Molecular phylogenetics and systematics of two enteric helminth parasites (*Baylisascaris laevis* and *Diandrya vancouverensis*) in the Vancouver Island marmot (*Marmota vancouverensis*)

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**ABSTRACT**

Island biogeography can promote rapid diversification and speciation via geographic isolation and novel selection pressures. These same factors can threaten the persistence of island endemics by limiting gene flow and suitable habitat. Host-parasite interactions on islands introduce another dimension of complexity as both species must simultaneously adapt to exogenous and endogenous factors. One example of host-parasite island biogeography is the critically endangered Vancouver Island (VI) marmot (*Marmota vancouverensis*) which is endemic to VI, Canada, and hosts two enteric helminth parasites: *Baylisascaris laevis*, an ascarid nematode common in tribe Marmotini, and *Diandrya vancouverensis*, an anoplocephalid cestode endemic to the VI marmot. Here, we aligned novel sequences from *B. laevis* (six genes) and *D. vancouverensis* (two genes) with congeneric sequences from GenBank. Phylogenies reconstructed using Bayesian and maximum parsimony approaches consistently placed *B. laevis* in a morphoclade, and *D. vancouverensis* in a monophyletic clade sister to *D. composita*. Mean pairwise sequence divergence between *D. vancouverensis* and *D. composita* (9.06 ± 1.94%) surpassed commonly accepted thresholds for species delimitation, whereas divergence between VI and mainland populations of *B. laevis* (1.12 ± 0.78%) was comparable to (or sometimes greater than) pairwise divergence values between other *Baylisascaris* species. Disparity in the genetic divergence of each parasite may reflect differences in their life cycle, host specificity, virulence, and the chronological extent of their isolation. Detailed descriptions of the population genetic structure and effects of both parasites on their shared host are crucial next steps in understanding the history of *B. laevis* and *D. vancouverensis* on VI and informing conservation efforts for the VI marmot and its enteric helminth parasites.

1. Introduction

Island biogeography is a major driver of speciation, with geographically isolated ecosystems providing hotspots of endemism. The dynamic factors that promote speciation and persistence of insular endemics include neutral models of evolution as well as adaptation to habitat-specific selection pressures (Whitehead and Jones, 1969; Eldridge et al., 2018). Isolated populations can diverge over generations by chance fluctuation in allele frequencies when gene flow is interrupted. The process of divergence is accelerated when small founder populations colonize islands, importing a small subset of the total genetic variation from their source population, and these events are an important driver of speciation on islands (Matzke, 2014). Host-parasite associations can introduce unique selection pressures, wherein factors such as frequency-dependent selection and co-adaptation contribute additional dimensions of evolutionary complexity (Gandon, 2002; Goater et al., 2014). Anthropogenic habitat destruction, pollution, and climatic influences threaten these fragile dynamics at an accelerating rate, increasing the need for formal description of biodiversity to inform conservation efforts (Leclerc et al., 2020). Vancouver Island (VI) constitutes one potential hotspot of cryptic biodiversity as several unique populations have been described (Walser et al., 2005; Chavez et al., 2014; Hessels et al., 2021), with at least two diverging to the extent of speciation (Swarth, 1911; Taylor et al., 2012).

The distribution of parasites within host taxa follows an oscillatory pattern, with co-speciation interspersed by periodic host-switching (Hoberg and Brooks, 2008). The extent to which parasites mirror host evolution is influenced by life cycle characteristics, including host...
specificity and mode of dispersal. In some cases, parasite phylogeography mirrors the host, as they colonize novel habitats together (Carlson et al., 2021), but parasites can undergo host-independent speciation events when their associations with hosts are unstable (Stefka et al., 2011). At the microevolutionary level, the frequency of host-switching events is negatively related to the extent of co-adaptation between a parasite and its hosts (Gandon, 2002).

Parasites impose unique selection pressures by influencing host fitness, and the nature of their influence is determined by qualities of the parasite itself (Goater et al., 2014). Differences in the specific reproductive and dispersal characteristics of parasite species, along with the biogeographic and climatic context of their association may influence their degree of co-adaptation and co-specialisation with hosts (Gandon, 2002). Enteric helminths, including monoeccious cestodes and dioecious nematodes, are endoparasites with diverse life cycles, which can exert a range of effects on intermediate and definitive hosts (Castro, 1996).

The Vancouver Island marmot (Marmota vancouverensis Swarth, 1911; VIM) is endemic to VI and is listed as Critically Endangered by the IUCN and Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2019). It is the youngest, and the only insular Marmota species, thought to have diverged rapidly from its sister species, the hoary marmot (M. caligata), following post-glacial colonization of VI 12–13 thousand years ago (Kya) (Nagorsen and Cardini, 2009; Kerhoula et al., 2015). The VIM is host to two enteric helminth parasites: an ascarid nematode Baylisascaris laevis Leidy, 1856 and an anoplocephalid cestode Diandrya vancouverensis Mace and Shepard (1981). Genetic sequence data have been unavailable for either species, hence both have been excluded from molecular phylogenetic analyses within their respective taxa. The effect of parasite burden on VIM health has yet to be examined in detail, and the extent to which these two helminth species have diverged from their mainland counterparts has never been investigated.

