi[c]	90.5% (93.5%)	89.8% (93.7%)	90.2% (93.7%)	89.4% (93.7%)	-	97.7% (99.1%)
U. hamiltoni[d]	92.0% (93.3%)	90.2% (92.8%)	90.9% (93.3%)	90.2% (93.3%)	97.7% (99.1%)	-

[a] GenBank accession no. AF194145 (Nadler et al. 2000b).
[b] GenBank accession no. HQ262052 (Nadler et al. 2013).
[c] GenBank accession no. HQ262132 (Nadler et al. 2013).
[d] GenBank accession no. HQ262104 (Nadler et al. 2013).

The phylogenetic analysis of the ITS-1 and ITS-2 rDNA regions was carried out using both MP and ML. The multiple, concatenated alignment for ITS-1 and ITS-2 of nine taxa resulted in a dataset of 698 characters. The number of parsimony-informative sites was 78. In the MP analysis, the single most parsimonious tree had a length of 114 steps, a consistency index of 0.83, and maximum statistical support (bootstrap values 100%) for three distinct clades. The same topology was also strongly supported by ML bootstrap values of 99-100% in the bootstrap ML consensus tree generated from 2000 bootstrap replicates (Figure 3.6). The phylogenetic analysis conducted herein clearly supports nestedness of the ursine hookworms U. rauschi and U. yukonensis with U. stenocephala. These three species formed a clade distinct from the monophyletic group including Uncinaria spp. from otariid pinnipeds. However, the two clades were poorly supported, with bootstrap values < 70% for both MP

and ML trees. The internal nodes before the terminal taxa of these two clades were polytomies, indicating either uncertainty about phylogenetic relationships or simultaneous divergence of the descendants.

Figure 3.6: Phylogenetic analysis of concatenated internal transcribed spacers of the nuclear ribosomal DNA for nine nematode taxa of the family Ancylostomatidae. The species *Ancylostoma duodenale* and *A. caninum* were used as outgroups (GenBank accession numbers EU344797 and JQ812694, respectively). The other sequences used include AF194145 and HQ262052 (*Uncinaria stenocephala*), HQ262132 (*U. lucasi*), HQ262104 (*U. hamiltoni*), and HQ262089 (*Uncinaria* sp.). The specimen identifiers X7206 and X7341 were used for *U. rauschi* and *U. yukonensis*, respectively. The displayed host is the type host from which the ITS sequences were obtained. The tree represents the results of both maximum parsimony (MP) and maximum likelihood (ML) analyses. MP and ML bootstrap support percentages are indicated above and below the lines, respectively.



3.3.3 Hookworm morphometry

Uncinaria rauschi and *U. yukonensis* specimens (n=430) were subjected to morphometric and statistical analyses. As determined by the Wilcoxon rank sum test with continuity correction, the various female morphotypes within the taxon *U. rauschi* did not show significant intra-specific morphometric differences relative to the number of vulvar knobs ($P > 0.05$). Similarly, morphometric traits of *U. yukonensis* females did not significantly differ when specimens with a linguiform vulvar flap were compared to specimens characterized by two vulvar knobs ($P > 0.05$).

The Wilcoxon rank sum test with continuity correction revealed intra-specific, host-related morphometric differences when *U. rauschi* from black bears were compared to the specimens obtained from grizzlies. Both male and female adult *U. rauschi* from black bears were significantly larger than those collected from grizzlies ($W = 9220$ and $P < 0.001$ for females; $W = 3064$ and $P < 0.001$ for males) (Figure 3.7). The same significant relationship

existed for the tail size of *U. rauschi* females ($W = 5569$ and $P < 0.001$ for tail width; $W = 5787$ and $P < 0.001$ for tail length) (Figure 3.8). Further measurements were not significantly different between *U. rauschi* from black bears and *U. rauschi* from grizzlies ($P > 0.05$). The finding of *U. yukonensis* from only one host did not allow statistical comparisons among specimens within the taxon. The statistical test was also applied for inter-specific comparisons between the collected *U. rauschi* and *U. yukonensis*. Highly significant differences were present between *U. rauschi* and *U. yukonensis*, with the body of the former significantly larger than the latter ($W = 1241$ and $P < 0.001$ for females; $W = 48$ and $P < 0.001$ for males) (Figure 3.7). Only female tail length and width were not statistically significant when *U. rauschi* and *U. yukonensis* were compared ($P > 0.05$) (Figure 3.8). However, while measurements of the reproductive traits for male specimens showed distinct, non-overlapping features between *U. rauschi* and *U. yukonensis* ($W = 0$ and $P < 0.001$) (Figure 3.9), the females of these two taxa lacked of such marked differences for any anatomic trait (Figure 3.7 and Figure 3.8). The results of the morphometric analysis are shown in Table 3.5 and Table 3.6. Historical data from Wolfgang (1956), Olsen (1968), and Rausch et al. (1979) have been included.

Figure 3.7: Body length for female (1) and male (2) hookworm specimens; comparison between U. rauschiin black bears (A), U. rauschiin grizzly bears (B), and U. yukonensis (C). Measurements are in micrometers. For the distribution of the data the thicker line indicates its median value, the box shows the distance between first and third quartile, the whiskers represent the lowest and the highest datum respectively within 1.5 interquartile range of the lower and upper quartile. Outliers are indicated by dots.

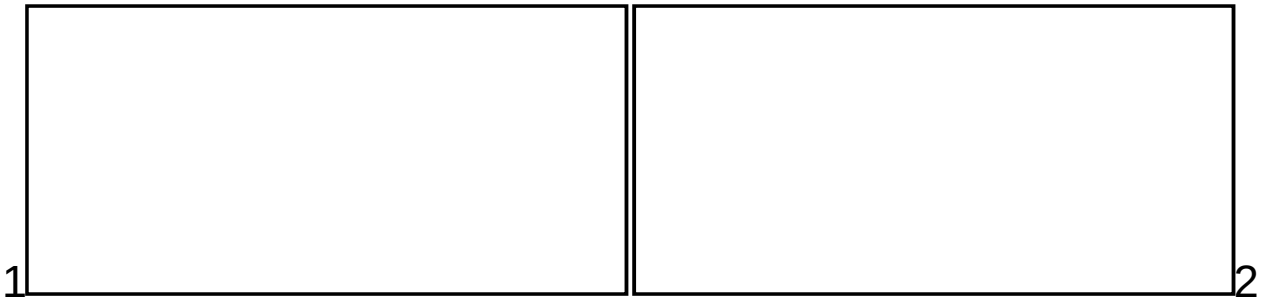


Figure 3.8: Tail length (1) and width (2) for female hookworm specimens; comparison between U. rauschiin black bears (A), U. rauschiin grizzly bears (B), and U. yukonensis (C). Measurements are in micrometers. For the distribution of the data the thicker line indicates its median value, the box shows the distance between first and third quartile, the whiskers represent the lowest and the highest datum respectively within 1.5 interquartile range of the lower and upper quartile. Outliers are indicated by dots.

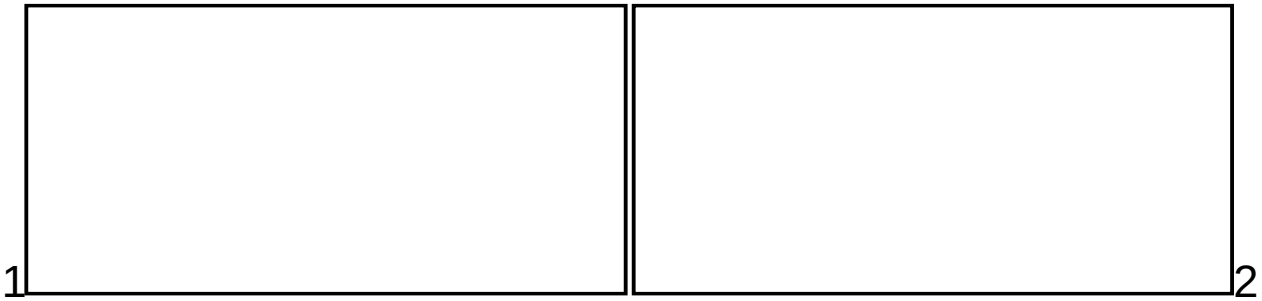


Figure 3.9: Gubernaculum length (1) and spicule length (2) in male specimens, comparison between U. rauschiin black bears (A), U. rauschiin grizzly bears (B), and U. yukonensis (C). Measurements are in micrometers. For the distribution of the data the thicker line indicates its median value, the box shows the distance between first and third quartile, the whiskers represent the lowest and the highest datum respectively within 1.5 interquartile range of the lower and upper quartile. Outliers are indicated by dots.

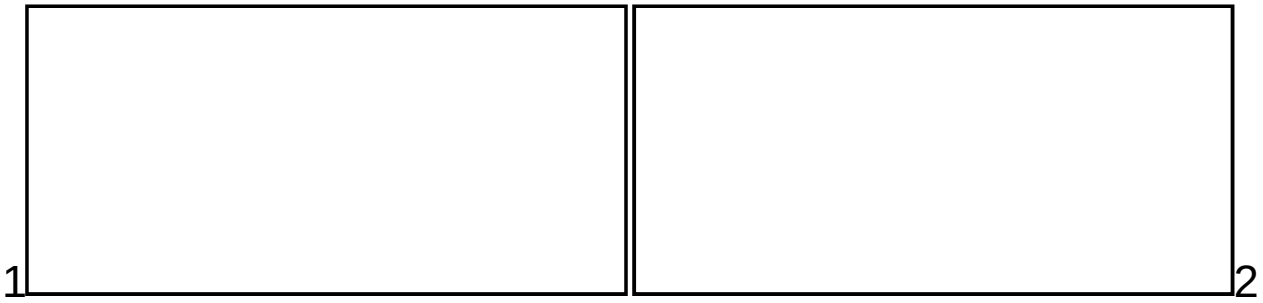


Table 3.5: Morphometrics of *Uncinaria* spp. males from *Ursus* hosts. Unless indicated, measurements of length (L) and width (W) are in micrometers, displayed as mean ± standard deviation with the range in parentheses. Only the range was available from Wolfgang (1956)[a], Olsen (1968), and Rausch et al. (1979)[b]. The number of analysed specimens is reported along with the parasite taxon.

