



Helminths of grizzly and black bears in Alberta and British Columbia, Canada

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20

21 **Abstract:** Between May 2011 and June 2013, we collected the carcasses and gastrointestinal tracts
22 of 40 American black bears (*Ursus americanus* Pallas, 1780) and 13 grizzly bears (*Ursus arctos* L.,
23 1758) from populations of Alberta and British Columbia, Canada. Specimens were examined for
24 helminths, which were identified to the species level by applying an integrated morphological and
25 molecular approach. Our goal was to investigate parasite biodiversity and infection parameters in
26 the sampled grizzly and black bears. We found seven parasite taxa: *Dirofilaria ursi* Yamaguti,
27 1941, *Baylisascaris transfuga* (Rudolphi, 1819), *Uncinaria rauschi* Olsen, 1968, *Uncinaria*
28 *yukonensis* (Wolfgang, 1956), *Taenia arctos* Haukisalmi, Lavikainen, Laaksonen & Meri, 2011,
29 *Diphyllobothrium dendriticum* (Nitzsch, 1824), and *Diphyllobothrium nihonkaiense* Yamane,
30 Kamo, Bylund & Wikgren, 1986. The statistical significance of infection prevalence, intensity, and
31 abundance for each helminth species was assessed relative to host species, gender, age class,
32 sampling season, and location. This is the first unequivocal report of the potentially zoonotic
33 tapeworms *D. dendriticum* and *D. nihonkaiense* in North American bears. Furthermore, we provide
34 insight into the biology and ecology of the nematodes *B. transfuga*, *D. ursi*, and species of
35 *Uncinaria* Frölich, 1789, and enrich the information available on the recently described tapeworm
36 *T. arctos*.

37

38 **Key words:** North America, black bear, *Ursus americanus*, grizzly bear, *Ursus arctos*, parasites,
39 zoonoses.

40 Introduction

41 Grizzly bears (*Ursus arctos* L., 1758) and American black bears (*Ursus americanus* Pallas,
42 1780) are iconic wildlife of North America. The two species have extensively overlapping
43 geographic ranges in northwestern USA and Canada. However, while black bears are mostly
44 abundant and viable throughout Canada, USA, and northern Mexico, the conservation of grizzly
45 bears is a high profile wildlife management issue in western North America (Mowat et al. 2005;
46 Proctor et al. 2012). The grizzly bear is classified as “Special Concern” by the Committee on the
47 Status of Endangered Wildlife in Canada (COSEWIC 2012). Moreover, the status of “Threatened”
48 has been proposed for its population in Alberta (AB), Canada (Festa-Bianchet 2010), and for some
49 insular populations in the southern portion of British Columbia (BC), Canada (Proctor et al. 2005).

50 To date, research on North American bears has focused primarily on habitat quality,
51 population dynamics, and anthropogenic disturbance (e.g., Mowat et al. 2005; Sawaya et al. 2012).
52 Declining grizzly bear metapopulations with diminishing demographic connectivity have been
53 documented in southwestern Canada and northwestern USA; major causes for their isolation are
54 habitat degradation and landscape fragmentation generated by increased resource-extraction
55 activities and human encroachment (Proctor et al. 2012). In this context, the inclusion of parasites
56 and diseases in order to assess bear habitat quality is still largely overlooked. Limited research has
57 been conducted on the parasite fauna of grizzly and black bears, with few studies available on
58 populations from western Canada (Choquette et al. 1969; Hair and Mahrt 1970; Dies 1979; Gau et
59 al. 1999; Johnson et al. 2013; Catalano 2014). Densely inhabited habitat fragments may increase the
60 opportunities for helminth transmission, therefore exposing the hosts to higher parasitic loads and to
61 infection by atypical pathogens (Hoberg and Brooks 2015). Changes in the helminth community
62 may affect host population dynamics and trade-offs in resource allocation with negative
63 consequences on individual reproductive physiology and population viability (e.g., Gulland 1992;
64 Stien et al. 2002). High parasitic infection levels may be responsible for poor body condition, with a
65 domino effect on female bear reproductive potential and rates of population growth: females may

66 forgo mating seasons because of the energetic stress, and persistent deferrals may lead to declining
67 population trends (Hilderbrand et al. 1999; Schwartz et al. 2003). Furthermore, malnourishment and
68 weakness can make bear cubs, yearlings and juveniles less vigilant and active, thus more vulnerable
69 to predators and susceptible to heavy parasite burdens (Rogers 1993).

70 Qualitative and quantitative parasitological surveys are relevant in improving conservation
71 strategies for species at risk of extinction in the face of interactive forces such as habitat loss and
72 global change. Nevertheless, no information is available on parasites of bears from BC and the
73 national parks of the Canadian Rocky Mountains. The objectives of our study were to document the
74 helminth fauna of grizzly and black bears from AB and BC, and to assess the statistical significance
75 of parasite infection prevalence, intensity, and abundance (see definitions in Rózsa et al. 2000) in
76 correlation with host life-history traits such as species, age, gender, sampling season, and location.

