Genetic Determination of sub-species classification for the Banff longnose dace (*Rhinichthys cataractae smithi*)

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Genetic examination of Banff longnose dace taxonomy revealed three evolutionary lineages of longnose dace common throughout extant North American populations. The Pacific lineage was not found in current Cave&Basin Marsh inhabitants and may be correlated with the loss of Banff longnose dace morphology.

Microsatellite DNA analysis revealed no significant differences between extant Marsh and Bow River longnose dace populations. Microsatellite DNA and otolith microchemistry results indicate gene flow between Marsh and Bow River dace.

mtDNA results do not support subspecies status. Regardless, Banff longnose dace represented a unique assemblage of fish that no longer exists in the Marsh. Biogeographic distinction of this population demonstrates it merited designation. However, designation of an extinct sub-species remains unresolved due to effects caused by the hot spring fed environment. I recommend that COSEWIC reassess the status of this sub-species from extinction of *R. c. smithi* to extirpation of *R. c. dulcis* from the Marsh.

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ABSTRACT

A morphologically unique population of longnose dace was known to exist in the Cave & Basin Marsh in Banff, Alberta. These fish were thought to be geographically separated and designated as a distinct sub-species, the Banff longnose dace. The traditional taxonomic traits used for this classification have been called into question and may not have accurately reflected phylogeny but resulted from genotype, phenotype, or a combination of both. I assessed the validity of the Banff longnose dace sub-species classification using molecular genetic techniques. I also used this approach in combination with otolith microchemistry for extant populations of Cave & Basin Marsh longnose dace to determine migration between the Bow River and the Marsh.

Historically, two different evolutionary mtDNA lineages (Great Plains and Pacific) of the longnose dace came into secondary contact in the Cave & Basin Marsh. None of these lineages proved to be unique or restricted to the Marsh. Instead haplotypes from both extant and archived Marsh populations were found in several other extant Western North America longnose dace populations. However, current longnose dace collections in the Marsh revealed only the Great Plains lineage; the Pacific lineage was not found and appears to have been swamped out and extirpated from the region by the more numerous longnose dace of Great Plains lineage. This suggests that the missing Pacific lineage and the loss of the Banff longnose dace morphotype may be correlated. Irrespective of the causes for the unique morphology, my mtDNA evidence does not support the morphological evidence of a distinct sub-species.

Microsatellite DNA analysis revealed extant longnose dace populations from the Bow River and Cave & Basin Marsh were not significantly different from one another. The otolith

microchemistry results complemented the genetic findings and indicated connectivity and movement of fish between the Marsh and the Bow River.

The lack of concordance between morphology and genetics, demonstrates the importance of using multiple criteria to determine taxonomy. My mtDNA results do not support the distinct subspecies status of the Banff longnose dace. Regardless of the subspecies status, the Banff longnose dace population represented a unique assemblage of fish that no longer exists in the Cave & Basin Marsh. The biogeographic distinction of this population demonstrates that it merited protection and designation. However, the designation of an extinct sub-species remains unresolved due to the unknown effects caused by the hot spring fed environment. Unless it can be proved that the morphological traits are heritable I would hesitate to use this evidence for designating subspecies status. I would, however, recommend that COSEWIC reassess the status of this sub-species from the extinction of *Rhinichthys cataractae smithi* to the extirpation of *Rhinichthys cataractae dulcis* from the Cave & Basin Marsh.

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INTRODUCTION

Extensive anthropogenic activities have led to unprecedented habitat degradation and serious declines in the Earth's biota. This biodiversity crisis is revealed in progressively increasing estimates of decline and the extinction of numerous populations and species worldwide (Wilson 1992; Myers 1993; Lawton and May 1995; Pimm et al. 1995). Species most vulnerable to human activities include those with specific habitat requirements and endemics with small geographic ranges (Pimm and Raven 2000). In order to prevent further loss of biodiversity, our societal strategy is to try and identify species at risk and protect them through the use of regulations. In Canada, an independent group of experts called the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) provides a single, official, classification of wildlife at risk. This information is then used by the federal government to determine whether the species merits listing under the Species at Risk Act (SARA).