Baylisascaris laevis is known to parasitize members of the Marmotini tribe, including Marmota, Urocitellus, and Otospermophilus, and has been recorded across Canada (ON, SK, BC) and the United States (AK, NY, PA, and CA) (Fig. 1; Berry, 1985; Sapp et al., 2017). It is the only member of the genus Baylisascaris with rodent definitive hosts, as well as the only species without an intermediate host — transmission occurs directly between definitive hosts via eggs ingested during grooming or feeding (Berry, 1985; Sapp et al., 2017) — although B. procyonis can also be transmitted vertically by the same means (Kazacos, 2001). Visceral pathology in naturally and experimentally infected hosts has been demonstrated as larvae migrate within host tissues (Babero, 1960). Camp et al. (2018) provides the most recent, and comprehensive, molecular systematic analysis of Baylisascaris, including seven of the 11 recognized species in the genus, but excluding B. laevis. The phylogenetic reconstruction shows two clades — the first containing species from guolrine mustelid, skunk, and raccoon hosts; the second encompassing species that parasitize marsupials, bears, and red panda (Ailurus fulgens) (Camp et al., 2018). Historically, Sprent (1968) placed B. laevis with B. columnaris (from skunks) and B. procyonis (from raccoons) based on morphology.

Diandrya vancouverensis differs from its sister species, D. composita Darrah (1930), based on morphology. No additional infections of D. vancouverensis have been reported in the literature since its description, with subsequent cestode infections in M. vancouverensis (perhaps erroneously) attributed to D. composita (Raverty and Black, 2001). Diandrya composita, on the other hand, is known to occur in four of the other five Marmota species in North America (all except the woodchuck M. monax), and has been reported in Alaska, Yukon, Washington, and Wyoming (Fig. 1; Rausch and Rausch, 1971). The complete life cycle of D. composita has not been described; however, it is hermaphroditic (Rausch, 1980), and is presumed to rely on free-living mites as intermediate hosts, as is common for cestodes of the family Anoplocephalidae (Rausch and Rausch, 1971; Denegri, 1993). The phylogenetic relationships among D. composita and other anoplocephalid cestodes have been resolved using genetic sequence data (Wickström et al., 2005), whereas the phylogenetic placement of D. vancouverensis has yet to be assessed beyond morphological comparisons.

Here, we compared newly generated nuclear and mitochondrial sequences from B. laevis and D. vancouverensis, collected from VI and mainland North America, to existing sequences from related species. We aimed to: 1) infer the phylogenetic placement of B. laevis within Baylisascaris; 2) assess the extent of divergence between B. laevis from VI and the mainland; and 3) resolve species-level relationships among D. vancouverensis and D. composita.

2. Materials and Methods

2.1. Specimen acquisition and DNA extraction

The Marmot Recovery Foundation provided adult specimens of Baylisascaris sp. and Diandrya sp. from VIM fecal samples. Mainland tissue samples of adult Baylisascaris sp. were collected during necropsy of Urocitellus columbianus in Idaho (Cook et al., 2017) and Marmota monax in Alaska (Table 1). Three Idaho samples were contributed by the Division of Parasites, Museum of Southern Biology, University of New Mexico (MSBP 24571, 24572, and 24594). The sole Alaskan sample was collected from the Marmot Recovery Foundation. Tissue Kit (Qiagen, Toronto, Ontario, Canada). For Diandrya, sections of proglottid were incubated at 56 °C in 180 μl Buffer ATL and 20 μl Proteinase K for 3 h. For Baylisascaris, sections of tail or midsection were incubated overnight, for enhanced digestion of cuticle-bound tissue. DNA extracts were resuspended in 100 or 200 μl of Buffer AE and stored at −20 °C.

2.2. PCR amplification and sequencing

Genes were selected for analysis based on availability of GenBank accessions from related species. Six genes had previously been used to identify relationships among seven Baylisascaris species (Camp et al., 2018) (refer to Table 1 for species details). These included three mitochondrial genes (12S rDNA, cytochrome oxidase subunits 1 and 2 (cox1...
Table 1
GenBank accession numbers for Baylisascaris and outgroup sequences included in the analysis. The length of each alignment is listed in nucleotide base pairs. Specimens sequenced in this study were contributed by the Marmot Recovery Foundation (MRF), the University of Alaska Museum of the North (UAM), and the Division of Parasites, Museum of Southwestern Biology (MSBP), University of New Mexico. Specimens from the MRF were submitted to the Royal British Columbia Museum (RBCM) after sequencing. Catalogue numbers are provided where applicable.