78 **Materials and methods**

79 **Specimen collection**

80 Between May 2011 and June 2013, we collected the entire carcass and/or the gastrointestinal
81 tract of 40 black bears and 13 grizzly bears from AB and BC. Carcasses (22 black bears, 7 grizzly
82 bears) were obtained from bears found dead within the provincial and national parks of AB.
83 Gastrointestinal tracts from BC (18 black bears, 6 grizzly bears) were submitted to our laboratory
84 after collection from animals killed by vehicle collision or by legal hunting within science-based
85 wildlife management and enforcement programs independent from the present study. The stomach
86 of 15 individuals (13 black bears, 2 grizzly bears) from BC was not provided due to logistical
87 issues. Information about species, gender, age class, date of death, and retrieval site of the carcass
88 was gathered for each individual host (Fig. 1 and Table 1). Information on the date of death (i.e.,
89 sampling season), was not available for 7 individuals (4 black bears, 3 grizzly bears). Age
90 classification was estimated either based on the actual birth date of each individual when monitored
91 since emergence from the den, or on morphological traits and dentition with an assumed birth date

92 at the beginning of February (Pelton 2003; Schwartz et al. 2003). Cubs were ≤ 1 year old, juveniles
93 were > 1 but < 4 years old, and adults were 4 or more years old.

94

95 **Laboratory analysis**

96 At necropsy, which in most cases was conducted on freshly collected bears, carcasses ($N =$
97 29) were examined for helminths, with inspection of body cavities, trachea, lungs, liver, spleen, and
98 kidneys. Gastrointestinal tracts ($N = 53$) were collected and stored frozen at -20° C. Once thawed,
99 the stomachs ($N = 38$) were dissected, and the contents and lining examined for parasites. Each
100 intestine was opened longitudinally and washed into a bucket; the contents were rinsed through a
101 sieve (500 μm pores), diluted into a graduated beaker and examined for helminths in a tray against a
102 black background. Intestinal helminths were counted to estimate infection abundance and intensity;
103 cestodes were counted based on the number of isolated scolices. All the isolated worms were
104 preserved in 70% ethanol at -20° C for morphological and molecular analyses.

105

106 **Parasite identification**

107 After rehydration in tap water and clearing with lactophenol, nematodes were
108 morphologically identified using an Olympus BX53 microscope and according to identification
109 keys (Anderson 2000). Cestodes were characterized to the genus level based on the morphology of
110 the scolex (Khalil et al. 1994); the anatomy of mature proglottids was examined only when
111 specimens were in an adequate state of preservation for staining. Two segments from two tapeworm
112 specimens were stained with Semichon's acetic carmine, dehydrated in ethanol, cleared in xylene,
113 and mounted in Canada balsam.

114 Molecular identification was required for hookworm nematodes and tapeworms. For
115 hookworms, we analysed the internal transcribed spacers (ITS-1 and ITS-2) of the nuclear
116 ribosomal DNA (rDNA). For tapeworms, we analysed the cytochrome c oxidase subunit 1 (*cox1*)
117 gene of the mitochondrial DNA (mtDNA). After rehydration in TE buffer, genomic DNA from

118 individual specimens was extracted using the Epicentre® MasterPure™ Complete DNA and RNA
119 Purification Kit (Epicentre Biotechnologies, Madison, Wisconsin, USA). DNA was eluted in 20 µL
120 TE buffer and stored at -20° C. DNA extracts from hookworm specimens were amplified for the
121 ITS-1 and ITS-2 genes using primers 93 and 264 for ITS-1, and primers 623 and 94 for ITS-2
122 (Nadler et al. 2000a; Nadler et al. 2000b). DNA extracts from cestode specimens were amplified for
123 a segment of the *cox1* gene using primers 2575 and 3021 for isolates of *Taenia* L., 1758 (Bowles et
124 al. 1992), and primers BW3 and BW4.5 for isolates of *Diphyllobothrium* Cobbold, 1858 (Wicht et
125 al. 2007). Enzymatic amplification and thermocycling protocols for Polymerase Chain Reaction
126 (PCR) were performed as described by Catalano et al. (2015) for hookworm specimens, and by
127 Lavikainen et al. (2011) for *Taenia* specimens. Amplification for *Diphyllobothrium* spp. was
128 performed in a 25 µL reaction mixture containing 2.5 µL 10X PCR buffer, 3 mM MgCl₂, 200 µM
129 deoxynucleoside triphosphates, 0.5 µM of each primer, 1 µL of bovine serum albumin (New
130 England Biolabs, Ipswich, Massachusetts, USA), 1 unit of *AmpliTaq* DNA polymerase, and 2 µL
131 DNA template. Cycling parameters consisted of an initial nucleic acid denaturation at 94° C for 5
132 min, followed by 33 cycles of 94° C for 45 sec, 50° C for 45 sec, and 45 sec at 72° C, with a final 5
133 min extension at 72° C.