The effectiveness of endangered species legislation is hotly debated (Mann and Plummer 1995; Gordon et al. 1997; Berger and Berger 2001; Male and Bean 2005), but it does provide the foundation for us to setup a series of steps to identify species at risk, implement recovery plans, and evaluate effectiveness. One of the problems of this approach, however, is determining what scale and method are appropriate to effectively designate imperilled forms of a species. There is abundant evidence of intraspecific variation over geographic range (Burnett 1983; Benitez-Diaz 1993; Keivany and Nelson 2000). Increasingly the use of genetics has enabled scientists to define geographic populations at a much finer and more objective scale (Templeton et al. 1995).

Reproductive isolation creates genetic divergence. Straying among populations, however, disrupts isolating effects and tends to homogenize genotypes of populations where gene flow exists. Locally adapted traits that are influenced by fitness would further maintain genetic

differences due to natural selection. Consequently, animals tend to differ morphologically and genetically across and within geographic regions (Avise 1994).

Several species of North American freshwater fish show considerable intraspecific divergence revealed in distinct geographic lineages (Murdoch and Hebert 1994; Wilson and Hebert 1988; Turgeon and Bernatchez 2001). These groups often exhibit morphological differences that form the traditional basis for sub-species classification. For example, the longnose dace, *Rhinichthys cataractae* (Valenciennes), exhibits a number of these geographic races whose subspecific status remains largely unresolved. Bartnick (1972) described the distribution of two sub-species of longnose dace; *R. c. cataractae* east of the Continental Divide and *R. c. dulcis* on both sides of the Continental Divide. However, *R. c. dulcis* was named from a Missouri River tributary and is not likely to exist on the west slope of the continental divide (McPhail 2007). This information emphasizes the confusion surrounding longnose dace taxonomy arising from two separate geographic races sharing the same name. What makes the longnose dace a particularly attractive species to study is the presence of a fourth morphologically distinct form that differs from the other putative sub-species. This morphologically unique, geographically isolated population was discovered in a small marsh fed by the Cave & Basin Hotsprings in Banff National Park, Alberta. The first specimens were collected in 1892 (Eigenmann 1895) and described as a distinct sub-species, the Banff longnose dace (*R. c. smithi*) in 1916 (Nichols 1916).

Surprisingly, the fact that the distribution of the Banff longnose dace was entirely within a National Park provided very little protection for this putative sub-species. Human influences posed serious threats to the continued existence of Banff longnose dace. The Cave & Basin public baths, first constructed in the late 1800s, provided a source for continued eutrophication

and chlorination of the marsh through waste disposal (Lanteigne 1988). Public baths also caused a periodic reduction of inflow which may have limited suitable habitat for longnose dace (Renaud and McAllister 1988). Additionally, tropical fish competed for marsh resources with the native longnose dace. In 1924, the mosquitofish (*Gambusia affinis* Baird and Girard) was introduced to control the extensive mosquito population and quickly established a breeding population and thrived in the marsh (Nelson 1983). At present, it is the most abundant species found in the marsh (personal observation). The live bearing mosquitofish produces broods throughout the year and indiscriminately preys on small fish and eggs (Sublette et al 1990). Other tropical fish, introduced by aquarium enthusiasts in the 1960s, also competed for resources with native Marsh fish. Two of these introduced species, the sailfin molly (*Poecilia latipinna* Lesueur) and the jewelfish (*Hemichromis bimaculatus* Gill), are still abundant in the marsh today (personal observation). The collection of Banff longnose dace, especially when the population became endangered, likely also had negative impacts on the population. Locals were known to take fish for their aquariums by dip netting and many longnose dace have been removed since 1892 for the purpose of scientific studies (Nelson 1983).