| Species | Host | Location | 12S (464 bp) | coxl (904 bp) | cox2 (506 bp) | 28S (933 bp) | ITS (756 bp) | ardl (637 bp) |
|---------|------|----------|-------------|-------------|-------------|-------------|-------------|-------------|
| B. laevis VI | Marmota vancouverensis | VI, CAN | ON994381 | ON982731 | ON988167 | ON994376 | ON982744 | ON988164 |
| B. laevis AE | M. monax | Alaska, USA | ON994382 | ON982732 | ON988168 | ON994377 | ON982755 | ON988165 |
| B. laevis ID1 | Urocitellus columbianus | Idaho, USA | ON994383 | ON982733 | ON988169 | ON994378 | ON988170 | ON988166 |
| B. laevis ID2 | U. columbianus | Idaho, USA | ON994384 | ON982734 | ON988170 |
| B. laevis ID3 | U. columbianus | Idaho, USA | ON994385 | ON988171 | ON994378 |
| B. columaris CT | Mephitis mephitis | Connecticut, USA | MG937785 | MG979514 | MG969662 | MG927772 | MG030594 | MG900134 |
| B. columaris IL | M. mephitis | Illinois, USA | MG937786 | MG979514 | MG969663 | MG927773 | MG030595 | MG900135 |
| B. procyonis CT | Procyon lotor | Connecticut, USA | MG937787 | MG979514 | MG969664 | MG927774 | MG030596 | MG900136 |
| B. procyonis CA | P. lotor | California, USA | MG937789 | MG979515 | MG969665 | MG927775 | MG030597 | MG900137 |
| B. deivi | Pekania pennanti | Ontario CAN | MG937789 | MG979515 | MG969666 | MG927776 | MG030598 | MG900138 |
| B. Schroederi | Alluropoda melanoleuca | Sichuan, CHN | MG937790 | MG979515 | MG969667 | MG927777 | MG030599 | MG900139 |
| B. aliiari | Aliauraria fulgens | Sichuan, CHN | MG937791 | MG979515 | MG969668 | MG927778 | MG030600 | MG900140 |
| B. transfuga ALB | Ursus arctos | Alberta, CAN | MG937792 | MG979514 | MG969669 | MG927779 | MG030601 | MG900141 |
| B. transfuga WV | U. americanus | West Virginia, USA | MG937793 | MG979515 | MG969670 | MG927780 | MG030602 | MG900142 |
| B. tasmaniensis | Sarcophilus harrisii | Tasmania, AUS | MG937794 | MG979516 | MG969671 | MG927781 | MG030603 | MG900143 |
| A. scarus | Sus scrofa domesticus | Louisiana or Michigan, USA | MG937795 | MG979517 | MG969672 | MG927782 | MG030604 | MG900144 |
| Parascaris equorum | Equus ferox caballus | Louisiana, USA | MG937796 | MG979518 | MG969673 | MG927783 | MG030605 | MG900145 |
| Toxascaris leonina | Volpes vulpes | South Dakota, USA | MG937797 | MG979519 | MG969674 | MG927784 | MG030606 | MG900146 |

and cox2) and three nuclear genes (large-subunit rDNA (28S), internal transcribed spacers 1–2 (ITS), and the exon–primer intron–crossing (EPIC) locus alcohol/ribitol dehydrogenase (ard1)). For Diodranya, we selected cox1 and ITS1 because these genes have previously been sequenced for D. composita and related species by Wickstrom et al. (2005) (Table 2). We designed new primers in Primer3 v2.3.7 (Untergasser et al., 2012) based on congeneric DNA sequence alignments to improve amplification success (Tables 1–3). Each gene was amplified via polymerase chain reaction (PCR) containing 12.5 μl of 2x TopTaq Master Mix (Qiagen, Toronto, Ontario, Canada), 200 μM deoxynucleotide triphosphates, 0.4 μM of forward and reverse primer, 3 mM total MgCl2, 3 μl (~200–600 ng) of template genomic DNA (gDNA), and ribonuclease-free water, to a final volume of 25 μl. We increased the concentration of the forward primer to 0.8 μM for cox2 following Nadler and Hudspeth (2000) and increased the amount of gDNA and total MgCl2 (3.5–5 mM) when our extractions yielded lower concentrations of DNA. Table 3 lists all primers and annealing temperatures (Ta) used for amplification and sequencing. Cycling parameters for Baylisascaris 12S, cox1, cox2, and ITS, and Diodranya ITS1, comprised 4 min denaturation at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at Ta, 70 s at 72 °C, and a final extension for 7 min at 72 °C. Annealing time was increased to 45 s for Baylisascaris ard1 and 28S, as well as Diodranya cox1, while the number of cycles was increased to 45 for extractions with low concentrations of DNA. Amplicons were Sanger sequenced in both directions at the University of Alberta’s Molecular Biology Services Unit. Consensus sequences, for each sample at each gene, were extracted from overlapping forward and reverse sequences after trimming low quality ends and primer regions, using Geneious v10.2.6 (Kearse et al., 2012). For Diodranya ITS1, consensus sequences were extracted from alignments produced with both primer pairs listed in Table 3, although the primer pair DvITS1F-2 and DvITS1R-2 generally reproduced the consensus sequence. Newly generated GenBank accession numbers for B. laevis and D. vancouverensis are available in Tables 1 and 2.

2.3. Alignment and phylogenetic analyses

Phylogenetic analyses were conducted for individual genes as well as concatenated sequence datasets of mitochondrial, nuclear, and/or combined loci, where sequences were available. Only Baylisascaris specimens from Idaho failed to produce sequence data at every gene, and thus were excluded from certain concatenated and gene-specific analyses. Outgroup taxa for Baylisascaris were Aescus suum, Parascaris equorum, and Toxascaris leonina, following Camp et al. (2018).