134 PCR products were sequenced using an Applied Biosystems 3730xl DNA Analyzer with
135 BigDye Terminator™ chemistry (Perkin-Elmer, Waltham, Massachusetts, USA) after purification
136 with the E.Z.N.A.™ MicroElute Cycle-Pure Kit (Omega Bio-Tek, Norcross, Georgia, USA).
137 Sequencing reactions were double-stranded using the original PCR primers. Contig assembly and
138 editing were performed with CodonCode Aligner (CodonCode Corporation, Centerville,
139 Massachusetts, USA). Obtained sequences were compared by alignment with previously published
140 data available on the National Center for Biotechnology Information (NCBI) GenBank database.

141

142 **Statistical analysis**

143 The statistical significance of infection prevalence, intensity, and abundance for each
144 parasite species was analysed using Pearson's chi-squared (χ^2) test and Kruskal-Wallis rank sum
145 test ($P < 0.05$ for statistical significance). Parasitological data from the seven bears for which
146 information on the sampling season was not available were not included in the statistical analysis.
147 The non-normal distribution of the data, assessed by the Shapiro-Wilk W-test, justified the
148 application of Kruskal-Wallis rank sum test. Pearson's χ^2 test was used to assess significantly
149 different infection prevalence relative to host species, gender, age class, sampling season, and
150 location. Kruskal-Wallis rank sum test was used to assess significantly different median abundance
151 and median intensity relative to host species, gender, age class, sampling season, and location.
152 Statistical analyses were run using the software R (R Core Team 2013).

153

154 Results

155 The isolated parasites included the nematode species *Dirofilaria ursi* Yamaguti, 1941,
156 *Baylisascaris transfuga* (Rudolphi, 1819), *Uncinaria rauschi* Olsen, 1968, and *Uncinaria*
157 *yukonensis* (Wolfgang, 1956), and the cestode species *Taenia arctos* Haukisalmi, Lavikainen,
158 Laaksonen & Meri, 2011, *Diphyllobothrium dendriticum* (Nitzsch, 1824), and *Diphyllobothrium*
159 *nihonkaiense* Yamane, Kamo, Bylund & Wikgren, 1986. The results of the parasitological analysis
160 are summarized in Table 2, with helminth infection prevalence and intensity grouped by host
161 species and age class. The nematode *D. ursi* was recovered at necropsy from the subcutaneous
162 tissues, peri-tracheal fascia, and/or peritoneal fascia of 7/22 black bears (31.8%) and 2/7 grizzly
163 bears (28.6%). The gastric contents were analysed for 38 bears (27 black bears, 11 grizzly bears):
164 two grizzly bears from Owikeno Lake, BC (51°42'30" N, -126°54'52" W), harboured *B. transfuga*
165 juveniles in their stomachs. Helminth intestinal infections were found in 49 out of 53 bears (92.5%):
166 38/40 black bears (95%) and 11/13 grizzly bears (84.6%). We isolated *U. rauschi* in 36 hosts (29/40
167 black bears, 7/13 grizzly bears), while only one adult female grizzly bear was co-infected with both
168 *U. rauschi* and *U. yukonensis*.

169 Morphological and molecular analyses of *U. rauschi* and *U. yukonensis* confirmed the
170 presence of polymorphism at the vulvar region (Catalano et al. 2015); all the hosts infected with *U.*
171 *rauschi* harboured its female phenotypes characterized by either one or two knobs at the vulvar
172 opening. In contrast, the poor quality of the *Taenia* isolates did not allow for their morphological
173 characterization. The specimens were identified as *T. arctos* based on the similarity of the obtained
174 *cox1* sequences (396 base pairs) with data previously reported (Lavikainen et al. 2011; Catalano et
175 al. 2014). *Taenia arctos* infected 4/40 black bears (10%) and 1/13 grizzly bear (7.7%), of which one
176 black bear was from Vancouver Island, BC (49°21'35" N, -124°31'2" W), while the remaining *T.*
177 *arctos*-positive bears were from the national parks of the Canadian Rocky Mountains within AB.
178 The molecular analysis was not successful for the only *Taenia* specimen found in a yearling female
179 black bear from Jasper National Park, AB (52°51'35" N, -118°4'35" W); in this case, the infection
180 with *T. arctos* was assumed since the host came from the same area as the other *T. arctos*-positive
181 bears. The three *T. arctos*-positive black bears from AB were collected between late May and late
182 July, whereas the only positive black bear from BC and grizzly bear were sampled at the end of
183 October and in mid-May, respectively. The cestodes *D. dendriticum* and *D. nihonkaiense* were also
184 identified based on the sequences of their *cox1* gene (368-397 base pairs). Two grizzly bears, one
185 from Owikeno Lake and the other from Kitwancool Lake, BC (55°24'32" N, -128°8'51" W), hosted
186 *D. dendriticum* and *D. nihonkaiense*, respectively. Before the genetic analysis, two segments from
187 two adult tapeworms later classified as *D. nihonkaiense* were stained for morphological
188 characterization (Fig. 2), but identification to the species level was unattainable.