By the early 1980s the number of longnose dace found in Marsh fish collections had greatly diminished and the population was considered endangered (McAllister et al. 1985). In an attempt to confirm the sub-species classification for the Banff longnose dace, Renaud and McAllister (1988) examined morphological differences among archived Banff longnose dace specimens and extant longnose dace populations from Western North America. They found that Banff longnose dace collected before 1941 had fewer lateral line scales (48-50) and dorsal fin rays (7-8) than extant longnose populations from both side of the Continental Divide which had 58-74 lateral line scales and 8-9 dorsal fin rays. This examination also revealed that longnose

dace from the Cave & Basin Marsh became progressively more similar to Bow River longnose dace until they became indistinguishable by the 1980s. Despite the continued presence of longnose dace in the Marsh, this morphological evidence was used by COSEWIC to designate the Banff longnose dace extinct in 1987.

Renaud and McAllister (1988) proposed three hypotheses for the unique morphology of the Banff longnose dace. The first was a phenotypic hypothesis whereby the morphological differences were caused by changing environmental conditions over time. They also proposed a genotypic hypothesis whereby the Banff longnose dace (*R. c. smithi*) introgressively hybridized with the longnose dace (*R. c. cataractae*) from the Bow River. Their final postulate was an admixture hypothesis whereby the proportion of longnose dace from the Bow River to Banff longnose dace increased over time until the Banff longnose dace was extirpated from the Marsh. They concluded that *R. c. smithi* was a distinct sub-species endemic to the marsh that had undergone almost complete introgression with its closest relative *R. c. cataractae* until it became extinct.

Rarity of this population of fish, geographic isolation, and morphological uniqueness suggest the Banff longnose dace was a separate sub-species. Additionally, it has been suggested that a Banff-Jasper refugium existed during the Wisconsin era (Crossman and McAllister 1986) where the Banff longnose dace may have survived the last ice age and subsequently evolved as a unique lineage. The distinct sub-species designation of the Banff longnose dace, however, is not without controversy. Traditional taxonomic traits including the numbers of fin rays and lateral line scales do not always accurately reflect genotypic variation and phylogenies because their origin may be genetic, environmental, or some combination of both (Billerbeck et al. 1997). Phylogenetic patterns have verified morphologically based sub-species designations (Avise et al.

1984; Steppan 1998), however, designations are frequently not concordant with molecular genetic evidence (Larson 1997; Ball and Avise 1992; Avise and Nelson 1989; Williams et al. 2004; Zink 2004; Zink et al. 2004). Molecular techniques, therefore, offer an alternative method that did not previously exist to determine the sub-species status of this form of longnose dace. In this thesis I re-examine the taxonomy of the Banff longnose dace with the aid of molecular genetic techniques. My objectives were to:

1. determine the phylogeny and validity of the sub-species classification of the Banff longnose dace with the aid of mitochondrial DNA,
2. determine if gene flow exists between the extant populations of Cave & Basin Marsh longnose dace and Bow River longnose dace using genetics (microsatellite DNA) and chemical signatures (otolith microchemistry), and
3. comment on the mechanisms used to assign conservation values to species that may be at risk.