Table 2
GenBank accession numbers for Diodranya and outgroup sequences included in analysis. The length of each alignment is listed in nucleotide base pairs. Catalogue numbers for D. vancouverensis specimens submitted to the Royal British Columbia Museum (RBCM) are below each sample name.

| Species | Host | Location | cox1 (568 bp) | ITS1 (863 bp) |
|---------|------|----------|-------------|-------------|
| D. vancouverensis 1 | Marmota vancouverensis | VI, CAN | ON982735 | ON987264 |
| D. vancouverensis 2 | M. vancouverensis | VI, CAN | ON982736 | ON987265 |
| Diodranya composita 1 | M. caligata | Alaska, USA | AY181550 | AY755249 |
| D. composita 2 | M. broieri | Alaska, USA | AY568212 | AY755265 |
| D. composita 3 | M. caligata | Alaska, USA | AY181551 | AY755265 |
| Eurostomia gracilis | Microtus agrestis | Lahanka, FIN | AY396633 | AY396633 |
| Doutinina nordenskioldi | Dicrotomyx sp. | Victoria Island, CAN | AF558204 | AF314411 |
| Hymenolepis diminuta | Rattus sp. | Perth, AUS | NC_002767 | AF611125 |
| Andrya canicula | Oryctolagus cuniculus | Unspecified | AY189957 | AF314409 |
Table 3

| Locus       | Primer    | F/R | Sequence (5'-3') | Ta (°C) | Size (bp) | Reference          |
|-------------|-----------|-----|------------------|---------|-----------|--------------------|
| Baylisascaris |           |     |                  |         |           |                    |
| 12S         | 505       | F   | GTCCAGAATAATGGCTAGAC | 50      | 493       | Nadler et al. (2006) |
|             | 506       | R   | TCTACCTTCTACCTACTTC   |         |           |                    |
| cox1        | Blcos1F   | F   | TGGGTGTTGAGTCTAGTTGGA | 56      | 912       | This study         |
|             | Blcos1R   | R   | AGACCATCAGAGGCAACAA  |         |           |                    |
| cox2        | 211       | F   | TTTTCTTATAGTATAGTGGG   | 50      | 582       | Nadler and Hudspeth (2000) |
|             | 210       | R   | CCAACCTCCTAAATATCC    |         |           |                    |
| 28S         | BI28SF    | F   | AGTAACCTGAGGAAGGCA    | 60      | 943       | This study         |
|             | BI28SR    | R   | TGGCCCCCTATACAAGGCA   |         |           |                    |
| ITS         | dp617     | F   | CTGCAACGTTGCAAGCAGAC | 58      | 829       | Camp et al. (2018) |
|             | 94        | R   | TATGCTCCTTCTCCCTCGGC |         |           |                    |
| ard1        | Blard1F   | F   | TATGCGCCAGAGAAGGTCGA | 58      | 703       | This study         |
|             | Blard1R   | R   | CAGCGTATACGGCGAATTG   |         |           |                    |
| Diandrya    |           |     |                  |         |           |                    |
| cox1        | COX-F     | F   | GATGTTGCCTTATGATTATCTGGT | 51      | 640       | Haukisalmi et al. (2004) |
|             | COX-R     | R   | GCCACCAAATCTCAAGTATC  |         |           |                    |
| ITS1        | DvITSF1-1 | F   | GTCAAAGGTAGCTGTAGGG  | 56      | 642       |                  |
|             | DvITSR1-1 | R   | GGGATTCATATTATTTTGA   |         |           |                    |
|             | DvITSF1-2 | F   | AAGGTGAGTGGTGAAGGCC  | 56      | 664       | This study         |
|             | DvITSR1-2 | R   | TGTGAGTTCAAGAGAGTC    |         |           |                    |

Hymenolepis diminuta was selected as the outgroup taxon for Diandrya analyses, following Wickström et al.'s (2005) molecular phylogeny of anoplocephaline cestodes. Douthisitria nordenskioeldi, Andrya cuniculi, and Eurotzenia gracilis were included as ingroup species to improve the resolution of our Diandrya phylogeny. Wickström et al. (2005) placed D. nordenskioeldi as sister to Diandrya composita, whereas A. cuniculi was considered sister to D. composita prior to their analysis. Eurotzenia gracilis returned the best overall hits for Diandrya vancouverensis at cox1 and ITS1 in NCBI’s nucleotide database using their basic local alignment search tool (Johnson et al., 2008; Sayers et al., 2022).

Nucleotide sequences from B. laevis and D. vancouverensis were aligned with congeneric and outgroup sequences obtained from GenBank (Tables 1 and 2). Non-protein coding genes (12S, 28S, and ITS), the intron-crossing locus ard1, and concatenated sequences were aligned with ProbAlign v1.4 (Roshan and Livesay, 2006) using default parameters. Nucleotide sequences of protein coding genes cox1 and cox2 were aligned in Geneious using default parameters. To control for errors arising from alignment, nucleotide sequences of cox1 and cox2 were translated in silico with ExPasy (http://web.expasy.org/translate/) and the resulting amino acid sequences were aligned in Geneious. These amino acid alignments were uploaded as a scaffold for alignment of cox1 and cox2 nucleotide sequences in RevTrans v2.0 (Wernersson and Pedersen, 2003). The resulting alignments were identical to nucleotide alignments produced by Geneious. In addition, nucleotide alignments for all genes were tested for ambiguously aligned sites using Gblocks for non-protein coding genes ranging from 1% for B. laevis 28S to 58% for D. vancouverensis ITS1, and are reported in

3. Results

3.1. Sequencing success

All Baylisascaris amplicons from VI and Alaska produced high quality consensus sequences of sufficient length for analysis. Samples from Idaho were inconsistent in producing useable sequences (Table 1) therefore, Idaho ITS sequences were excluded from further analyses. All other single gene Baylisascaris datasets contained sequences from at least one Idaho specimen, and datasets for 12S and cox2 contained sequences from all Baylisascaris specimens listed in Table 1. Amplicons from both Diandrya specimens produced high quality consensus sequences at cox1 and ITS1 (Table 2). The proportion of ambiguously aligned sites identified by Gblocks for non-protein coding genes ranged from 1% for B. laevis 28S to 58% for D. vancouverensis ITS1, and are reported in

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Supplementary Table S3. No such sites were found for alignments of cox1 or cox2.