189 Pearson's χ^2 test indicated a significantly lower prevalence of *B. transfuga* in black bears
190 sampled during the spring ($\chi^2 = 4.22$, $P = 0.04$, odds ratio = 0.17). Kruskal-Wallis rank sum test
191 also found significantly lower relationships between black bears sampled during the spring and *B.*
192 *transfuga* median abundance (KW = 11.40, $P = 0.003$) and median intensity (KW = 6.83, $P = 0.03$).
193 Although not statistically significant, the infection values of *B. transfuga* in black bears were at
194 their highest in the fall, before hibernation [prevalence 7/8 (87.5%); median abundance 18.5;

195 median intensity 28], followed by summer [prevalence 11/16 (68.7%); median abundance 5; median
196 intensity 10] and spring [prevalence 4/12 (33.3%); median abundance 0; median intensity 3]. No
197 other statistical comparison between helminth infection values and host characteristics (species,
198 gender, age class, sampling season, and location) using Pearson's χ^2 test and Kruskal-Wallis rank
199 sum test resulted statistically significant ($P > 0.05$).

200 Voucher specimens were deposited in the U.S. National Parasite Collection (USNPC), U.S.
201 Department of Agriculture (USDA), under the accession numbers USNPC 106983, 106985, 107885
202 and 107886 for *U. rauschi*, USNPC 106982 for *U. yukonensis*, USNPC 106987 and 106988 for *B.*
203 *transfuga*, USNPC 106984 and 106986 for *T. arctos*, and USNPC 107884 for *D. nihonkaiense*.
204 Representative specimens were also deposited in the University of Alberta Parasite Collection
205 (UAPC) under the accession numbers UAPC 11582-11583 for *U. rauschi*, UAPC 11584-11585 for
206 *D. ursi*, and UAPC 11586-11587 for *B. transfuga*. The quality of *D. dendriticum* specimens was too
207 poor for submission to the archival collections. ITS rDNA sequence data from individual
208 hookworm specimens were deposited in the NCBI GenBank database under the accession numbers
209 KJ026495-KJ026519; mtDNA *cox1* sequence data from individual tapeworm specimens were
210 deposited in the NCBI GenBank database under the accession numbers KF356386-KF356387 and
211 KJ026488-KJ026489 for *T. arctos*, KJ026490 for *D. dendriticum*, KJ026491-KJ026494 for *D.*
212 *nihonkaiense*.

213

214 Discussion

215 This is the first report on the parasitological status of grizzly and black bears from BC and
216 the national parks of the Canadian Rocky Mountains. Bears from BC have never been investigated
217 for parasites in the past, whereas there are only two studies on black bears from northern AB
218 documenting helminth biodiversity (Dies 1979) and coccidian protozoa infections (Hair and Mahrt
219 1970). We confirm the common occurrence of *B. transfuga*, *D. ursi*, and the genus *Uncinaria*
220 Frölich, 1789 in North American bears, and provide further information on the recently described

221 cestode *T. arctos*. Furthermore, we unequivocally demonstrate that two grizzly bears harboured the
222 potentially zoonotic cestodes *D. dendriticum* and *D. nihonkaiense*. The only previous record of *D.*
223 *nihonkaiense* in bears was the characterization of its adult stages from Russian brown bears (*Ursus*
224 *arctos* L., 1758) using molecular techniques (Arizono et al. 2009a). In contrast, the identification of
225 *D. dendriticum* from bears has never been confirmed previously by molecular diagnostics.

226 *Dirofilaria ursi* is an arthropod-borne nematode of the family Onchocercidae, common in
227 bears of northern USA and Canada (e.g., Rogers and Rogers 1976; Addison et al. 1978; Michalski
228 et al. 2010). The life cycle of *D. ursi* involves larval development within black flies of the family
229 Simuliidae as the major intermediate hosts (Addison 1980). Bears act as natural definitive hosts of
230 *D. ursi*, but this filarial parasite also matures to its adult stage in humans, which can act as
231 incidental hosts (Beaver et al. 1987; Haldane et al. 1996). We detected higher *D. ursi* prevalence in
232 black bears (31.8%) and grizzly bears (28.6%) when compared to previous surveys on bears from
233 western Canada which reported 0.8-11.1% prevalence for *D. ursi* (Choquette et al. 1969; Dies 1979;
234 Johnson et al. 2013). In contrast, the occurrence of *D. ursi* in black bears of eastern Canada is much
235 higher (Frechette and Rau 1977; Addison et al. 1978; Duffy et al. 1994), likely due to wetter
236 habitats favouring black fly larval development and blood-feeding activity by adults.