MATERIALS & METHODS

SAMPLE COLLECTION

Extant Populations

Pelvic fin clips or whole specimens of *R. cataractae* were collected by minnow trapping or electro-shocking from seven locations in Western Canada (Figure 1). These locations were chosen based on longnose dace abundance, proximity to the Cave & Basin Marsh, and watershed connectivity. Longnose dace were collected throughout the Cave & Basin Marsh, which empties into the Bow River, and upstream of the Marsh in the Bow River adjacent to the Wolverine Creek confluence. Other collections acquired in Alberta included Jumpingpound Creek, a Bow River tributary approximately 100km downstream of the Marsh, and Callum Creek, an Oldman River tributary in the Oldman watershed. The Bow and Oldman watersheds join to form the South Saskatchewan River system. Collections in British Columbia occurred in two Pacific drainage streams in the Fraser watershed (Cale Creek and Blackwater River) and one Arctic drainage stream in the Peace watershed (Parsnip River). Additional specimens from four of these sites (Bow River, Cave & Basin Marsh, Jumpingpound Creek, and Callum Creek) were collected to increase samples size to approximately thirty, for analysis with microsatellite DNA. Twenty fish from the Bow River and twenty fish from the Cave & Basin Marsh were sacrificed and otoliths removed. Tissue samples for DNA analysis were stored in 95% ethanol. As an out-group species, anal fin clips of blacknose dace (*R. atratulus*) from Herring Run, Baltimore County, Maryland were provided by Dr. Jay A. Nelson, of Towson State University, Baltimore, MD.

Archived Samples

Archived Banff longnose dace (*Rhinichthys cataractae smithi*) specimens are part of several museum collections worldwide (Appendix I). The majority of these specimens have unknown preservation histories or have been formalin-fixed. Tissues fixed in formalin have proven to be difficult to reliably extract DNA. Several protocols have been demonstrated to successfully extract DNA from formalin fixed material (Shiozawa et al. 1992; Shedlock et al. 1997; Chase et al. 1998); however, when used on the archived Banff longnose dace specimens these protocols yielded poor success rates, highly inconsistent results, and low molecular weight DNA. Additionally, a comparative study using these protocols resulted in the unsuccessful extraction, amplification, and sequencing of specimens fixed in formalin for greater than 3 years (Chakraborty et al. 2006). Other protocols for DNA extraction (Klanten et al. 2003) use large amounts of tissue and thus, are not appropriate for use on archived museum specimens due to the destructive nature of the protocol. For these reasons four dried specimens from the Smithsonian's National Museum of Natural History (USNM) collection 44045 and eight ethanol fixed specimens from the University of Michigan, Museum of Zoology (UMMZ) collection 213828 were acquired. I was given permission to take tissue samples from all four USNM specimens and two UMMZ specimens. These UMMZ specimens were wrapped in ethanol soaked cheesecloth for transport. When the samples were unwrapped for examination, two small pieces of fins were found that had broken off of the fish. It was not possible to determine which fish the damaged tissue pieces originated from, however, DNA was also extracted from these fin fragments.

Individual samples from collection USNM 44045 were not given identification numbers by the museum and will be hereafter referred to as USNM 44045-1, 44045-2, 44045-3, and 44045-4. These four dace samples were recorded to be collected from cold and hot springs in

Banff by P. Macoun in 1891. The classification of these specimens was confirmed to be from the Genus *Rhinichthys* by the museum curator in 1892. Species classification, however, has changed since first collection. Originally the specimens were classified as blacknose dace (*R. atratulus*) but were later reclassified as *R. nasutus* and then *R. atronasus*. Ultimately, they were classified *as R. cataractae* at a later unrecorded date. The fact that only the latter of the four species is present (or has historically been present) in the Bow River drainage (Nelson and Paetz, 1992) indicates the specimens are most likely longnose dace. These samples were likely not fixed in formalin given the age of the collection (1891) and the fact that they were dried before arrival at the museum. Based on morphological information from Renaud and McAllister (1988), I confirmed that one of the four specimens, USNM 44045-1, could only be a Banff longnose dace based on the number (7), of dorsal fin rays. The other 3 specimens had 8 dorsal fin rays typical of both *R. c. cataractae* and *R. c. smithi*. Lateral line scales were not counted due to the lack of confidence in accurately counting scales of the dried and shrivelled specimens.