3.2. Molecular phylogenetics of Baylisascaris laevis

All BI and MP reconstructions across mt-trees, nr-trees, and combined trees consistently resolved B. laevis as a monophyletic group with high support (Figs. 2-4, Supplementary Figs. S1-S3). BI and MP single gene trees (Figs. S4-S15) reflected this relationship. All mt-trees, nr-trees, and combined trees supported the two clades resolved by Camp et al. (2018), splitting B. columnaris, B. procyonis, and B. devosi from B. Schroederi, B. transfuga, and B. ailuri (Figs. 2-4, S1-S3), although most trees placed B. tasmaniensis as sister to all other Baylisascaris species, apart from the BI mt-tree (Fig. 2). The mt-trees, nr-trees, and combined trees showed strong support for B. laevis as sister to the clade containing B. columnaris, B. procyonis, and B. devosi (Figs. 2-4, S1-S3). Most single gene trees supported this relationship, with the following exceptions: 1) the 12S MP and BI trees had an alternate branching pattern between B. laevis and B. devosi; 2) the cox1 MP tree placed B. laevis as sister to all Baylisascaris; and 3) the cox2 BI tree, and 28S trees from MP and BI, presented unresolved polytomies between B. laevis and the other species in this clade (Supplementary Figs. S3 and S4, S6, and S9-S11 respectively).

The mean pairwise sequence divergence (Mean ± SD) for B. laevis was 0.96 ± 0.70% between VI and Alaska; 1.12 ± 1.01% between VI and Idaho; and 1.12 ± 0.78% between VI and all mainland sequences (Table 4). The low divergence between VI and Alaska was consistent with topologies reconstructed in the BI and MP trees for individual nuclear genes, which showed VI and Alaska samples as sister taxa (Supplementary Figs. S10-S15). The trees for individual mitochondrial genes, and the concatenated mt-trees, were unable to resolve the relationships among B. laevis samples or placed the VI sample as sister to all other B. laevis samples (Fig. 2, S1, and S4-S9).

3.3. Molecular phylogenetics of Diandrya vancouverensis

BI trees for cox1 and combined genes placed D. vancouverensis as a monophyletic clade with high BPP support, whereas BPP support at ITS1 alone was less than 0.90 (Fig. 5). All MP trees strongly supported the monophyletic clade of D. vancouverensis (Supplementary Fig. S16). Mean pairwise sequence divergence between D. vancouverensis and D. composita was 9.06% (7.69% at cox1 and 10.44% at ITS1), and the two VI samples had identical sequences at both genes.

4. Discussion

Baylisascaris laevis and Diandrya vancouverensis represent two parasite species that have previously been classified solely based on morphology and ecology (Sprent, 1968; Mace and Shepard, 1981). Whereas B. laevis is common among ground squirrels and marmots across North America, D. vancouverensis has been described as endemic to the VIM. Until now, the extent to which their obligate association with this rare, island host has contributed to their genetic divergence and potential conservation concern was unclear. We reconstructed the phylogenetic relationships of both parasite species and estimated the degree of divergence between VI and mainland populations using new sequence data. Our results support the species designation of D. vancouverensis and present evidence for divergence of B. laevis on VI.

4.1. Systematics of Baylisascaris laevis

Camp et al. (2018) proposed two clades in Baylisascaris based on reconstructions across eight genes for seven of the 11 recognized species in the genus. Our reconstructions with additional samples and species supported these two clades; however, our analyses generally placed B. tasmaniensis as sister to all other Baylisascaris species, except in the cox1 and cox2 trees (Figs. S6, S7, and S9), the BI 28S tree (similar to the BI 28S tree from Camp et al. (2018; Fig. S11)), and the MP and BI ard1 trees (Figs. S12-S13). The proportion of unambiguous sites selected by Gblocks in our Baylisascaris alignments (Supplementary Table S3) were comparable to the proportion of characters retained by Camp et al. (2018; Supplementary Data) in datasets filtered based on a 60% posterior probability threshold with ProAlign (Löytynoja and Milinkovitch, 2003).

The topology of ingroup species (excluding B. laevis) in our BI mt-tree matched Camp et al. (2018), although B. procyonis from California and Connecticut were placed in a polytomy that was sister to B. columnaris (Fig. 2). Similarly, our BI nr-tree showed an alternate topology for Baylisascaris spp. in the clade with ursid and ailurid hosts and placed B. tasmaniensis as sister to all other Baylisascaris (Fig. 3). Our BI combined tree followed our mt-tree topology for the ursid and ailurid clade, but otherwise reflected our nr-tree arrangement of Baylisascaris spp. (Fig. 4). These differences may reflect the fact that our analyses excluded two nuclear genes included in the analysis by Camp et al. (2018).