237 *Baylisascaris transfuga* is a globally distributed ascarid commonly occurring in bears. In
238 North America, *B. transfuga* has been isolated in bears at both northern and southern latitudes
239 (Rogers and Rogers 1976; Catalano 2014). However, its lower prevalence in black bears of the
240 southeastern USA may result from limited egg survival as a consequence of a warmer climate,
241 and/or from reduced transmission potential induced by a lower host population density (Pence et al.
242 1983; Foster et al. 2004; Hristienko and McDonald 2007). For instance, environmental limitations
243 and inadequate host densities explained the decrease in prevalence of the nematode *Baylisascaris*
244 *procyonis* (Stefanski & Zarnowski, 1951) in raccoons (*Procyon lotor* (L., 1758)) from northern to
245 southern USA (see Kazacos 2001). Our study found similar *B. transfuga* prevalence in the intestine
246 of black bears (60%) and grizzly bears (53.8%), and significantly lower infection values in black

247 bears sampled during the spring. Although not statistically significant, the higher infection values of
248 *B. transfuga* in black bears sampled during the fall support an intriguing hypothesis about the
249 seasonal variation of *B. transfuga* incidence in black bears, and its elimination from the intestine as
250 winter hibernation approaches (Rausch 1961; Rogers 1975). Therefore, adult ascarids may not
251 overwinter in black bears as hypothesized by Frechette and Rau (1978).

252 To our knowledge, the hookworms *U. rauschi* and *U. yukonensis* are the only two *Uncinaria*
253 species infecting North American bears. A recent study confirmed the current species status and
254 reported morphological polymorphism for *U. rauschi* and *U. yukonensis* (Catalano et al. 2015).
255 Previously, information on ursine hookworms was limited to the taxonomic descriptions of *U.*
256 *yukonensis* in black bears from the Yukon Territory, Canada (Wolfgang 1956), and *U. rauschi* in
257 bears from Alaska, USA (Olsen 1968). Additional findings of *U. yukonensis* (Rausch 1961; Jonkel
258 and Cowan 1971; Frechette and Rau 1977) and *U. rauschi* (S. J. Kutz, personal communication
259 reported in Johnson et al. 2013) in North American bears have reported an infection prevalence \leq
260 32%. Only one survey in bears from northwestern Canada found 10/21 (47.6%) grizzly bears
261 infected with *U. yukonensis* (Choquette et al. 1969). Our analysis showed (i) higher prevalence of
262 ursine *Uncinaria* spp. (72.5% in black bears, 53.8% in grizzly bears) when compared to historical
263 studies; (ii) the more common occurrence of *U. rauschi* than *U. yukonensis* among the sampled
264 hosts; (iii) the potential for *U. rauschi-U. yukonensis* co-infections; and (iv) high infection
265 prevalence and intensity in all age classes of the hosts (Table 2). Although these findings are not
266 supported by any statistical test of significance, the relatively high hookworm infection values we
267 observed in bears of all age classes support the hypothesis of a mainly horizontal transmission route
268 for *U. rauschi* and *U. yukonensis*, similar to the transmission mechanisms described for the
269 phylogenetically close *Uncinaria stenocephala* (Railliet, 1884), a hookworm infecting canids and
270 felids (Anderson 2000; Catalano et al. 2015). Nevertheless, further data on hookworm infection in
271 grizzly and black bear cubs are necessary to confirm our assumption.

272 The cyclophyllidean cestode *T. arctos* was first described at its adult stage in the small
273 intestine of Finnish brown bears, and as larval stages in the skeletal muscle of cervids of the genus
274 *Alces* Gray, 1821 from Finland and Alaska (Haukisalmi et al. 2011). Recently, *T. arctos* was further
275 identified in North American grizzly and black bears using molecular techniques (Catalano et al.
276 2014). This finding has raised questions about the actual identity of previous records of *Taenia* spp.
277 in bears. To date, bears are the only definitive hosts for *T. arctos*. The morphological similarity
278 between *T. arctos* and *Taenia krabbei* Moniez, 1879 suggests that historical reports of *T. krabbei* in
279 North American bears may actually refer to *T. arctos* (Lavikainen et al. 2011; Kutz et al. 2012;
280 Catalano et al. 2014). Aside from any potential misidentifications, which were necessarily based on
281 morphological analyses, the prevalence of *Taenia* spp. in North American bears is reported as \leq
282 11% (e.g., Choquette et al. 1969; Frechette and Rau 1977; Dies 1979). In particular, *T. arctos*
283 prevalence in Finnish brown bears was 1.9% (Lavikainen et al. 2011). Only two studies on nine
284 black bears (Rogers 1975) and 12 black bears (Duffy et al. 1994) found taeniid specimens in 28.6%
285 and 25% of the examined hosts, respectively. However, the small sample size and the collection of
286 the carcasses from a relatively limited area may have affected the proportion. We recorded a
287 relatively low total prevalence of 9.4% (5/53 positive hosts) and intensity (range 1-3) of *T. arctos* in
288 the bears we analysed, which is in agreement with earlier studies. The low values of *T. arctos*
289 infections in bears may be the consequence of their omnivorous diet and of limited predatory
290 activity on ungulates, increased only during late spring and early summer (Pelton 2003; Schwartz et
291 al. 2003; Munro et al. 2006).