The eight longnose dace specimens from UMMZ 213828 were assigned individual museum numbers and will be hereafter referred to as those same numbers: UMMZ 213828-1 to UMMZ 213828-8. These samples were collected by Eigenmann in 1892 and fixed in ethanol. Longnose dace from this collection were found to have whitish eyes, indicating a very good possibility that they were never fixed in formalin. Specimens from UMMZ 213828 were formerly part of Indiana University's collection IU 4409 and were previously identified as Banff longnose dace by Renaud and McAllister (1988) based on morphology. A small piece of hypaxial tissue (1 mm x 1.5 mm) was excised from the left side of two fish (UMMZ 213828-5 and UMMZ 213828-7). DNA was also extracted from the fin fragments, hereafter referred to as UMMZ 213828-P1 and UMMZ 213828-P2. See Appendix IV for a list of the samples.

MITOCHONDRIAL DNA (mtDNA)

mtDNA Amplification and Sequencing of Extant Samples

DNA was extracted from either muscle or fin tissue using the DNeasy Tissue Kit (QIAGEN, Mississauga, Ontario) according to the manufacturer's tissue protocol. A 730 bp segment of cytochrome *b* and an 850 bp segment of the control region were amplified in a PTC-100 Programmable Thermal Controller (MJ Research Inc., Waltham, MA). Cytochrome *b* was amplified with the primers CB3H (5'-GGC AAA TAG GAA RTA TCA TTC-3') and gluDG (5'-TGA CTT GAA RAA CCA YCG TTG-3'; Palumbi et al. 1991) and the control region was amplified with the primers LPro (5' - AAC TCT CAC CCC TAG CTC CCA AAG – 3'; Jäger et al. 1992) and MRT-2 (5' - TTA GCA TCT TCA GTG CTA TGC – 3'; Ptacek and Breden 1998). A 25 µL reaction volume contained 1X PCR reaction buffer (50 mM KCl, 20 mM Tris-Cl (pH 8.4)), 2 mM $MgCl_2$, 200 µM of each dNTP, 1 unit of Taq DNA polymerase (Invitrogen, Burlington, ON), 0.4 µM of each primer and approximately 10 ng of DNA. Amplification was performed with a thermal cycling parameter consisting of an initial denaturing step at 94°C for 4 minutes, followed by a 1 minute annealing cycle at 48°C and a 2 minute elongation cycle at 72°C. This was followed by 94°C for 30s, 48°C for 30s, and 72°C for 90s, repeated 34 times. A final extension at 72°C for 6.5 minutes was followed by cooling to 4°C until the product was removed. The PCR products were purified by ethanol precipitation containing 3 M ammonium acetate and then separated and visualized by gel electrophoresis on a 1.5% agarose gel containing 5 µl/100 mL ethidium bromide to determine DNA concentration. These cleaned PCR products were cycle-sequenced in both directions with the same primers as those used for the initial amplification. Sequencing reactions were analysed on a Beckman Coulter CEQ 8000

genetic analysis system (Fullerton, California) using a Beckman Dye Terminator Cycle sequencing kit (DTCS) Quick Start Kit.

mtDNA Amplification and Sequencing of Archived Samples

Initially, DNA extraction of archived specimens with the primer pairs LPro & MRT2 and gluDG & CB3H resulted in multiple failures with a single exception, the successful DNA extraction of USNM 44045-4. This likely indicated that most of these specimens did not preserve well resulting in poor quality DNA. It has been demonstrated that PCR can reconstruct intact DNA from severely degraded fragments of less than 100 base pairs in mitochondrial control region with the aid of multiple primer pairs, which amplify overlapping segments (Paabo 1989; Paabo et al. 1989). Hence, multiple longnose dace cytochrome *b* specific primers were designed using Primer Express v. 2.0.0 (Applied Biosystems, Foster City, CA) based on the aforementioned extant sequences from Western Canada (Table 1). These overlapping cytochrome *b* primers amplified products as large as 619 bp (Table 2). All primer pairs amplified products with extant samples, however, certain primer pairs were chosen due to better performance (Table 2). Species-specific primers designed to amplify a shorter segment for the control region of longnose dace (236 bp; 5'-ACCCCTGGCTCCCAAAGC-3' and 5'-GGTCTATGTACGTCTTAG-3') were used to amplify archived samples according to the previously published protocol of Girard and Angers (2006a). The concentrations of the initial PCR products of many of the archived samples were so low that they were not visible on a gel. Hence, these PCR products were re-amplified using either the same primers or nested primer pairs resulting in a visible product that was then sequenced (Figure 2). Amplification parameters were identical to the conditions previously mentioned with the exceptions of the