Parasite species	*U. rauschi* (n=115)	*U. rauschi* (n=31)	*U. yukonensis* (n=20)	*U. rauschi* (n=NA)	*U. yukonensis* (n=2[a]; n=3[b])
Host	*U. americanus*	*U. a. horribilis*	*U. a. horribilis*	*U. americanus* *U. a. horribilis*	*U. americanus*[a] *U. arctos*[b]
Reference	Present study	Present study	Present study	Olsen (1968)	Wolfgang (1956)[a] Rausch et al. (1979)[b]
Body L (mm)	10.20 ± 0.77 (7.39–12.14)	8.63 ± 1.14 (6.90–10.71)	12.28 ± 0.76 (10.56–13.44)	7.40–9.10	10.50–12.94[a] 13.40–15.10[b]
Body W at bursa	275.6 ± 30.8 (165.7–354.2)	271.8 ± 33.2 (209.5–355.1)	381.0 ± 18.3 (355.0–426.0)	243–297	520[a] 556–576[b]
Buccal capsule L	152.8 ± 9.0 (128.4–177.0)	162.1 ± 12.2 (141.6–188.8)	162.9 ± 15.2 (135.7–187.5)	213–297	230–280[a] 196–222[b]
Buccal capsule W	120.7 ± 8.3 (101.4–150.2)	120.6 ± 9.1 (104.0–137.7)	129.5 ± 10.7 (109.4–152.2)	133–186	150–190[a] 131–150[b]
Oesophagus L	819.8 ± 38.2 (731.4–956.6)	817.7 ± 44.4 (736.5–915.8)	931.0 ± 32.5 (871.1–974.1)	763–856	860–1150[a] 949–982[b]
Oesophagus W	166.6 ± 16.9 (115.1–221.5)	165.2 ± 19.4 (113.2–197.3)	184.7 ± 21.4 (128.5–207.3)	167–191	196–222[b]
Body W at oesophageal base	262.8 ± 22.8 (211.6–335.9)	275.7 ± 33.6 (196.5–366.7)	290.4 ± 12.6 (270.6–312.5)	-	-
Nerve ring L*	363.0 ± 42.6 (221.8–483.4)	349.6 ± 40.5 (255.1–470.9)	374.3 ± 30.4 (331.0–428.7)	445–583	640–750[a] 576–602[b]
Excretory pore L*	711.4 ± 74.5 (537.1–898.9)	685.9 ± 55.6 (584.0–788.9)	770.3 ± 37.0 (681.3–839.3)	-	655–766[b]
Bursa L	566.6 ± 55.5 (399.5–680.9)	544.2 ± 80.0 (386.0–689.3)	813.5 ± 111.5 (642.5–1064.4)	-	-
Bursa W	616.6 ± 114.6 (426.9–908.1)	598.3 ± 76.2 (467.2–771.8)	919.1 ± 175.9 (644.6–1360.2)	-	-
Spicules L	913.4 ± 48.7 (799.6–1172.9)	912.8 ± 35.6 (786.9–973.8)	1641.4 ± 86.2 (1320.4–1763.7)	816–954	1650–1750[a] 1600–1800[b]
Gubernaculum L	111.9 ± 5.8 (90.5–120.6)	116.5 ± 6.0 (108.7–128.4)	185.0 ± 3.8 (176.0–190.6)	114–146	200[a] 196–222[b]
Gubernaculum W posteriorly	18.0 ± 2.0 (11.8–22.5)	17.8 ± 1.8 (13.6–20.4)	28.2 ± 2.1 (22.8–30.4)	18–26	-
Dorsal ray L	190.4 ± 16.2 (156.2–232.8)	190.2 ± 12.5 (172.0–221.8)	292.7 ± 19.4 (246.6–316.2)	-	-

Parasite species	U. rauschi (n=115)	U. rauschi (n=31)	U. yukonensis (n=20)	U. rauschi (n=NA)	U. yukonensis (n=2[a]; n=3[b])
Host	U. americanus	U. a. horribilis	U. a. horribilis	U. americanus U. a. horribilis	U. americanus[a] U. arctos[b]
Reference	Present study	Present study	Present study	Olsen (1968)	Wolfgang (1956)[a] Rausch et al. (1979)[b]
Dorsal ray base W	55.1 ± 5.9 (42.6–80.1)	52.9 ± 7.9 (40.7–66.5)	77.6 ± 7.0 (67.2–89.3)	-	-
Dorsal ray bifurcation W	45.0 ± 6.6 (31.2–59.5)	46.3 ± 6.3 (33.9–56.0)	65.2 ± 7.1 (49.7–76.5)	-	-

‰ Wolfgang (1956)[a], Olsen (1968), and Rausch et al. (1979)[b] made this measurement at the maximum body width.

Š Measurements made at the maximum width of the oesophagus.

* Measurements made to the anterior end of the oesophagus; Wolfgang[a] (1956), Olsen (1968), and Rausch et al. (1979)[b] measured these distances to the anterior end of the body.

Table 3.6: Morphometrics of *Uncinaria* spp. females from *Ursus* hosts. Unless indicated, measurements of length (L) and width (W) are in micrometers, displayed as mean ± standard deviation with the range in parentheses. Only the range was available from Wolfgang (1956)[a], Olsen (1968), and Rausch et al. (1979)[b]. The number of analysed specimens is reported along with the morphotype.

Parasite species	U. rauschi			U. rauschi			U. yukonensis		U. rauschi	U. yukonensis
Host	U. americanus			U. a. horribilis			U. a. horribilis		U. americanus U. a. horribilis	U. americanus[a] U. arctos[b]
Reference	Present study			Present study			Present study		Olsen (1968)	Wolfgang (1956)[a] Rausch et al. (1979)[b]
Morphotype¶	0 vk (n=2)	1 vk (n=98)	2 vk (n=70)	0 vk (n=5)	1 vk (n=27)	2 vk (n=28)	1 vf (n=27)	2 vk (n=12)	(n=NA)	(n=5[a]; n=3[b])
Body L (mm)	14.20 ± 0.83 (13.62–14.79)	14.27 ± 0.89 (10.67–16.17)	13.94 ± 1.69 (8.16–16.47)	10.95 ± 2.25 (8.40–12.98)	11.51 ± 1.66 (8.97–15.23)	10.57 ± 1.71 (7.77–13.91)	15.23 ± 0.96 (12.89–16.71)	15.61 ± 0.81 (13.72–16.89)	7.2–10.5	14.25–17.12[a] 19.4–19.9[b]
Body W at vulva¥	454.6 ± 9.6 (447.8–461.4)	430.0 ± 38.8 (318.4–528.3)	409.0 ± 55.7 (222.3–497.0)	382.1 ±78.7 (283.1–465.5)	413.0 ± 45.9 (342.1–515.4)	363.8 ± 64.5 (254.8–482.6)	437.3 ± 35.4 (373.0–498.8)	438.2 ± 38.3 (355.0–485.7)	275–381	490–600[a] 628–884[b]
Buccal capsule L	165.2 ± 14.3 (155.1–175.3)	171.7 ± 10.4 (139.1–197.5)	171.2 ± 13.3 (140.2–195.9)	171.5 ± 10.7 (161.6–188.3)	173.3 ± 15.7 (141.2–208.3)	178.0 ± 18.5 (141.7–217.6)	177.7 ± 9.0 (164.4–203.8)	183.8 ± 10.1 (165.7–202.3)	215–282	230–280[a] 183–229[b]
Buccal capsule W	148.6 ± 7.6 (143.2–154.0)	140.9 ± 11.5 (106.5–176.7)	143.9 ± 10.1 (119.2–165.3)	143.5 ± 7.1 (138.1–154.7)	139.9 ± 9.8 (117.5–159.4)	142.8 ± 12.1 (106.2–160.5)	145.6 ± 11.3 (128.8–168.6)	146.9 ± 15.2 (120.7–171.1)	133–186	150–190[a] 117–150[b]
Oesophagus L	883.8 ± NA (NA)	902.3 ± 58.6 (784.8–1149.1)	895.6 ± 37.0 (813.1–988.5)	884.1 ± 89.2 (831.6–1042.2)	903.6 ± 51.0 (785.7–1012.2)	888.4 ± 54.2 (763.5–1006.9)	997.4 ± 39.8 (897.8–1067.1)	994.1 ± 39.4 (936.1–1062.6)	742–954	860–1150[a] 940–1000[b]
Oesophagus W*	181.4 ± 12.9 (172.2–190.6)	196.6 ± 21.1 (137.4–250.1)	197.8 ± 22.9 (155.4–241.8)	186.8 ± 14.6 (162.4–200.3)	193.7 ± 22.9 (131.4–227.1)	188.5 ± 16.3 (150.6–215.2)	197.6 ± 24.3 (136.5–234.9)	200.0 ±22.0 (163.3–230.2)	159–201	229–242[b]
Body W at oesophageal base	316.0 ± 18.8 (302.7–329.4)	311.6 ± 30.9 (240.5–395.0)	316.6 ± 28.8 (256.4–398.1)	316.1 ± 42.2 (278.1–382.4)	352.3 ± 40.3 (299.2–451.7)	330.8 ±32.7 (274.2–394.0)	333.0 ± 21.3 (292.8–396.8)	338.4 ± 18.7 (309.7–368.6)	-	-