292 Pseudophyllidean cestodes of the genus *Diphyllobothrium* are intestinal parasites infecting
293 fish-eating birds and mammals as definitive hosts (including humans), and using copepod
294 crustaceans and freshwater, anadromous, or catadromous fish as first and second intermediate hosts,
295 respectively (Dick et al. 2001; Scholz et al. 2009). Human diphyllbothriasis represents an
296 important fish-borne zoonosis since the second half of the 19th century (Arizono et al. 2009b;
297 Scholz et al. 2009). We applied molecular techniques to identify and report *D. dendriticum* and *D.*

298 *nihonkaiense* in North American bears. Bears are final hosts of various *Diphyllobothrium* spp., but,
299 before our study, unequivocal molecular identification was only achieved for *D. nihonkaiense*
300 specimens in brown bears from the Kamchatka Peninsula, Russia (Arizono et al. 2009a). We
301 confirmed the great difficulty in characterizing *Diphyllobothrium* spp. based on morphological
302 criteria alone (e.g., de Vos et al. 1990). Nevertheless, the finding of the two *Diphyllobothrium* spp.
303 in the Canadian wilderness is not surprising. *Diphyllobothrium nihonkaiense* has already been
304 reported in western Canada in one human case record of a tourist who ate infected raw sockeye
305 salmon (*Oncorhynchus nerka* Walbaum, 1792), and in a study on wolves (*Canis lupus* L., 1758)
306 from the coastal areas of BC (Wicht et al. 2008; Bryan et al. 2012). Bryan and colleagues (2012)
307 found eggs of *Diphyllobothrium* spp. in 142 out of 1558 wolf scats (9.1% prevalence), but
308 molecular identification of *D. nihonkaiense* resulted from the genetic analysis of a small subset of
309 nine fecal samples containing tapeworm eggs. *Diphyllobothrium dendriticum* has been frequently
310 reported from a broad range of hosts in circumpolar regions of North America (Andersen et al.
311 1987; Dick et al. 2001). However, *D. dendriticum* infection has been confirmed by molecular
312 methods only in one case of a woman who had visited Alaska (Kuchta et al. 2013). The actual
313 prevalence of *D. dendriticum* is probably underestimated and morphology-based species records of
314 *Diphyllobothrium* in North America should be carefully interpreted (Dick et al. 2001; Kuchta et al.
315 2013).

316 In conclusion, we provided original data on parasite biodiversity in North American grizzly
317 and black bears, and insight into the biology, ecology, and taxonomy of the isolated helminths.
318 Furthermore, we observed a remarkable similarity in the helminth fauna of the two host species,
319 which may be the result of the overlap of home ranges and ecological niches. Despite the new
320 information acquired, the impact of parasites on the health of bears is still poorly understood, and
321 the integration of parasitic diseases into wildlife management and conservation programmes is
322 limited (Cleaveland et al. 2002). The importance of habitat quality for the regulation of bear
323 populations has been extensively discussed (e.g., Mowat et al. 2005; Sawaya et al. 2012), but efforts

324 in research and surveillance on ursine parasites and diseases are also crucial given the potentially
325 detrimental effects of infectious agents on the viability of bear populations. The outcome of higher
326 parasitic infections can be dramatic for bears, in particular if constrained in degraded and crowded
327 habitats. In the last decade, habitat loss and fragmentation led to disease emergence and mortality in
328 giant pandas (*Ailuropoda melanoleuca* (David, 1869)) parasitized by the intestinal nematode
329 *Baylisascaris schroederi* (McIntosh, 1939) (Zhang et al. 2008). The progressive human-induced
330 bear population fragmentation in northwestern USA and western Canada (Mowat et al. 2005;
331 Proctor et al. 2012) may increase niche overlap and trophic relationships between grizzly and black
332 bears in smaller patches of habitat, with positive density-dependent effects on pathogen
333 transmission, spread and persistence.

334

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346

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542 **Table 1.** Host gender, age class, and sampling
 543 season for specimens of black bears (*Ursus*
 544 *americanus*) and grizzly bears (*Ursus arctos*)
 545 from Alberta (AB) and British Columbia (BC),
 546 Canada.

Host traits	Host species	
	Black bears	Grizzly bears
Gender		
Males	32	6
Females	8	7
Age class		
Cubs	7	1
Juveniles	19	5
Adults	14	7
Province		
AB	16	7
BC	24	6
Season*		
Spring	12	3
Summer	16	2
Fall	8	5

547 *For four black bears and three grizzly bears
 548 information on the sampling season is not
 549 available.

Draft

550 **Table 2.** Infection prevalence (%) and intensity (median and range in parentheses; NA if not applicable) for helminths of black bears (*Ursus americanus*) and grizzly bears (*Ursus arctos*) from
 551 Alberta and British Columbia, Canada. For each group (C for cubs, J for juveniles, A for adults), the number of examined hosts is reported in parentheses.