annealing temperature ranging from 48°C to 52°C and the addition of 0.8 μg/μL of bovine serum albumin (BSA). Bovine serum albumin has been widely used to prevent inhibition of PCR reactions (Akane et al. 1993; Hoss et al. 1992; Hoss and Paabo 1993; Gibbs and Siebenmann 1998). Research benches and tools were cleaned before and after every DNA extraction and amplification with RNAse Away (Fisher Scientific, Ottawa, ON) to prevent contamination.

Archived DNA sequences were run in both directions and often with overlapping primer pairs to ensure accuracy. Additionally, specimens from the USNM collection were extracted on two separate occasions to ensure precision and accuracy. The archived tissue samples were soaked in ultrapure water before the second USNM extraction. UMMZ 213828-7 was extracted and amplified at a separate time from all other specimens once permission to take a tissue sample was granted. Replication of the entire process resulted in the same sequences where they overlapped verifying the DNA sequence. Nested primer pairs also revealed shorter but identical nucleotide sequences.

mtDNA Alignment and Analyses

Alignments of sequences were performed using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, Michigan) and checked visually. Sequences were used from two regions of the mtDNA molecule: 457 bp segments of cytochrome *b* and 189 bp segments of the control region. Within population genetic diversity was estimated using nucleotide (π) and haplotype diversity (h). The genetic differentiation between populations was quantified using the F_{ST} (Weir and Cockerham 1984) statistic computed for both haplotype frequencies and kimura-2 distance (corrected for gamma distribution) using the program ARLEQUIN v. 3.1.1 (Excoffier et al. 2005). Statistical significance levels were determined using 1000 Monte Carlo simulations.

Significance levels were not corrected because of the small number of populations sampled and the high likelihood of Type II errors. Pairwise sequence divergences between haplotypes were determined with the Kimura two-parameter model (Kimura 1980) that was implemented in MEGA 4.0 (Tamura et al. 2007). The program MEGA 4.0 was also used to construct phylogenetic trees with neighbour joining, maximum parsimony, and minimum evolution algorithms. A likelihood approach implemented in Modeltest version 3.06 (Posada and Crandall 1998) was used to determine the best fit model of evolution for the data. The resulting estimates of the shape parameter of the gamma distributions of the cytochrome *b* ($\alpha = 0.2727$) and combined mtDNA sequences ($\alpha = 0.2791$) were used in the analyses. Phylogenetic confidence was measured by bootstrapping (Felsenstein 1985) with a 65% cut-off value. Analyses of phylogenetics were also conducted with the inclusion of sequences obtained from Genbank (samples I to XV, accession numbers AH015666-80; Girard and Angers 2006a) and unpublished sequences provided by J.D. McPhail, University of British Columbia, to aid in determination of glacial refuge of origin.

Evolutionary and potential ancestor–descendant relationships among longnose dace haplotypes were represented with a minimum spanning tree (MST). Trees were generated with the program TCS v. 1.13 (Clement et al. 2000) according to the methods of Templeton (1992).

An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was conducted using ARLEQUIN v. 3.1.1 (Excoffier et al. 2005) which computed the proportion of variation among populations and within populations. Diversity was based on both frequency differences of haplotypes and a molecular distance matrix (haplotypes corrected for gamma shape parameters).