Our analyses placed B. laevis in the clade with (but also sister to) species that parasitize skunks, raccoons, and guloine mustelids (B. columnaris, B. procyonis, and B. devosi, respectively). This relationship confirms the morphological grouping assigned by Sprent (1968).
and constitutes the first molecular phylogenetic placement of *B. laevis*. It has previously been suggested that the direct life cycle of *B. laevis* is the result of a capture event in its evolution, during which an ancestral form with a carnivoran host was able to develop to adulthood within its rodent intermediate host (Anderson, 2000). The position of *B. laevis* as sister to one clade, but not the other, suggests that this change likely occurred after the common ancestors of each clade diverged.

The pairwise sequence divergence between the VI and mainland samples of *B. laevis* ranged from 0.11 to 2.86% (Table 4), with the greatest divergence observed between VI and Idaho at *ardI*. However, variation between VI and Idaho was surpassed by variation between Idaho and Alaska at the same locus (3.39%). Locus *ardI* is an EPIC marker selected to capture variability between closely related species (Camp et al., 2018); it is highly variable by design, and thus likely not representative of mean divergence for the purpose of species delimitation. ITS and *cox1*, on the other hand, have been used to estimate interspecific divergence and prospect for cryptic species within nematode genera (Powers et al., 1997; Blouin, 2002).

Pérez Mata et al. (2016) used ITS to classify the novel species *Baylisascaris venezuelensis* and reported interspecific pairwise divergences of 2.8–10.0% among *B. venezuelensis*, *B. schroederi*, *B. transfuga*, and *B. procyonis*. Clearly, the extent of interspecific divergence within *Baylisascaris* encompasses a range of values depending on both the genes and species pairs examined. *Baylisascaris columnaris* and *B. procyonis* are considered distinct species with shallow genetic divergence (Franssen et al., 2013; Camp et al., 2018). In our analyses, these species formed a sister clade to *B. laevis*, and are thus a reasonable comparison for interspecific divergence values in closely related species.

The mean pairwise divergences between VI and mainland *B. laevis* at ITS and *cox1* (2.08 and 0.72%, respectively) were comparable to (or greater than) the divergences we calculated between *Baylisascaris* and *B. procyonis* in their alignments. These discrepancies may be due to differences in either the length of alignments (e.g., ITS sequences from Camp et al. (2018) were $889–975$ bp long, whereas our alignment was truncated to 756 bp), or the

![Baylisascaris phylogram](image-url)

**Table 4**

| Locus     | VI-AK | VI-ID | VI-ML | AK-ID | ID-ID |
|-----------|-------|-------|-------|-------|-------|
| 12S       | 0.44  | 0.22  | 0.33  | 0.22  | 0.00  |
| 28S       | 0.11  | 0.75  | 0.43  | 0.86  | –     |
| *ardI*    | 1.25  | 2.86  | 2.05  | 3.39  | –     |
| *cox1*    | 0.66  | 0.77  | 0.72  | 0.33  | 0.00  |
| *cox2*    | 1.19  | 0.99  | 1.09  | 0.20  | 0.00  |
| ITS       | 2.08  | –     | 2.08  | –     | –     |
| Mean ± SD | 0.96 ± 0.70 | 1.12 ± 1.01 | 1.12 ± 0.78 | 1.00 ± 1.36 | –     |

![Baylisascaris phylogram](image-url)
Vilas et al. (2005) reported pairwise sequence divergences of 0.1-10.44%, respectively. Hence, this analysis supports the species classification of D. composita as originally proposed based on its unique morphology (Mace and Shepard, 1981).

4.2. Systematics of Diandrya vancouverensis

Our combined and cox1 trees separated D. vancouverensis and D. composita with high support (Fig. 5 and Fig. S16). Wickstrom et al. (2005) placed D. composita in a clade with Paranoplocephala spp. and Vilas et al. (2005) reported pairwise sequence divergences of 0.1–5.9% and 5.2–18.4% at ITS1 and cox1, respectively, among species pairs from Paranoplocephala. These values are comparable to the divergences between D. vancouverensis and D. composita for these genes (7.69 and 10.44%, respectively). Hence, this analysis supports the species classification of D. vancouverensis originally proposed based on its unique morphology (Mace and Shepard, 1981).

4.3. Factors influencing the divergence of helminths in the VIM

Host biogeography and metapopulation dynamics can play substantial roles in the phylogeography and evolution of parasitic species; however, the extent of co-phylogeny between parasites and their hosts is variable and inversely related to parasite characteristics such as virulence and host specificity (Gandon, 2002). Although they are associated with the same endemic insular host, D. vancouverensis appears to have diverged substantially more from its mainland counterpart than B. laevis. The observed disparity between the genetic divergences of these species may be explained by parasite-specific factors that affect their extent of co-speciation with their shared host.