Parasite species	U. rauschi			U. rauschi			U. yukonensis		U. rauschi	U. yukonensis
Host	U. americanus			U. a. horribilis			U. a. horribilis		U. americanus U. a. horribilis	U. americanus[a] U. arctos[b]
Reference	Present study			Present study			Present study		Olsen (1968)	Wolfgang (1956)[a] Rausch et al. (1979)[b]
Morphotype¶	0 vk (n=2)	1 vk (n=98)	2 vk (n=70)	0 vk (n=5)	1 vk (n=27)	2 vk (n=28)	1 vf (n=27)	2 vk (n=12)	(n=NA)	(n=5[a]; n=3[b])
Nerve ring L*	361.7 ± NA (NA)	386.1 ± 43.7 (294.9-498.5)	386.8 ± 40.2 (320.2-493.7)	370.2 ± 52.9 (316.8-451.8)	379.4 ± 34.2 (284.5-435.8)	381.3 ± 43.0 (331.7-509.8)	420.9 ± 63.2 (359.7-634.2)	401.2 ± 37.5 (371.5-494.3)	318-614	640-750[a] 576-713[b]
Excretory pore L*	907.5 ± 181.8 (778.9-1036.0)	762.2 ± 74.0 (588.6-1001.9)	772.6 ± 71.1 (657.5-1077.3)	748.9 ± 123.2 (647.3-950.7)	749.89 ± 63.1 (646.6-883.9)	757.1 ± 67.0 (665.9-939.9)	847.4 ± 70.8 (714.6-979.2)	849.7 ± 82.8 (723.2-993.1)	488-700	674[b]
Vulva-end (mm)	5.27 ± 0.14 (5.17-5.36)	5.19 ± 0.45 (4.06-6.24)	5.06 ± 0.72 (2.78-6.10)	3.73 ± 1.17 (2.32-4.77)	3.93 ± 0.64 (2.89-5.52)	3.56 ± 0.82 (2.19-5.09)	5.45 ± 0.43 (4.64-6.19)	5.39 ± 0.43 (4.73-6.11)	2.4-3.5	7.1-7.9[b]
Tail L‰	162.7 ± 19.3 (149.1-176.4)	187.1 ± 23.2 (127.5-244.7)	190.9 ± 19.3 (140.8-242.9)	185.6 ± 26.8 (149.8-210.2)	173.3 ± 18.1 (140.3-201.3)	177.6 ± 23.7 (137.2-233.6)	191.1 ± 24.0 (118.0-238.7)	189.0 ± 28.0 (131.5-247.5)	127-170	230-250[a] 189-255[b]
Tail W	136.01 ± 12.43 (127.22-144.8)	141.2 ± 19.0 (103.5-225.4)	139.8 ± 13.9 (106.5-168.7)	128.9 ± 11.7 (114.1-140.4)	135.3 ± 11.6 (118.3-160.3)	133.7 ± 22.1 (111.0-208.8)	144.2 ± 14.7 (114.3-182.4)	146.8 ± 15.6 (120.7-175.2)	-	-
Egg LŠ	70.9 ± 0.3 (70.7-71.1)	77.6 ± 4.9 (69.0-90.0)	77.1 ± 5.8 (66.8-87.0)	78.4 ± 5.5 (72.9-83.8)	81.4 ± 4.5 (72.3-89.1)	80.6 ± 5.0 (71.8-89.3)	80.3 ± 4.5 (70.1-88.8)	80.4 ± 4.1 (73.4-86.4)	72-80	75-83[a] 79-86[b]
Egg WŠ	38.6 ± 3.8 (35.8-41.3)	45.5 ± 4.0 (38.5-57.3)	45.4 ± 4.8 (38.0-56.7)	47.9 ± 3.3 (44.1-50.2)	45.6 ± 3.9 (40.4-53.9)	45.7 ± 3.5 (39.5-53.5)	44.9 ± 3.9 (40.5-54.5)	44.3 ± 3.4 (38.1-48.3)	35-45	60-68[a] 43-49[b]

¶ Number of observed vulvar knobs (vk) or flaps (vf).

¥ Measurements made at the maximum width of the body.

% Measurements made at the maximum width of the oesophagus.

* Measurements made to the anterior end of the oesophagus; Wolfgang[a] (1956), Olsen (1968), and Rausch et al.[b] (1979) measured these distances to the anterior end of the body.

‰ Tail length from anus to posterior end.

Š Measurements made from eggs in the uterus.

3.4 Discussion

3.4.1 Hookworm identity

This study reports morphological features and genetic data from the ITS rDNA regions of *U. rauschi* and *U. yukonensis* to provide new insights into their systematics. The variability of vulvar morphology for the isolated hookworm females has the potential to confuse even the most expert taxonomist about the presence of additional species other than the two currently known ones. Wolfgang (1956) and Olsen (1968) described a prominent ventral flap covering the vulva for *U. yukonensis* females, whereas female *U. rauschi* lacked a pronounced pre-vulvar flap. These authors indicated the anatomy of the vulva as a species-specific feature to distinguish between *U. rauschi* and *U. yukonensis*. Together with *U. yukonensis* females having the typical linguiform flap, the present research recovered specimens without, or with reduced, obvious vulvar knobs. Even higher variability was observed for female hookworms within each *U. rauschi*-positive host, with isolates having either one, two, or no vulvar knobs. Nevertheless, the distinctive male reproductive traits (i.e., spicules, gubernaculum, and dorsal ray of the bursa) allowed the unequivocal identification of *U. rauschi* and *U. yukonensis* in the sampled bears. The morphological examination suggested rejecting the hypothesis of more than two species of *Uncinaria* infecting Canadian grizzlies and black bears, and in contrast supported the presence of phenotypic plasticity for *U. rauschi* and *U. yukonensis*. Previous studies on parasites within the order Strongylida have shown how polymorphism among adults can be common for both males and females (Lancaster and Hong 1981; Lichtenfels and Hoberg 1993; Drozd 1995; Lichtenfels et al. 1997; Hoberg et al. 1999, 2001, 2012). In particular, the vulva is a variable structure for many species of strongylids. Careful analyses showed the presence of morphovariants where it was once thought there were multiple species infections; vice versa, they suggested the presence of different species where it was believed there was a single species infection (e.g., Lancaster and Hong 1990; Hoberg et al. 1993; Lichtenfels et al. 1994; Newton et al. 1998).

Morphological observations were supported by genetic data from the ITS rDNA loci. The molecular analysis unambiguously confirmed the female polymorphism hypothesis. The ITS-1 and ITS-2 sequences for *U. rauschi* and *U. yukonensis* constantly showed a total of 12 nucleotide differences at fixed sites. The analysed specimens represented the various morphovariants

isolated during the study in both grizzlies and black bears from different collection localities in Alberta and British Columbia. The ITS dataset delineated the presence of two distinct, independent evolutionary lineages, with no intra-specific variability. With the development and the application of molecular techniques to parasite systematics, many cases of phenotypic plasticity were confirmed on a molecular basis. In many instances for strongylid nematodes, and other parasitic taxa, "distinctive" species were in fact one and the same, but studies on single species concealing a complex of genetically different taxa are also abundant (e.g., Stevenson et al. 1995, 1996; Dallas et al. 2000, 2001; Hung et al. 1997, 1999; Leignel et al. 2002). U. rauschi and U. yukonensis, the analysis of the ITS regions confirmed their species status, and the presence of female polymorphism within both taxa. The ITS regions were further demonstrated to be reliable genetic markers to discriminate among nematode species; their low level (typically *ƒ* 1%) of intra-specific variability, along with higher rates of evolution than functional rRNA genes, makes ITS-1 and ITS-2 valuable molecular markers for species delineation (Baldwin et al. 1995; Stevenson et al. 1995, 1996; Dallas et al. 2000; Gasser and Newton 2000; Nadler et al. 2000a, 2000b; Nadler 2002; Chilton 2004; Ngui et al. 2012; Ramos et al. 2013).

3.4.2 Hookworm phylogeny

Pairwise sequence divergence of Uncinaria spp. showed a close relationship among U. rauschi, U. yukonensis, and U. stenocephala. Surprisingly, genetic distance was higher between the ursine hookworms U. rauschi and U. yukonensis (98.8% for ITS-1 and 97.3% for ITS-2) than between U. yukonensis and U. stenocephala, a parasite of canids and felids (98.8% for ITS-1 and 98.1% for ITS-2). Similarity scores were lower when U. rauschi and U. stenocephala were compared (98.6% for ITS-1 and 97.0% for ITS-2). However, phenograms based on pairwise similarity were not inferred. Distance methods for phylogenetic approaches ignore variation in rates of sequence evolution among lineages and confound interpretations on evolutionary divergence of taxa (Nadler 2002). The phylogenetic analysis of the ITS-1 and ITS-2 sequence datasets confirmed nestedness between the ursine hookworms U. and stenocephala and evolutionary distance between this clade and the one formed by hookworms of otariid pinnipeds. The concordant MP and ML phylogenetic trees displayed the presence of three monophyletic clades with maximum statistical support. The evolutionary distance separating Uncinaria spp. of

terrestrial mammalian hosts, Uncinaria spp. of marine mammalian hosts, and species of the genus Ancylostoma was supported by MP and ML bootstrap values of 100%. However, bootstrap percentages < 70% did not allow the full resolution of the clades composed by members of the genus Uncinaria, preventing further inference regarding host-parasite co-evolution. Uncertainty regarding the phylogenetic relationship among U. rauschi, U. yukonensis, and U. stenocephala would require the analysis of multiple, more rapidly evolving genes to shed light on their evolutionary relatedness.

The compliance between MP and ML phylogenetic analyses of U. rauschi and U. yukonensis provides insights into evolutionary processes that otherwise would not be apparent from investigations based on morphology alone (Blaxter 2003; Nadler and Pérez-Ponce de León 2011). Evolutionary closeness has been interlaced with life-cycle features for A. duodenale and A. caninum (Loukas and Prociv 2001; Jex et al. 2009), and it seems to be valid also for Uncinaria spp. of pinniped hosts (Nadler et al. 2013). In fact, sister species such as A. caninum and A. duodenale, and as Uncinaria spp. in northern fur seals and California sea lions, not only are phylogenetically related, but also share common life-cycle features such as transmammary transmission of their larval stages. Hookworm infections acquired after birth via milk-borne larvae were reported as a primary cause of debilitation and mortality in pups of New Zealand sea lions (Castinel et al. 2007a), northern fur seals (Lyons et al. 1997; Lyons et al. 2011b), and California sea lions (Spraker et al. 2004, 2007; Lyons et al. 2005). The present study observed that the ursine Uncinaria spp. are phylogenetically distant from hookworms in otariid pinnipeds. In contrast, it found phylogenetic nestedness of U. rauschi and U. yukonensis with U. stenocephala, one of the least pathogenic hookworm species (Weller 1968; Bowman et al. 2010). A mainly oral infection route has been demonstrated for U. stenocephala with a series of experimental infections (Gibbs 1961), whereas no data supported the transmammary transmission of its larvae from the mother to the newborns (Walker and Jacobs 1982, 1985; Traversa 2012). Corroborated by the observation of bear adults as the most hookworm-infected age classes (see Chapter 2), the clustering of U. rauschi, U. yukonensis, and U. stenocephala within a distinct evolutionary lineage strongly suggests a similar life-cycle for these hookworm species. No support for U. rauschi and U. yukonensis as sister species of the pinniped

hookworms, therefore for potential heavy intestinal infections in bear cubs caused by milk-borne larvae (i.e., transmammary transmission route), has been provided by this study.