Helminth species	Black bears				Grizzly bears			
	C (N = 7)	J (N = 19)	A (N = 14)	Total (N = 40)	C (N = 1)	J (N = 5)	A (N = 7)†	Total (N = 13)
Nematodes								
<i>Baylisascaris transfuga</i>	42.9% (3; 1-6)	73.7% (8; 1-78)	50% (11; 4-40)	60% (7.5; 1-78)	100% (6; NA)	40% (10.5; 4-17)	57.1% (10.5; 4-26)	53.8% (9; 4-26)
<i>Diriofilaria ursi</i> *	25% (NA)	20% (NA)	50% (NA)	31.8% (NA)	NA	—	40% (NA)	28.6% (NA)
<i>Uncinaria rauschi</i>	71.4% (21; 5-740)	57.9% (24; 2-739)	92.9% (158; 2-506)	72.5% (118; 2-740)	100% (20; NA)	40% (96.5; 22-171)	57.1% (47; 30-79)	53.8% (46; 20-171)
<i>Uncinaria yukonensis</i>	—	—	—	—	—	—	14.3% (140; NA)	7.7% (140; NA)
Cestodes								
<i>Diphyllobothrium dendriticum</i>	—	—	—	—	—	—	14.3% (3; NA)	7.7% (3; NA)
<i>Diphyllobothrium nihonkaiense</i>	—	—	—	—	100% (27; NA)	—	—	7.7% (27; NA)
<i>Taenia arctos</i>	—	5.3% (1; NA)	21.4% (2; 1-3)	10% (1.5; 1-3)	—	20% (1; NA)	—	7.7% (1; NA)

552 *The total number of carcasses examined for *D. ursi* was 29: 7/22 black bears (1/4 cubs, 2/10 juveniles, and 4/8 adults) and 2/7 grizzly bears (0/2 juveniles, 2/5 adults) resulted positive.

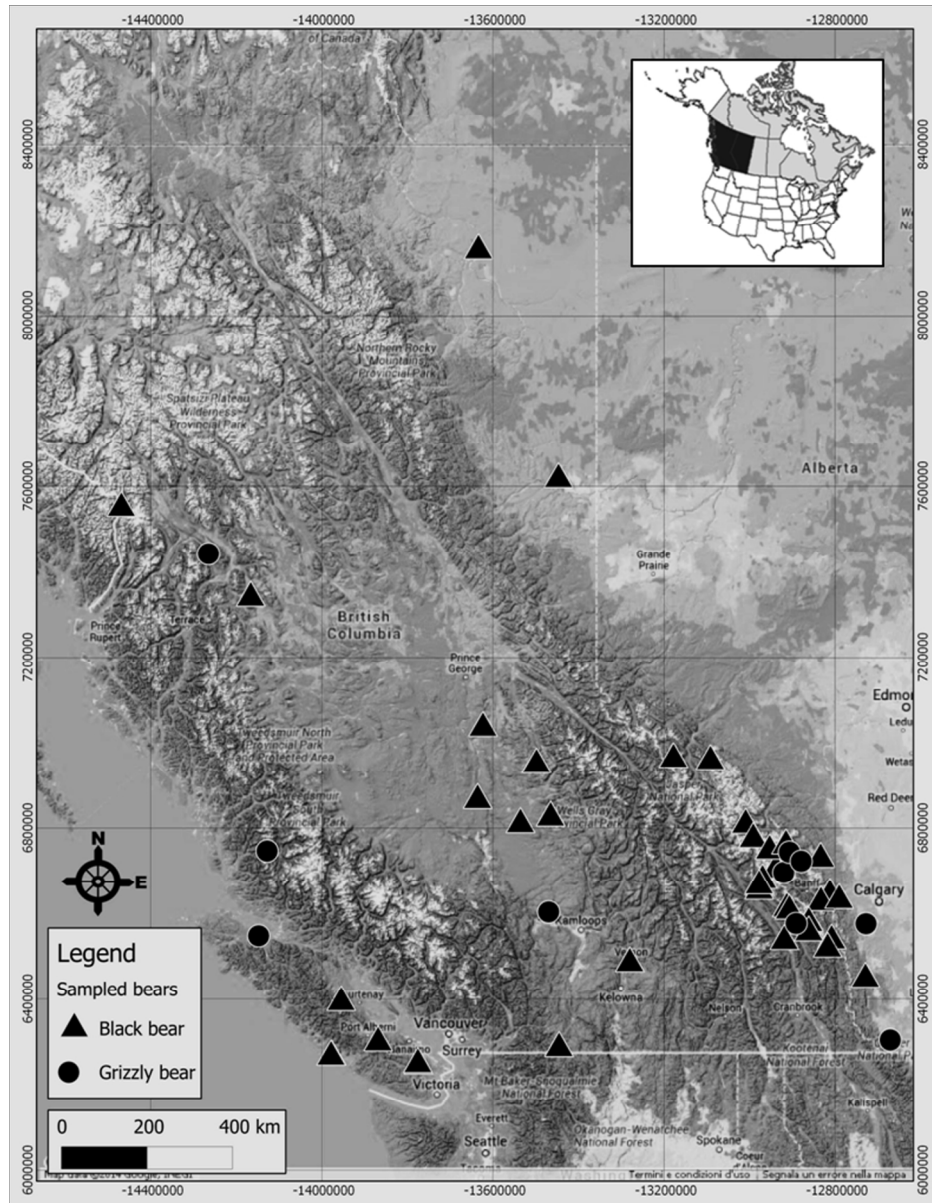
553 †One host was co-infected with *U. yukonensis* and *U. rauschi*, of which 140 and 48 specimens were isolated, respectively.