Fossil evidence suggests that marmots have colonized VI multiple times during the Pleistocene, most recently following the last glacial maximum 12–13 Kya (Ward et al., 2003; Nagorsen and Cardini, 2009). Prolonged geographic isolation of the VIM, since its likely recolonization of VI, would provide an opportunity for extended divergence between marmot parasites on VI and those on the mainland. Molecular phylogenetic analyses from mitochondrial data have estimated divergence of the VIM from its sister species, the hoary marmot (M. caligata) between 0.4 and 1.2 million years ago (Mya) (Steppan et al., 2011). However, recent nuclear analyses have suggested earlier divergence followed by introgression of hoary mtDNA to the VIM 0.73 Mya (Kerhoulas et al., 2015, Kerhoulas, 2017). It is likely that shifting glacial barriers during the Pleistocene produced geographic isolation of these species, interspersed by periods of sympathy (Kerhoulas, 2017). Intermittent gene flow between VI and hoary marmots since their initial divergence may have provided transient opportunities for host-switching events, which would have disproportionately favoured gene flow among parasites with lower host specificity.

In general, the phylogenetic relationships within Baylisascaris do not closely mirror the biogeography or phylogeny of their host taxa, and colonization of novel hosts is thus expected to factor prominently in their diversification (Camp et al., 2018). For example, Zhou et al. (2013) reported an absence of population genetic structure in a metapopulation of B. Schroederi from the giant panda (Ailuropoda melanoleuca), despite high levels of host differentiation across their fractured range. Furthermore, Baylisascaris spp. with hosts in the superfamly Musteloidea (gulonine mustelids, skunks, raccoons, and the red panda) are polyphyletic — B. ailuri from the red panda groups with species parasitizing members of the Ursidae. We note that B. laevis seems to have evolved after the split of the two major clades. This is intriguing as both clades

Fig. 5. Bayesian consensus tree from Diandrya, related genera, and outgroup alignments of cox1, ITS1, and concatenated sequences from GenBank and this study (D. vancouverensis). Branch labels represent Bayesian posterior probabilities. Branch lengths are scaled to expected number of substitutions per site. See also Table 2.
generally represent species with hosts from Carnivora, whereas *B. laevis* is associated with hosts from Rodentia. This provides further evidence for a capture event during which *B. laevis* evolved the ability of direct transmission in an intermediate host (see section 4.1). These incongruences between the phylogeography of *Baylisascaris* species and their hosts suggests relatively low host specificity within the genus, which corresponds to low population genetic structure (Nadler, 1995).

Nematodes are dioecious and tend to evolve faster than other parasite lineages, despite the increased fecundity of monocious species, such as cestodes (Anderson et al., 1998). *Baylisascaris laevis* is unique among its congeners in that it does not rely on intermediate hosts for transmission (Sapp et al., 2017). This feature of its life cycle likely facilitated its persistence on VI and may have conferred a dispersal advantage compared to *D. vancouverensis*, which, as an anoplocephalid cestode is expected to rely on a mite intermediate host for transmission (Denegri, 1990). Low host specificity in *Baylisascaris* could have contributed to increased gene flow among populations from different Marmota species during secondary contact events and may explain the weak divergence of *B. laevis* compared to *D. vancouverensis* (e.g., Hache et al., 2017). Each parasite will have had its own unique phylogeographic history involving colonization of a modern VIM or an ancestor, subsequent isolation, and episodic opportunities for gene flow, culminating in their present host-parasite assemblage (Hoberg and Brooks, 2008). The relative contributions of historical biogeography and life cycle characteristics to the divergence of these parasites alongside their shared host may be more clearly understood in light of their specific effects on marmot fitness, as well as direct analyses of their population genetic structure (e.g., Carlson et al., 2021).

4.4. Conservation implications

Antagonistic coevolution occurs when an adaptation in one symbiont (e.g., to increase exploitation by the parasite), elicits an evolutionary response by the other (e.g., to resist exploitation by the host) (Goater et al., 2014). Host-parasite competition can result in oscillatory fitness of parasite resistance genes, as described by the Red Queen hypothesis, when an adaptive response in the host becomes maladaptive as it is met by the reciprocal response from its parasite (Dybå and Lively, 1998). Under Red Queen dynamics, new adaptations become most effective when they are least common, which can drive genetic variation by selecting for rare phenotypes via negative frequency-dependent selection (Hamilton and Zuk, 1982). In terms of population fitness, the result of selection pressures on phenotypic diversity may depend on the existing genetic variance in the host population.

*Baylisascaris laevis* and *Diandyra* spp. have been associated with observable pathology in experimental and natural hosts, but neither have been directly implicated as cause of death (Babero, 1960; Raverty and Black, 2001). Detailed descriptions of their impact on VIM health will be a crucial next step in understanding their relevance within the VIM ecosystem. The VIM has exceedingly low genetic diversity (Barrett et al., 2022) which may limit its future capacity for novel adaptation, making it more vulnerable to infection as its adaptive responses lag behind those of its parasites.

Parasite lineages currently represent a major source of biodiversity loss worldwide (Carlson et al., 2020). Until relatively recently, eradicating parasites during host species conservation has stunted research on their ecosystem relevance. Much like apex predators, parasites can serve important roles as trophic regulators (Dougherty et al., 2016). Discovery and delimitation of cryptic parasite species is a critical challenge in documenting parasite biodiversity which must be guided by rigorous systematic methodology (Pérez-Ponce de León and Nadler, 2010).