3.4.3 Hookworm morphometry

Measurements on the ursine hookworms showed strong sexual dimorphism in agreement with previous morphological studies on *U. rauschi* (Olsen 1968), *U. yukonensis* (Wolfgang 1956; Rausch et al. 1979), and other species of *Uncinaria* (e.g., Gibbs 1961; Nadler et al. 2000a; Castinel et al. 2006; Ramos et al. 2013). The original descriptions of *U. rauschi* and *U. yukonensis* also reported total body length as a discriminating feature, with the latter species distinctly larger than the former for adults of both genders (Wolfgang 1956; Olsen 1968). The present study confirmed this only significant differences for body size and other anatomic structures between *U. rauschi* and *U. yukonensis*, but it also highlighted the great morphometric overlap between the two hookworm species. The morphological examination of 376 *U. rauschi* adult found a wider morphometric range for almost all the analysed anatomic structures than the original study by Olsen (1968). A larger size was particularly remarkable for body length in both *U. rauschi* males [6.90–12.14mm in the current study, 7.40–10.10mm in Olsen (1968)] and females [7.77–16.47mm in the current study, 7.20–13.50mm in Olsen (1968)]. The statistical analysis demonstrated that bigger dimensions were not significantly correlated with the number of vulvar knobs for female *U. rauschi* isolates. On the other hand, morphometric data on 59 adult *U. yukonensis* from a grizzly closely resembled previous measurements on specimens from black bears of the Yukon Territory (Wolfgang 1956), whereas the same hookworm species was much larger in Russian brown bears (Rausch et al. 1979). The body length for *U. yukonensis* males [10.56–13.44mm in the current study, 10.50–12.94mm in Wolfgang (1956), 13.40–15.10mm in Rausch et al. (1979)] and females [12.18–18.89mm in the current study, 14.25–17.12mm in Wolfgang (1956), 19.40–19.90mm in Rausch et al. (1979)] considerably differs between North American reports and the study on brown bears from the Russian Far East.

This study observed marked host-related intraspecific differences within the taxon *U. rauschi*, with specimens from black bears significantly larger than those from grizzlies. The isolation of *U. yukonensis* from only one grizzly bear precluded a similar statistical comparison. However, as previously stated, the morphometric data on *U. yukonensis* showed close similarity

to the original measurements (Wolfgang 1956), and a smaller body size than isolates from Russian brown bears (Rausch et al. 1979). Such differences may be related to more recent host-parasite associations conditioning fitness and growth of *U. rauschi* and *U. yukonensis* within North American bears. The apparent origin of *U. yukonensis* in the Palearctic (Rausch et al. 1979) and of *U. rauschi* in the Nearctic (no reports from Eurasia) endorses the hypothesis of host-switching events during postglacial time, with *U. rauschi* and *U. yukonensis* colonizing the grizzly and the black bear, respectively. The history of grizzly and black bears began with their divergence from common ancestry approximately 5.05 mya, at the Mio–Pliocene boundary (Krause et al. 2008). Thereafter, black bears settled in North America, whereas the ancestors of the modern grizzly bears colonized it at the beginning of the Wisconsin glaciation (~100–70 ka) through the Bering Land Bridge, used as a refugium until the recession of the Wisconsin ice sheets, ca. 10 ka (Kurtén and Anderson 1980). Glacial refugia in the Beringian nexus may have favoured genetic divergence and speciation of grizzlies and Kodiak bears from their Eurasian precursors (Waits et al. 1998). During the Wisconsin glaciation, black bears used multiple refugia in the Pacific Northwest, isolated from Beringia by the Cordilleran and Laurentide continental ice sheets (Shields and Kocher 1991; Shafer et al. 2010). Therefore, the ancestors of grizzlies and black bears have been geographically separated for millions of years, and became sympatric merely after the recession of the ice sheets (Kurtén and Anderson 1980; Krause et al. 2008). The phylogeography of North American bears seems to support the hypothesis of ancient *U. americanus*–*U. rauschi* and *U. arctos*–*U. yukonensis* associations. A relatively recent colonization event may be the rationale for the smaller size of *U. rauschi* in grizzly bears, hypothesised as the most recent host–parasite relationship. Similarly, smaller *U. yukonensis* from North American bears may reflect both a recent host-switching event in black bears, and the migration with subsequent speciation of the grizzly bear in the Nearctic ecozone. Similar hypotheses have been formulated for other host–parasite systems (e.g., Gemmill et al. 2000; Dare et al. 2008). Particularly fitting is host-induced size differences for *U. lucasi* in northern fur seals and Steller sea lions (Olsen 1952; Nadler et al. 2013). Adult *U. lucasi* from the two pinniped hosts were found to be significantly larger in Steller sea lions than in northern fur seals, suggesting that Steller sea lions are the most ancient host for *U. lucasi*, allowing the parasite to maximise its growth (Nadler et al. 2013). Parasite body size has been reported as a reliable

measure of parasite fitness and reproductive output for female parasites within the host (Skorping et al. 1991; Poulin and Morand 2000; Poulin 2007). The potentially different biogeographical history of *U. rauschi* and *U. yukonensis* in ursid hosts seems to suggest an important role for fitness pulses and ecological adaptations in the diversification of these host–parasite assemblages (Dare et al. 2008; Hoberg and Brooks 2010).

3.5 Conclusions

The present study reports a variety of morphotypes characterized by different vulvar appendages and wide morphometric traits never described previously for *U. rauschi* and *U. yukonensis*. Such documentation of phenotypic plasticity for the two hookworm species legitimized the application of molecular tools to improve our understanding of Uncinaria biodiversity in North American bears. ITS rDNA sequence data provides undisputable support for the monophyly of each of the ursine hookworm species, indicating that *U. rauschi* and *U. yukonensis* have been evolving independently. The combination of morphology and genetics is an invaluable resource for both initial prospecting of parasite species and further integration of characters useful to delimit and diagnose parasite taxa (Pérez-Ponce de León and Nadler 2010; Nadler and Pérez-Ponce de León 2011). The concerted morphological and molecular approach allowed testing of hypotheses concerning the phylogeny, biogeography, and life history traits of *U. rauschi* and *U. yukonensis*. Nevertheless, the life-cycle of these hookworms remains enigmatic, and the relatedness of *U. rauschi* and *U. yukonensis* with *U. stenocephala* needs to be resolved. A comprehensive multi-gene phylogenetic hypothesis for hookworm species of terrestrial mammals would be a useful framework for further investigations of host–parasite relationships. Molecular tools perfectly complement morphological and ecological observations on host–parasite systems; phylogenetic closeness as a proxy for similar life history traits has already been inferred for hookworm species (Lukas and Prociv 2001; Jex et al. 2009). Phylogenetic studies of hookworms are central for the understanding of their epidemiology and population genetics, and can have important practical implications in relation to infection control; more information could provide important insights into host affiliations, gene flow and transmission patterns for hookworm species (Hu et al. 2002; Jex et al. 2009; Nadler and Pérez-Ponce de León 2011).

CHAPTER FOUR: FIRST FINDING OF TAENIA ARCTOS FROM A GRIZZLY AND A BLACK BEAR OF KANANASKIS COUNTRY, ALBERTA, CANADA

4.1 Introduction

Tapeworm parasites of the genus *Taenia* are typical of terrestrial mammals. They develop as adult strobilate stages in the small intestine of a carnivore definitive host, and as cystic larval forms (metacestodes) in the body cavity and tissues of intermediate hosts. North American bears are reported to be final hosts for several taeniid species, particularly *T. krabbei* and *T. hydatigena*, for which canids and cervids respectively act as primary definitive and intermediate hosts (Jones and Pybus 2001). However, the recent characterization of *T. arctos* has raised doubts on the identity of historical records of *Taenia* spp. from ursid hosts (Lavikainen et al. 2011; Kutz et al. 2012). The taxonomic classification of *T. arctos* was achieved after the morphological and molecular investigation of adult tapeworms harboured in the small intestine of brown bears from Finland (Haukisalmi et al. 2011). Larval stages of the parasite, reported in the skeletal muscle of Finnish Eurasian elk (*Alces alces*) and Alaskan moose, confirmed *Alces* spp. as intermediate hosts of *T. arctos* (Lavikainen et al. 2010, 2011). Recently, muscle cysts of *T. o. krabbei* were reported in a muskox from an introduced population in the Kangerlussuaq area, west Greenland (Raundrup et al. 2012). However, Raundrup et al. (2012) actually detected *T. arctos*, as demonstrated by their molecular data. The report of *T. arctos* in a muskox from west Greenland expands the host spectrum and the geographic range of the parasite, but the ecology of this parasite in the Kangerlussuaq area remains unexplored. Importantly, the application of molecular tools to discriminate between *T. arctos* and other taeniid species is required given the considerable overlap in the number and size of large and small rostellar hooks, and the subtle differential morphological traits of mature proglottids (Haukisalmi et al. 2011). Previous reports on tapeworm infections in North American bears and moose were largely ascribed to *T. krabbei* and *T. hydatigena* (e.g., Samuel et al. 1976; Addison et al. 1978; Dies 1979; Seesee and Worley 1986). However, classification criteria were necessarily based on the morphology of the rostellar hooks and on differences in the anatomy of proglottids when specimens were in an adequate state of preservation for staining. The only detailed morphological characterization of *T. krabbei* from grizzly bears (Seesee and Worley 1986) is

remarkably similar to the description of *T. arctos* from Finnish brown bears (Haukisalmi et al. 2011). To date, unequivocal genetic characterization of *Taenia* isolates in bears has only been carried out for *T. arctos* from Finnish brown bears and for *T. soliu*m larval stages (*Cysticercus cellulosae*) found in the skeletal muscle of black bears from California, USA (Theis et al. 1996).

The focus of our study was to report the identity of the tapeworms isolated from a grizzly and a black bear from Alberta, Canada. These findings represent the first report of *T. arctos* in North American bears, extending the host range of the parasite, and confirming its Holarctic distribution and its specificity for ursids as definitive hosts.