554 **Fig. 1.** Map of the locations where carcasses of black bears (*Ursus americanus*) and grizzly bears
555 (*Ursus arctos*) were retrieved in Alberta and British Columbia, Canada. Map developed using the
556 software ArcGis 10.1 ESRI®.

557

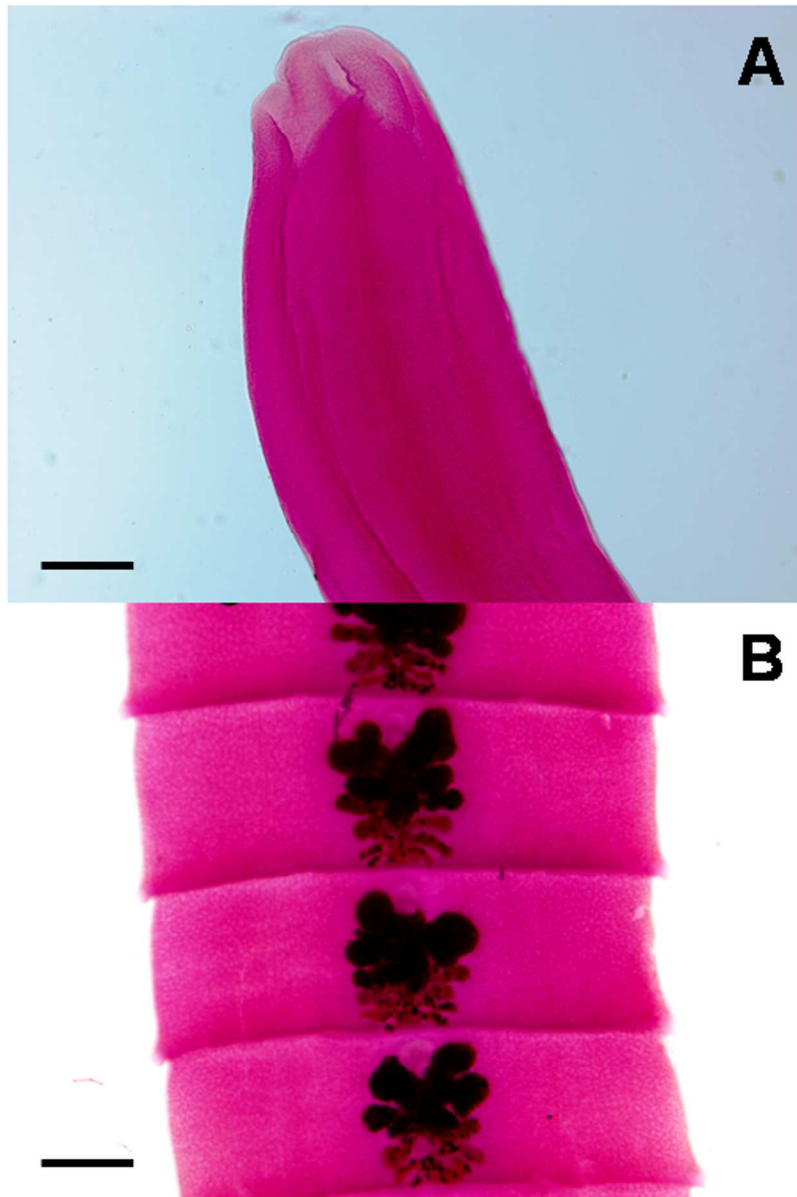
558 **Fig. 2.** Scolex (A) and proglottids (B) of *Diphyllbothrium nihonkaiense* recovered from a grizzly
559 bear (*Ursus arctos*) of British Columbia. The photographs were made after a staining process using
560 Semichon's acetic carmine. The scale bars represent 200 µm (A) and 500 µm (B).

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Map of the locations where carcasses of black bears (*Ursus americanus*) and grizzly bears (*Ursus arctos*) were retrieved in Alberta and British Columbia, Canada. Map developed using the software ArcGis 10.1 ESRI®.

110x142mm (300 x 300 DPI)



Scolex (A) and proglottids (B) recovered from a grizzly bear (*Ursus arctos*) of British Columbia. The photographs were made after a staining process using Semichon's acetic carmine. The scale bars represent 200 μm (A) and 500 μm (B).
128x192mm (300 x 300 DPI)