The critically endangered VIM is itself host to a cryptic community of endemic biodiversity. It has already been argued that *D. vancouverensis* must share the endangered status of the VIM (VIMRT, 2008). If future analyses find support to designate *B. laevis* on VI as a unique species, then this status should extend to both parasites. *Diandyra vancouverensis* and *B. laevis* represent the first examples of an otherwise uncharacterized community of endemic biodiversity within the VIM. Similarly, other symbionts may constitute cryptic species and are, in any case, intimately dependent on the fate of the VIM. Conservation efforts for the biotic community of the VIM must be informed by an appreciation of their systematics and unique associations with their host.

This study represents a crucial first step in characterizing the complex coevolutionary dynamics between the VIM and two of its endoparasites. Based on novel genetic sequences for each species, our analysis exceeds commonly accepted thresholds for species delimitation of *D. vancouverensis* and presents evidence for divergence of *B. laevis* on VI. Molecular phylogenies reconstructed using BI and MP algorithms reflect the relationships predicted by the morphology of each parasite species (Sprent 1968; Mace and Shepard, 1981; Camp et al., 2018). Unfortunately, we were unable to secure specimens of *B. laevis* from *M. caligata*, the sister species to *M. vancouverensis*. This comparison is a necessary next step in resolving the phylogeography of *B. laevis* on VI. Hopefully, further analyses of population genetic structure in *B. laevis* and *D. vancouverensis*, along with long-term monitoring of parasite burden in the VIM will contribute to an impactful appreciation of these relationships and will inform conservation efforts for all three species.

Data availability

Parasite specimens collected from *M. vancouverensis* were submitted to the Royal British Columbia Museum and catalogue numbers are available in Tables 1 and 2, along with GenBank accession numbers for newly generated sequences.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2022.11.006.

References

Anderson, R.C., 2000. Nematode Parasites of Vertebrates: Their Development and Transmission. CABI Publishing, New York, USA, p. 650.
Steel, M., Penny, D., 2000. Parsimony, likelihood, and the role of models in molecular phylogenetics. Mol. Biol. Evol. 17, 839–850.
Štefaňová, J., Hooek, P.F., Keller, L.F., Smith, V.S., 2011. A hitchhiker’s guide to the Galápagos: co-phylogeography of Galápagos mockingbirds and their parasites. BMC Evol. Biol. 11, 284.
Stephan, J.J., Kenagy, G.J., Zawadzki, C., Robles, R., Lyapunova, E.A., Hoffmam, R.S., 2011. Molecular data resolve placement of the Olympic marmot and estimate dates of trans-Beringian interchange. J. Mammal. 92, 1028–1037.
Swarth, H.S., 1911. Two new species of marmots from northwestern America. Univ. Calif. Publ. Zool. 7, 201–204.
Swofford, D.L., Sullivan, J., 2012. Phylogeny inference based on parsimony and other methods using PAUP*. In: Lemey, P., Salemi, M., Vandamme, A.-M. (Eds.), The Phylogenetic Handbook: a Practical Approach to Phylogenetic Analysis and Hypothesis Testing, second ed. Cambridge University Press, pp. 267–312.
Taylor, E.B., Harris, L.N., Spice, E.K., Docker, M.F., 2012. Microsatellite DNA analysis of parapatric lamprey (Entosphenus spp.) populations: implications for evolution, taxonomy, and conservation of a Canadian endemic. Can. J. Zool. 90, 291–303.
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S. G., 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res. 40, e115.
Vancouver Island Marmot Recovery Team, 2008. Recovery Strategy for the Vancouver Island Marmot (Marmota vancouverensis) in British Columbia. Prepared for the B.C. Ministry of Environment, Victoria, BC, p. 25.
Vilas, R., Criscione, C.D., Blosum, M.S., 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. Parasitology 131, 839–846.
Walser, J.C., Holderegger, R., Gugerli, F., Hoebbe, S.E., Scheidegger, C., 2005. Microsatellites reveal regional population differentiation and isolation in Lobaria pulmonaria, an epiphytic lichen. Mol. Ecol. 14, 457–467.
Ward, B.C., Wilson, M.C., Nagorsen, D.W., Nelson, D.E., Driver, J.C., Wigen, R.J., 2003. Port Eliza cave: North American West Coast interstadial environment and implications for human migrations. Quat. Sci. Rev. 22, 1383–1388.
Wernersson, R., Pedersen, A.G., 2003. RevTrans: multiple alignment of coding DNA from aligned amino acid sequences. Nucleic Acids Res. 31, 3537–3539.
Whitehead, D.R., Jones, C.E., 1969. Small islands and the equilibrium theory of insular biogeography. Evolution 23, 171–179.
Wickstrom, L.M., Haukisalmi, V., Vartio, S., Hantula, J., Hettonnen, H., 2005. Molecular phylogeny and systematics of anoplocephaline cestodes in rodents and lagomorphs. Syst. Parasitol. 62, 83–99.
Yang, Z., 2015. The BPP program for species tree estimation and species delimitation. Curr. Zool. 61, 854–855.
Zhou, X., Xie, Y., Zhang, Z.-H., Wang, C.D., Sun, Y., Gu, X.-B., Wang, S.-H., Peng, X.-R., Yang, G.-Y., 2013. Analysis of the genetic diversity of the nematode parasite Baylisascaris schroederi from wild giant pandas in different mountain ranges in China. Parasites Vectors 6, 233.