4.2 Materials and Methods

In May 2012, during the post-mortem examination of a grizzly and a black bear found dead within the boundaries of Kananaskis Country, southwestern Alberta, Canada, adult cestodes were isolated from their small intestine. The poor quality of the tapeworm isolates did not allow the analysis of proglottids by classical staining techniques. Morphological characterization was therefore limited to the genus level and to the measurement of the rostellar hooks when present. The molecular diagnosis was based on the analysis of the mtDNA *cox1* gene. Genomic DNA from each isolate was extracted using Epicentre® MasterPure™ Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI). Enzymatic amplification and thermocycling protocol were performed as described in Kanzaki et al. 2011). The PCR products were purified with E.Z.N.A.® MicroElute Cycle-Pure Kit (Omega Bio-Tek, Norcross, GA), then sequenced using an Applied Biosystems 3730xl DNA Analyzer with BigDye Terminator™ chemistry (Perkin-Elmer, Waltham, MA). The sequences were double-stranded. Obtained mtDNA fragments were edited and compared to previously published *cox1* sequence data for various species of *Taenia* using the software Geneious 5.5 created by Biomatters (www.geneious.com).

4.3 Results

The grizzly and the black bear respectively hosted one and two adult stages of *Taenia* tapeworms in the first half of the small intestine. Only one specimen from the black bear had five small rostellar hooks, whose shape and mean total length of 96.8 µm (92.3–98.4 µm range)

resembled those of other taeniids (Haukisalmi et al. 2011). The three isolates were molecularly identified as *T. arctos* based on the similarity of the obtained cox1 sequences (396 base pairs) with data previously reported (Lavikainen et al. 2011). Two cox1 haplotypes with one nucleotide difference were detected in the two bear species, whereas the two tapeworms harboured by the black bear had identical cox1 sequences (Table 1). Our isolates were 99.75–100% similar to the cox1 gene fragment available for *T. arctos* from Alaskan moose (GenBank accession no. JF261319), and 98.74–99.24% (corresponding to 3–5 nucleotide differences) to the cox1 data of *T. arctos* from a Finnish brown bear (JF261318) and Eurasian elk (GU252130 and GU252131). Our cox1 sequences showed 97.98–98.94% similarity (4–5 nucleotide differences) when compared to the cox1 data (377 base pairs) provided by Raundrup and co-authors (2012).

Voucher specimens were archived in the U.S. National Parasite Collection (USNPC), U.S. Department of Agriculture, under the accession numbers USNPC 106984 for *T. arctos* from the grizzly bear, and USNPC 106986 from the black bear. The two corresponding mtDNA cox1 sequences were deposited in the genetic sequence database at the National Centre for Biotechnology Information (NCBI GenBank) under the accession numbers KF356386 and KF356387.

Table 4.1: Sites of polymorphism within the mitochondrial DNA cytochrome c oxidase subunit 1 sequences (396/377 base pairs) of *Taenia arctos*. Aligned sequences are available from the GenBank database, except for the isolate from Raundrup (2012).

GenBank accession no.	Host species	Location	mtDNA cox1 sequence variation positions						
			10	15	25	42	159	183	369
KF356386	U. americanus	Alberta, Canada	C	A	A	C	A	T	T
KF356387	U. a. horribilis	Alberta, Canada	C	A	G	C	A	T	T
JF261319[b]	A. americanus	Alaska, USA	C	A	A	C	A	T	T
JF261318[b]	U. arctos	Finland	A	A	A	T	G	C	T
GU252131[a]	A. alces	Finland	A	A	A	T	A	C	T
GU252130[a]	A. alces	Finland	C	G	A	T	A	C	T
Not available[c]	O. moschatus	West Greenland	C	G	A	T	A	C	-

[a] Isolates of Lavikainen et al. (2010).
[b] Isolates of Lavikainen et al. (2011).
[c] Isolate of Raundrup et al. (2012).

4.4 Discussion

This study is the first report of *T. arctos* from grizzly and black bears in North America. The only previous unequivocal characterization of this parasite on the continent was the detection of larval forms in Alaskan moose using molecular techniques (Lavikainen et al. 2011). Our results further support the specificity of *T. arctos* for ursids as definitive hosts, and potentially expand the geographic distribution of the parasite to ecosystems of the Nearctic where bears and moose are sympatric. The apparent strict specificity of *T. arctos* for bear species as final hosts was suggested by the failure to isolate the tapeworm from the intestinal tract of Finnish and Swedish wolves (*Canis lupus*) (Lavikainen et al. 2011). Similarly, metacestode stages of *T. arctos* have not been detected in semi-domesticated reindeer (*Rangifer tarandus tarandus*), Grant's caribou (*Rangifer tarandus granti*), or domestic sheep (*Ovis aries*), potential intermediate hosts sampled in regions where *T. arctos* is known to occur (Lavikainen et al. 2008, 2010, 2011).

The taxonomic description of *T. arctos* is recent. Before the identification of distinctive morphological features of its mature proglottids (Haukisalmi et al. 2011), *T. arctos* was considered as a cryptic species within *T. krabbei* given their marked overlap in the dimensions, number, and shape of the rostellar hooks (Lavikainen et al. 2010). However, anatomic similarity does not correspond to genetic relatedness. The two tapeworms are phylogenetically distant: *T. arctos* clusters with *T. solium* whereas *T. krabbei* is a sister species to *T. multiceps* (Lavikainen et al. 2011). In the light of a significant morphological similarity between *T. arctos* and *T. krabbei* at both metacestode and adult stages, historical reports of *T. krabbei* infections in North American bears and moose may actually refer to *T. arctos* (Lavikainen et al. 2011; Kutz et al. 2012). Within Alberta, Samuel and colleagues (1976) and Dies (1979) described *T. krabbei* from the skeletal muscle of moose and from the intestinal tract of black bears, respectively. These records should be carefully interpreted given our isolation of *T. arctos* from bears in the same general region in Canada. However, a molecular investigation using the mtDNA *cox*1 region unambiguously diagnosed *T. krabbei* infection in moose from Alaska (Lavikainen et al. 2011), demonstrating that *Alces* spp. can be competent intermediate hosts for both *T. arctos* and *T. krabbei*.

Apart from *T. krabbei*, several taeniid species have been reported in North American bears. The misidentification of *T. hydatigena* is unlikely, given its markedly different morphological features from *T. arctos* (Haukisalmi et al. 2011). By contrast, descriptions of other species of *Taenia* are uncertain, with deceptive classification criteria in some cases [reviewed in Lavikainen et al. (2011)]. Molecular analysis of ursine taeniid specimens deposited in museums and collections would be necessary to clarify the identity of *Taenia* spp. in bears. For example, representative specimens classified as *T. krabbei* from grizzly (Seesee and Wong 1986) and black bears (Addison et al. 1978) are present at USNPC (accession numbers 074817 and 079053). If suitable for molecular analyses, these archived cestodes may offer the opportunity to reassess historical records on tapeworm infections in bears, highlighting once more the importance of archival collections and their contribution to our knowledge on parasite biodiversity (Hoberg 2002).

Further interpretation of *T. arctos* life-history traits and evolutionary ecological processes may come from paleoecological data and phylogenetic hypotheses (Hoberg and Brooks 2008; Hoberg et al. 2012). While the predecessor of the black bear colonized North America around 5.3 million years ago, at the Miocene–Pliocene boundary (Krause et al. 2008), the ancestors of modern grizzlies and moose entered the Nearctic through the Beringia Land Bridge approximately 100,000 and 10,000 years ago, respectively at the beginning and at the end of the Wisconsin glaciation. The latter two species used Beringia as a refugium until the recession of the ice sheets by the early Holocene (Kurtén and Anderson 1980; Hundertmark et al. 2002). During the Wisconsinan, black bears inhabited multiple refugia in the Pacific Northwest, south of the Cordilleran and Laurentide ice sheets (Shafer et al. 2010). With the recession of the ice sheets and the subsequent expansion of boreal forests, grizzly bears and moose radiated towards the south-east, while black bears broadly expanded across most of the continent; as a consequence, the three species became sympatric (Kurtén and Anderson 1980; Hundertmark et al. 2002). Grizzly and moose ancestors, during their Pleistocene migration from Eurasia to North America, may have perpetuated the life-cycle of *T. arctos* within the Beringian refugia. The parasite could have been subsequently acquired by black bears during the postglacial period. Testing these co-phylogeographic hypotheses would help to infer the nature of *T. arctos* life-cycle patterns and its evolution within *Ursus* and *Alces* species (Waltari et al. 2007; Hoberg and Brooks 2008; Hoberg

et al. 2012). However, the finding of *T. arctos*, misidentified as *T. o. krabbei*, in a muskox from west Greenland (Raundrup et al. 2012) possibly suggests a more complex history for this taeniid species. In the absence of ursids in the Kangerlussuaq area, only the abundant arctic fox (*Vulpes lagopus*) may serve as definitive host for *T. arctos*.

Our study first reports *T. arctos* infections in a grizzly and an American black bear, further supporting the Holarctic distribution of this taeniid species and its life-cycle specificity for Ursus species. The *cox1* gene has again proven to be a particularly valuable marker for the characterization of taeniids, with molecular diagnostics as an essential tool to shed light on the identity of *Taenia* species (Lavikainen et al. 2011). Our results confirm previous findings of a lower nucleotide variability (*p* 0.25%) among the Alaskan and Canadian specimens if compared to the Palearctic haplotypes (*p* 0.76%), which originate from a more restricted area (Lavikainen et al. 2011). Nevertheless, further sequence data from geographically diverse specimens are needed to better understand *T. arctos* phylogeography and population genetics across its Holarctic distribution. The historical biogeography of *T. arctos* and its occurrence in a muskox from west Greenland (Raundrup et al. 2012) remain enigmatic, particularly in the Kangerlussuaq area where arctic foxes are the only potential carnivore definitive host. In mainland North America, given the different evolutionary history of black bears, grizzlies, and moose, we hypothesized that the presence of *T. arctos* in black bears may represent a host-switching event after the radiation of grizzly bear and moose ancestors across the Nearctic. Testing assumptions on the historical biogeography of *T. arctos* and its hosts may serve as an excellent indicator of current patterns of host-parasite association (Waltari et al. 2007; Hoberg and Brooks 2008; Hoberg et al. 2012).

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS

5.1 Ursine Parasites and their Impact on Host Health

Grizzly and black bears are highly iconic species in North America (Simberloff 1999). Black bear populations are abundant through Canada, USA, and Mexico (Garshelis et al. 2008). However, they have lost approximately 48% of their historical range (Pelton and Van Manen 1994), and the viability of some insular, isolated populations (e.g., the Louisiana black bear) raise concern (Pelton 2003). The grizzly bear is listed as Special concern, under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2012), and the status of Threatened, has been suggested in Alberta (Festa-Bianchet 2010). The mortality rate for Albertan grizzly bears is extremely high (up to 47% for cubs). Moreover, individual body condition scores and reproductive rates of some grizzly populations in Alberta are at the lowest for the species because of habitat degradation and anthropogenic disturbance (Garshelis et al. 2005). Body mass and fat content are of primary importance for the reproduction of bears: the energetic stress can affect the reproductive potential of female bears, with lower fitness leading to a declining populations trend (Hilderbrand et al. 1999). In this context, causes of natural mortality and the impact of parasites on ursid species is still largely undetermined.

This is a major dearth of knowledge if we consider that parasitic infections may harm bears in several ways:

- High parasite burdens may be responsible for poor body fat content (Hochberg et al. 1992), with consequent negative effects on female bear reproductive potential and rates of population growth.
- The progressive fragmentation of the Central Rockies Ecosystem (Nielsen et al. 2006; Proctor et al. 2012) may favour higher densities of grizzly and black bears in a limited, patchy suitable habitat, with positive density-dependent effects on pathogen transmission.
- Ursine hookworms may cause significant cub mortality if their life-cycle was similar to *Uncinaria* spp. in otariid pinnipeds, the evolutionary closest relatives of modern bears (Davis et al. 2004; Higdon et al. 2007).

This study attempted to address the issue of a limited understanding of parasitic infections in bears. As previously stated, bear reproduction is directly related to body fat content;

when energetically stressed, female bears may forgo reproduction, with persistent deferrals causing a declining population trend (Noyce and Garshelis 1994; Hilderbrand et al. 1999; Schliebe et al. 2006). Furthermore, the progressive fragmentation of the Central Rockies Ecosystem (Nielsen et al. 2006; Proctor et al. 2012) may be increasing niche overlap and trophic relationships between grizzly and black bears. Confined into smaller patches of suitable habitat, higher densities of bear populations would favour pathogen transmission. Nevertheless, specific studies on the pathology of *B. transfuga* in North American bears are absent. Hundreds of adult *B. transfuga* can cause the death of bears by obstructing the lumen of the digestive tract (Mozgovoi 1953; Testini et al. 2011). In China, the degradation and patchiness of bamboo forests constrained wild giant pandas in the remnant areas of their habitat. The high density favoured the transmission of the ascarid *B. schroederi*. Pandas were found to harbour hundreds of adult roundworms; by clogging the pylorus and the superior segment of the duodenum of the host, *B. schroederi* became a major cause of mortality (Xie 1987; Zhang et al. 2008, 2011). The prospect of parasites as regulators and drivers of host population dynamics has never been investigated in *Ursus* species. The role of parasites in ursids will remain unknown if we do not start collecting baseline information. Historical data on the helminth fauna of grizzlies and black bears from Alberta and British Columbia are scarce (Hall and Mahrt 1970; Dies 1979). This study reports the first recent data on prevalence and intensity of parasitic infections in bear populations from western Canada. The analysis of the parasite infection values for different age classes suggested that the burden of *B. transfuga* and *Uncinaria* sp. increases with the age of the bears. Nonetheless, research and surveillance of *Baylisascaris* spp. in bears is necessary to improve our understanding of this host–parasite system.

The ursine hookworms *U. rauschi* and *U. yukonensis* are a further potential threat to the viability of bears. Hookworms are among the most widespread parasites of mammals, causing anaemia and a significant health impact in all hookworm-infected host species (Bowman et al. 2003; Hotez 2009; Spraker and Lander 2010). The transmammary transmission route (i.e., milk borne larvae transmitted from the mother to the offspring during nursing) is frequent for hookworm species of canids, and it is the main infection route for *Uncinaria* spp. in otariid pinnipeds. This infection route potentially results in high pup mortality rates from peracute/acute blood loss (Lyons et al. 1997; Procter 1998; Castinel et al. 2007a). Despite the lack of

information on the life cycle and pathology of *Uncinaria* spp. in bears, I hypothesized potential clinical effects in infected bear cubs, those similarity with the host-parasite interaction described in canids and otariids since these host share common ancestry with ursids (Davis et al. 2004; Higdon et al. 2007). In contrast, higher hookworm infection values in adult grizzly and black bears were found. These data strongly differ from the ones on hookworm species transmitted by transmammary route, where parasites persist in the intestine of the pup for few months before either disappearing or causing the death of the host (Lyons et al. 2003; Lyons et al. 2011a). In the past, *U. rauschi* and *U. yukonensis* were rarely isolated but already recorded in adult bears (e.g., Choquette et al. 1969). The phylogenetic analysis based on the ITS rDNA regions of the two ursine hookworm species also supported the rejection of the initial hypothesis. In fact, the molecular analysis revealed nestedness of *U. rauschi* and *U. yukonensis* with *U. stenocephala*, a hookworm of canids and felids particularly prevalent at northern latitudes. The species *U. stenocephala* is characterized by a mainly oral transmission of its infective larvae, and it is considered one of the least pathogenic hookworms (Gibbs 1961; Prociv 1998; Bowman et al. 2010). Although only experimental infections can prove with certainty the cycle of life of *Uncinaria* spp. in bears, the present study reported evidence supporting a low pathogenicity for *U. rauschi* and *U. yukonensis* in bear species.

In summary, this thesis does not resolve the cycle of life of *U. rauschi* and *U. yukonensis* in grizzlies and black bears, nor states if the ascertained parasitic levels of infection are sustainable for the viability of bear populations in Alberta and British Columbia. Nevertheless, the study is an important step towards a better understanding of parasitic infections in bear species. The roundworm *B. transfuga* has not been found at a high intensity of infection. The hookworms *U. rauschi* and *U. yukonensis* may be responsible for negligible blood loss in ursid hosts given their peak of infection levels in adult bears, and their evolutionary relatedness with *U. stenocephala*. Other parasites found in the sampled bears were the cestodes *Taenia* sp., *D. dendriticum*, and *D. nihonkaiense*. While Rausch (1955) speculated that infection was the cause of death of a young black bear experimentally infected with *Diphyllobothrium* species, the low prevalence and intensity of tapeworm infections reported by my study do not seem to be of any pathological significance.

5.2 Parasite Biodiversity in North American Bears

This research project provides new insights on the gastrointestinal parasites of grizzly and black bears from Alberta and British Columbia. The highlights of the survey on the parasite biodiversity in the digestive tract of bears from western Canada can be summarized as follows

- Discovery of novel parasite species from North American bears.
- Phenotypic plasticity for females of *U. rauschi* and *U. yukonensis*.
- Potential misidentification of *T. arctos* in historical reports on taeniid taxa infecting North American bears.

Over the last 25 years a few surveys have been conducted on the helminth fauna of North American bears (Duffy et al. 1994; Gau et al. 1999; Kutz 2003a, 2003b, 2004a; Foster et al. 2004). These more recent reports, together with preceding studies, show how much lower the parasite biodiversity in North American bears at northern latitudes is when compared to studies on bear population of southern USA. Typically, the parasitological surveys on the gastrointestinal tract of Kodiak bears, grizzlies, and American black bears from Canada and the northern states of USA have isolated four nematode species (*B. transfuga*, *P. rara*, *U. rauschi*, *U. yukonensis*), three cestodes (*Diphyllobothrium* sp., *T. hydatigena*, *T. krabbei*), and two trematodes (*A. americana*, *E. revolutum*) (reviewed in Rogers and Rogers 1976; see also Table 1.1, Chapter 1). While the nematode *B. transfuga* was often isolated and reported, other parasite species seem to be less common and seem to be characterized by lower prevalence and intensity of infection.

Cestodes of the genus *Diphyllobothrium* have often been reported as *D. ursi* when isolated as adult stages in the intestine of ursid hosts (e.g., Choquette et al. 1969; Worley et al. 1976; Frechette and Rau 1977). In the past, parasites of this genus were classified in one species or another mostly depending on the taxonomist who analysed the specimens. In the current study, the report of *D. dendriticum* and *D. nihonkaiense* was achievable only with the application of molecular tools. The characterization of these two tapeworm species widens their host specificity and geographical distribution. The species *D. dendriticum* was already considered to have a broad range of intermediate and definitive hosts (Andersen et al. 1987). Andersen et al. (1987) reexamined specimens from bears of British Columbia based on their morphology pointing out the potential misidentification of *D. dendriticum* with *D. ursi*. That study

synonymized *D. ursi* with *D. dendriticum*, but the former has been recently recognized as a valid species with support of both morphological and molecular data (Yamasaki et al. 2012). The present research is the first indisputable record of *D. dendriticum* adult stages in the intestinal tract of a bear, expanding the host spectrum of this parasite to ursid species as potential definitive hosts. The other isolated pseudophyllidean cestode is *Diphyllobothrium nihonkaiense*. Although never described from North American bears previously, this species was already reported in Russian brown bears based on morphology and on the molecular analysis of the mtDNA cox1 gene (Arizono et al. 2009).

The present survey also characterized *Taenia arctos* in both grizzlies and black bears from Alberta and British Columbia as definitive hosts. The only previous unequivocal identification of this parasite in mainland North America was the detection of larval forms in moose from Alaska using molecular techniques (Lavikainen et al. 2011). North American bears are reported to be final hosts for several taeniid species, particularly *T. krabbei* and *T. hydatigena* (Jones and Pybus 2001). However, Lavikainen et al. (2010, 2011) demonstrated that *T. arctos* and *T. krabbei* are undistinguishable based on the morphology of their rostellar hooks. The isolation of *T. arctos* from Canadian bears, its apparently narrow host-specificity for bears as definitive hosts, and the remarkable morphological similarity between *T. arctos* and *T. krabbei* suggest the possibility that this tapeworm species might have gone undetected in the past, when molecular tools were not available. My findings, as well as previous studies (Lavikainen et al. 2011; Kutz et al. 2012), recommend the careful interpretation of historical records on tapeworm infections in ursids, since they may actually refer to *T. arctos*.

Finally, this research combined classical and molecular parasitology to shed light on the presence of polymorphism for females of *U. rauschi* and *U. yukonensis* in both grizzlies and black bears. Results confirmed the taxonomic classification of *U. rauschi* and *U. yukonensis*, and added molecular data supporting monophyly for the two hookworm species. The present study reported a variety of morphotypes characterized by different vulvar appendages and wide morphometric traits never described previously for *U. rauschi* and *U. yukonensis*. However, although female morphology may be misleading for species-specific diagnoses, the anatomical traits of male hookworms allow the identification of *U. rauschi* and *U. yukonensis*. The two ursine hookworm species are a further example of female morphometric plasticity for nematodes

within the order Strongylida. The development of molecular techniques greatly benefitted parasite systematics; many cases of phenotypic plasticity have been unmasked where it was once thought there were multiple species infections (e.g., Lancaster and Hong 1981; Lichtenfels et al. 1994, 1997; Dallas et al. 2000, 2001; Leignel et al. 2002). Furthermore, the morphological and molecular approaches allowed the formulation of assumptions on the phylogeny, biogeography, and life-history traits of *U. rauschi* and *U. yukonensis*. Nevertheless, the life-cycle of these hookworms remains enigmatic, and their closeness to *U. stenocephala* needs further investigations in order to be elucidated.

5.3 Conclusions and Future Directions

Ascarids, hookworms, and the parasitic community as a whole may play an important role as determinant of host population dynamics, and as driver of biodiversity within ecosystems (Bye and Halvorsen 1983; Combes 1996; Albon et al. 2002; Poulin 2007). Many studies (e.g., Gulland 1992; Hochberg et al. 1992; Hudson et al. 1992, 1998; Catchpole et al. 2000; Martin et al. 2002; Stien et al. 2002; Luikart et al. 2008) demonstrated that parasites can have severe effects of malnutrition and upset host resource allocation with consequent negative effects not just on individual physiology (i.e., lower reproductive potential), but also on population fitness and viability (i.e., instability and population decline). This is particularly of concern for endangered and/or genetically depleted animal populations, where reduced health and increased genetic drift may cause a serious risk of extinction (Spielman et al. 2004; Whiteman et al. 2006).

In the course of progressive climate, landscape, and human-induced changes impacting not only the geographical range and size of wildlife populations, but also the distribution and ecology of their parasites, it is more important than ever to adequately consider the effects of pathogens on host communities (Daszak et al. 2000; Harvell et al. 2002; Wood et al. 2007). Habitat alterations and latitudinal shifts of host and parasite distributions can lead to overlapping ranges, higher densities of potential hosts, therefore higher abundance of infective larvae and increased opportunities to colonize new host species and/or populations (Peters 1992; Cleaveland et al. 2002; Tompkins and Poulin 2006). In the Arctic and Subarctic, where temperatures have increased at almost twice the rate of the rest of the world in the past two decades (Parkinson and Butler 2005), the outcome of these events may be tremendous. Baseline data on the health status

of mammals in northern ecosystems are scant; the potential emergence of an array of new parasites and pathogens would have a greater impact on naive host populations (Kutz et al. 2004b; Hoberg 2005; Burek et al. 2008).

The current "global change" (see Walker and Steffen 1999; Sutherst 2000) is altering ecology and habits of polar bear populations, even if these modifications significantly vary among the species range. Climate warming is stressing polar bear feeding behaviour, obliging them to scavenge or to eat the digestive tract and other viscera of the prey (Derocher et al. 2004). Simultaneously, the increasing average temperature of the globe is reducing the sea ice extent and its seasonal presence, with an increasing number of polar bears forced ashore for longer periods (Peacock et al. 2011). In northern Canada and Alaska there are areas where the ranges of polar and grizzly bears overlap (Schliebe et al. 2006; Doupé et al. 2007). Interbreeding between the two species, with the production of viable and reproductive offspring, has been demonstrated (Gray 1972; Kelly et al. 2010). Hybridization may contribute to create a genetic and ecological bridge (see Floate and Whitham 1993) allowing parasite exchange and host switching between the two bear species.

In this scenario of marked changes, the range expansion of pathogens and their emergence may be facilitated, and infectious disease outbreaks may occur (Kutz et al. 2005; Burek et al. 2008). The identification of any potentially vulnerable host-parasite ecosystem complex, the more profound knowledge of parasite infection patterns, and the correct identification of parasite species are critical information for clinical diagnostics, for disease transmission surveys, and for designing strategies to control infections and outbreaks (Sutherst 2001; Criscione et al. 2005; Leung et al. 2009). In this context, the morphological identification of parasites alone may have limited usefulness and reliability; the development of molecular methods reduces the proportion of unidentified specimens (or unidentifiable, i.e., cryptic species), enhancing the specificity of the diagnosis and the possibility to compare taxa by using sequences available in open access databases (Dare et al. 2008; Lavikainen et al. 2011; Nadler and Pérez-Ponce de León 2011). Furthermore, the increased number of phylogenetic studies has expanded our understanding of parasite evolution and host genealogical history (Page 2003; Whiteman and Parker 2005). Parasites can be used as witnesses of host migratory events, range

shifts, and timing of colonization of new ecosystems/animal populations (Ostfeld et al. 2005; Nieberding and Olivieri 2007).

Causes of natural mortality and the impact of infectious agents are largely unknown for wild grizzly and black bears, with even less information available on polar bears. The conservation of black, grizzly and polar bears is a high profile wildlife management issue in North America. Grizzly and black bears are iconic species in the ecosystems they inhabit, with polar bears playing the same role in the Canadian High Arctic (Simberloff 1999; Garshelis et al. 2005; Peacock et al. 2011). The potential detrimental consequences of *Uncinaria* spp. and other parasitic infections on bear health deserve serious consideration, since they may impair conservation strategies. The overall effects of parasitic diseases on the well-being of existing populations of bears in North America require further investigation, especially because the current climate warming is facilitating the shifting of southern bear populations to the Canadian Arctic. Although the present thesis advances our knowledge on parasite biodiversity and infection patterns in grizzly and black bears, their impact on the health of ursids is not yet understood. Efforts to increase research and surveillance must be made since infectious diseases are strong biotic forces that can threaten biodiversity, with global change acting as a catalyst (Daszak et al. 2000; Harvell et al. 2002; Hudson et al. 2006). These efforts should be directed towards the:

- Development of comprehensive databases on bear species and their parasites.
- Study of the life-history traits of ursine parasites and of disease patterns.
- Parasitological investigation of northern populations of bears.
- Collection of baseline information on the infection status of wild polar bears, with particular focus on their southernmost distribution range.

In conclusion, despite the acknowledged role of parasites within ecosystems, their effects on wildlife health are not yet effectively integrated into natural environmental management and conservation programmes (Cleaveland et al. 2002; Lafferty and Kuris 2005). Such knowledge can provide a powerful tool to limit the potential impact of climate change and emerging infectious agents, and to drive prevention and conservation actions for the stability of wildlife populations (Hoberg 1997; Burek et al. 2008). This is particularly true for polar bear populations, whose primary scientific concern for their conservation became the effects of global change

(Schliebe et al. 2006; Peacock et al. 2011). Global warming and the loss of optimal habitat are already affecting the viability of polar bear populations. The potential emergence of parasites able to survive in Arctic and Subarctic ecosystems and to expand their host spectrum to polar bears may definitively compromise conservation efforts.

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APPENDIX A: PROTOCOL FOR THE POST-MORTEM EXAM OF BEARS, EXAMINATION AND COLLECTION OF ORGANS

All specimens come with a CCWHSC submission form including: species, date of necropsy, date of death, location, reason for collection, pathological diagnosis

Weight	Weight of the carcass prior to necropsy
Length (crown of head to end of rump)	Measure of the body length from crown of the head to end of the rump
Sex	Male or Female
Body Condition	1 to 5 (poor/fair/moderate/good/excellent)
Paw size	Right front and rear foot, length and width
Estimated Age	Cub, Juvenile, Subadult, Adult

FORMALIN	FREEZER -20°C	70% ETHANOL
-	Hair	-
Lungs	Lungs	-
-	Mammary gland/Abdominal wall	-
Kidney right/left	Kidney right/left	-
Liver (one piece per lobe)	Liver (one piece per lobe)	-
Spleen	Spleen	-
Lymph nodes (Mandibular, Retropharyngeal, Prescapular, Axillary Tracheobronchial, Gastric, Hepatic, Mesenteric)	Lymph nodes (Mandibular, Retropharyngeal, Prescapular, Axillary Tracheobronchial, Gastric, Hepatic, Mesenteric)	-
-	Tongue/Diaphragm/Intercostal muscle (*Trichinella* spp.)	-
-	Right/Left ear wax	-
-	Intestinal tract	-
-	Intra-cardiac blood sample/Intra-abdominal transudate	-
-	Jaw	-
-	Brain tissue	-
-	-	Faeces
Tonsils	-	-
Gall bladder	-	-
Gonads	-	-
Oesophagus (one segment)	-	-
Trachea (one segment)	-	-
Thyroid	-	-
Adrenal glands	-	-

APPENDIX B: DATA BASE OF THE INFORMATION COLLATED ON THE BEAR CARCASSES INCLUDED IN THE STUDY

Bear ID#	Species	Sex	Age class	Date of death	Province	Location	Notes
BB1	Black bear	M	Cubs	28-May-12	BC	Smithers	Lat. 54.881674 Long. -127.259789
BB2	Black bear	M	Adults	19-Oct-11	BC	Sierra Yoyo Desan Access Road km 101, Fort Nelson	Lat. 58.869489 Long. -122.477818
BB7	Black bear	M	Juveniles	20-Oct-11	BC	Lake Cowichan, Vancouver Island	Lat. 48.810403 Long. -124.080620
BB8	Black bear	M	Juveniles	20-Oct-11	BC	Somenos, Vancouver Islanc	Lat. 48.826662 Long. -123.734894
BB9	Black bear	M	Juveniles	20-Oct-11	BC	Somenos, Vancouver Islanc	Lat. 48.845029 Long. -123.748348
BB12	Black bear	M	Juveniles	3-Jul-12	BC	Ucluelet, Vancouver Island	Lat. 48.945556 Long. -125.561779
BB13	Black bear	M	Juveniles	24-Jul-12	BC	Ucluelet, Vancouver Island	Lat. 48.946728 Long. -125.572089
BB14	Black bear	F	Adults	3-Jun-12	BC	Cariboo River Provincial Park Quesnel	Lat. 52.815650; Long. 121.258185; no cubs
BB17	Black bear	M	Juveniles	-	BC	Stewart	Lat. 55.954087 Long. -129.974495
BB18	Black bear	M	Juveniles	1-Nov-11	BC	Fort Saint John	Lat. 56.290330 Long. -120.795450
BB19	Black bear	M	Juveniles	9-Jun-12	BC	Vernon	Lat. 50.215561 Long. -119.300451
BB20	Black bear	M	Adults	11-May-12	BC	Swift River Forest Road, Quesnel	Lat. 53.011205 Long. -122.044512
BB21	Black bear	M	Adults	15-May-12	BC	Victoria Creek Road, Quesne	Lat. 53.025359 Long. -122.252555
BB24	Black bear	M	Adults	22-Oct-12	BC	Dashwood, Vancouver islanc	Lat. 49.359833 Long. -124.517197
BB26	Black bear	M	Cubs	4-Jan-13	BC	Invermere	Lat. 50.514827 Long. -116.052103
BB27	Black bear	M	Juveniles	18-Jan-13	BC	Quesnel	Lat. 52.969715 Long. -122.492323
BB41	Black bear	F	Adults	17-May-12	BC	Victoria Creek Road, Quesne	Lat. 53.006335 Long. -122.212923
BB43	Black bear	M	Cubs	22-Jan-13	BC	Manning Park	Lat. 49.064647 Long. -120.781581
GB7	Grizzly bear	M	Adults	3-Jun-13	BC	Port McNeill, Vancouver Island	Lat. 50.588459 Long. -127.091603
GB9	Grizzly bear	F	Cubs	2-Nov-12	BC	Kitwancool Lake, Kitwanga	Lat. 55.408909 Long. -128.147449
GB10	Grizzly bear	F	Adults	-	BC	Owikeno Lake	Lat. 51.708399 Long. -126.914492
GB11	Grizzly bear	F	Juveniles	-	BC	Owikeno Lake	Lat. 51.708399 Long. -126.914492

Bear ID#	Species	Sex	Age class	Date of death	Province	Location	Notes
GB12	Grizzly bear	F	Juveniles		BC	Owikeno Lake	Lat. 51.708399 Long. -126.914492
GB13	Grizzly bear	M	Juveniles	18-Jul-12	BC	Intersection Deadman Cach Creek Road/Deadman Videtl Road, Walhachin	Lat. 50.905578 Long. -121.000564
BB4	Black bear	M	Cubs	22-Sep-11	AB	Lake Louise	Lat. 51.466150 Long. -116.231094
BB5	Black bear	M	Juveniles	9-Jul-11	AB	Jasper National Park	Lat. 52.882732 Long. -118.381744
BB6	Black bear	F	Juveniles	30-Jul-11	AB	Jasper National Park	Lat. 52.859822 Long. -118.076501
BB10	Black bear	M	Juveniles	4-May-12	AB	Bow Valley Provincial Park	Lat. 51.080526 Long. -115.091593
BB11	Black bear	M	Juveniles	28-Apr-12	AB	Canmore	Lat. 51.071389 Long. -115.304775
BB15	Black bear	M	Adults	20-May-12	AB	Canmore	Lat. 51.115817 Long. -115.374856
BB16	Black bear	M	Adults	11-Jul-12	AB	Highway93 5km south of Rampart Hostel	Lat. 52.042733 Long. -116.862944
BB22	Black bear	F	Adults	17-Aug-12	AB	Highway93 2.5km from Silverhorn Mountain	Lat. 51.743634 Long. -116.514065
BB25	Black bear	F	Adults	25-Jul-12	AB	Highway1, Banff	Lat. 51.194057 Long. -115.559282
BB28	Black bear	M	Juveniles	3-Sep-12	AB	Camp Chief Hector YMCA, Bow Valley Provincial Park	Lat. 51.064514 Long. -115.069700
BB29	Black bear	M	Juveniles	24-Aug-12	AB	Highway40, Barriet Lake, Bow Valley Provincial Park	Lat. 51.022492 Long. -115.057626
BB30	Black bear	M	Juveniles	27-Jul-12	AB	Highway1, Yoho National Park	Lat. 51.287935 Long. -116.582558
BB31	Black bear	M	Cubs	7-Aug-12	AB	Highway93, Bow Pass (alsc called Bow Summit)	Lat. 51.718253 Long. -116.493005
BB32	Black bear	M	Juveniles	1-Jun-12	AB	Yoho National Park	Lat. 51.377558 Long. -116.512547
BB33	Black bear	M	Adults	9-Jul-12	AB	Highway93 2.5km north of Simpson Monument, Kootenay National Park	Lat. 50.972697 Long. -115.947304
BB34	Black bear	F	Juveniles	1-Jul-12	AB	Highway1 between Castle Junction and Lake Louise	Lat. 51.321883 Long. -116.020432
BB36	Black bear	M	Juveniles	11-Aug-12	AB	Highway1, Yoho National Park	Lat. 51.236240 Long. -116.580161
BB37	Black bear	M	Adults	30-Jun-12	AB	Highway22 closeby Blairmore	Lat. 49.609559 Long. -114.181423
BB38	Black bear	M	Adults	7-Jul-12	AB	Highway1, Yoho National Park	Lat. 51.304768 Long. -116.579981
BB39	Black bear	M	Adults	26-May-12	AB	Highway1 between Canmore andHarvie Heights	Lat. 51.118071 Long. -115.378332
BB40	Black bear	F	Cubs	26-Sep-12	AB	Kananaskis Trail	Lat. 50.877470 Long. -115.145035

Bear ID#	Species	Sex	Age class	Date of death	Province	Location	Notes
BB42	Black bear	F	Cubs	10-Jun-13	AB	1988 Olympicway Suite 100 Canmore	Lat. 51.090336 Long. -115.385535
GB1	Grizzly bear	M	Adults	29-Sep-11	AB	Lake Louise	Lat. 51.430107 Long. -116.185351
GB2	Grizzly bear	F	Adults	10-Aug-11	AB	Highway1 between Banff and Lake Louise	Lat. 51.276190 Long. -115.962744
GB3	Grizzly bear	F	Adults	28-May-11	AB	Banff National Park	Lat. 51.184864 Long. -115.750923
GB4	Grizzly bear	M	Juveniles	15-May-12	AB	Highway68 8km south of Highway1	Lat. 51.044334 Long. -114.699969
GB5	Grizzly bear	F	Adults	28-Sep-12	AB	Pincher Creek	Lat. 49.176279 Long. -113.831234
GB6	Grizzly bear	M	Adults	15-Oct-12	AB	Pincher Creek	Lat. 49.482809 Long. -113.988819
GB8	Grizzly bear	M	Juveniles	5-Oct-12	AB	Banff National Park	Lat. 51.157271 Long. -115.688610

APPENDIX C: DATABASE OF THE GASTROINTESTINAL PARASITES HARBOURED BY THE ANALYSED BEARS

Bear ID#	Gastric parasites	Intestinal parasites	Uncinaria spp. total	B. transfuga total	Tapeworms	No. Scolices
BB1	No	Yes	740 U. rauschi	3	No	-
BB2	No	Yes	118 U. rauschi	28	No	-
BB7	No	Yes	82 U. rauschi	47	No	-
BB8	Stomach absent	Yes	-	45	No	-
BB9	Stomach absent	Yes	-	3	No	-
BB12	Stomach absent	Yes	-	12	No	-
BB13	Stomach absent	Yes	-	6	No	-
BB14	Stomach absent	Yes	506 U. rauschi	-	No	-
BB17	No	Yes	3 U. rauschi	1	No	-
BB18	Stomach absent	Yes	-	29	No	-
BB19	No	Yes	2 U. rauschi	3	No	-
BB20	Stomach absent	Yes	506 U. rauschi	-	No	-
BB21	Stomach absent	Yes	158 U. rauschi	-	No	-
BB24	Stomach absent	Yes	3 U. rauschi	9	T. arctos	1
BB26	Stomach absent	Yes	21 U. rauschi	-	No	-
BB27	Stomach absent	Yes	-	78	No	-
BB41	Stomach absent	Yes	132 U. rauschi	-	No	-
BB43	Stomach absent	No	-	-	No	-
GB7	Stomach absent	No	-	-	No	-
GB9	Stomach absent	Yes	20 U. rauschi	6	D. nihonkaiense	27
GB10	B. transfuga juveniles	Yes	-	26	D. dendriticum	3
GB11	B. transfuga juveniles	Yes	-	17	No	-
GB12	No	Yes	-	4	No	-
GB13	No	Yes	22 U. rauschi	-	No	-
BB4	No	Yes	41 U. rauschi	-	No	-
BB5	No	Yes	284 U. rauschi	1	No	-
BB6	No	Yes	221 U. rauschi	10	T. arctos	1
BB10	No	Yes	11 U. rauschi	-	No	-
BB11	No	Yes	3 U. rauschi	-	No	-
BB15	No	Yes	376 U. rauschi	4	No	-
BB16	No	Yes	271 U. rauschi	40	No	-
BB22	No	Yes	275 U. rauschi	-	No	-
BB25	No	Yes	121 U. rauschi	-	No	-

Bear ID#	Gastric parasites	Intestinal parasites	Uncinaria spp. total	B. transfuga total	Tapeworms	No. Scolices
BB28	No	No	-	-	No	-
BB29	No	Yes	-	5	No	-
BB30	No	Yes	739 U. rauschi	1	No	-
BB31	No	Yes	8 U. rauschi	-	No	-
BB32	No	Yes	24 U. rauschi	-	No	-
BB33	No	Yes	62 U. rauschi	5	No	-
BB34	No	Yes	303 U. rauschi	-	No	-
BB36	No	Yes	10 U. rauschi	10	No	-
BB37	No	Yes	2 U. rauschi	11	T. arctos	3
BB38	No	Yes	295 U. rauschi	14	No	-
BB39	No	Yes	-	-	T. arctos	2
BB40	No	Yes	5 U. rauschi	6	No	-
BB42	No	Yes	-	1	No	-
GB1	No	Yes	30 U. rauschi	12	No	-
GB2	No	Yes	79 U. rauschi	9	No	-
GB3	No	Yes	188 Uncinaria spp.	-	No	-
GB4	No	Yes	-	-	T. arctos	1
GB5	No	Yes	46 U. rauschi	4	No	-
GB6	No	No	-	-	No	-
GB8	No	Yes	171 U. rauschi	-	No	-