MICROSATELLITE DNA

Microsatellite Amplification and Fragment Analysis

Extracted DNA from longnose dace samples was amplified using primers for nine microsatellite loci that were previously shown to be variable in the Genus *Rhinichthys*: *Rhca*16, *Rhca*20, *Rhca*24, *Rhca*31 (longnose dace, *Rhinichthys cataractae*, Girard and Angers 2006b), *Lco*1, *Lco*3, *Lco*4, *Lco*5 (common shiner, *Luxilus cornutus*, Turner et al. 2004), and *Ca*12, (central stoneroller, *Campostoma anomalum*, Dimsoski et al. 2000). These loci were chosen because the PCR product amplified easily and demonstrated variability when screened using my samples. Other primers were screened but not chosen for fragment analysis due to stuttering, inconsistent amplification, and low variability (Table 3).

PCR amplifications were conducted in a PTC-100 Programmable Thermal Controller (MJ Research Inc, Waltham, MA) according to previously published methods (Dimsoski et al. 2000; Turner et al. 2004; Girard and Angers 2006b) with modified annealing temperatures as outlined in Table 4. A 25 μL reaction volume contained 1X PCR reaction buffer (50 mM KCl, 20 mM Tris-Cl (pH 8.4)), 200 μM of each dNTP, 1 unit of Taq DNA polymerase (Invitrogen), 0.4 μM of each primer, approximately 10 ng of DNA, and variable concentrations of $MgCl_2$ and BSA (Sigma; Table 4). Fragment sizes were determined using fluorescently labelled primers and assayed on a Beckman Coulter CEQ 8000 (Fullerton, CA) automated sequencer.

Microsatellite loci from archived dace samples amplified poorly. Successful amplification of fragment polymorphisms ranged from 1 locus in samples USNM 44045-3 and UMMZ 213828-P1 to 7 loci in UMMZ 213828-5 (Table 5). Only three archived samples, USNM 44045-4 (M1), UMMZ 213828-5 (C1), and UMMZ 213828-7 (M1) were considered for population assignment analysis because they had five or more successful amplifications.

Microsatellite DNA Statistical Analyses

Genotypic linkage disequilibrium within pairs of loci among populations was calculated using default Markov chain method values in the program GENEPOP v. 3.4 (Raymond and Rousset 1995). This program was also used to detect departures from Hardy-Weinberg equilibrium (HWE) for each locus-population combination using an exact test in which P-values were estimated using a Markov chain method. In the case where a significant deviation from Hardy-Weinberg equilibrium was detected, I used the program MICROCHECKER v. 2.2.3 (Van Oosterhout et al. 2004) to evaluate the probable cause of deviation. Sample size (N), number of alleles, observed (H_O) and expected heterozygosity (H_E) were compiled and population sub-structure (F_{ST} and R_{ST}; Slatkin 1995) was examined in ARLEQUIN v. 3.1.1 (Excoffier et al. 2005). This program was also used to conduct an analysis of molecular variance.

Population Assignment

I used Geneclass v. 2.0 (Piry et al. 2004) to assign extant individual dace to one of the three populations of origin: Bow River / Cave & Basin Marsh, Jumpingpound Creek and Callum Creek. The Bow River / Cave & Basin Marsh were considered one population because the pairwise F_{ST} and R_{ST} values were neither substantial nor significantly different between the two sampling locations. Extant genotype likelihoods were calculated for each individual in each population following Paetkau et al. (1995) with the exception of $L = L_h$ which was used as the test statistic because not all source populations for immigrants were sampled (reviewed in Paetkau et al. 2004). In order to generate critical values to determine if an individual was born in its sampled population, the Monte Carlo re-sampling method of Paetkau et al. (2004) was

performed. Individual dace that were not assigned to their population of origin (F_0 migrants) were removed from further analysis. Archived Banff longnose dace were then assigned to or excluded from the extant populations and their critical values generated according to Paetkau et al. (2004), with a threshold p-value of 0.01.

ELEMENTAL ANALYSIS

Otolith Extraction

The heads of frozen longnose dace were individually placed in a petri-dish filled with ultrapure water and macerated. Using a dissecting microscope, otoliths were located and removed and cleaned, air-dried in a laminar flow hood and stored in polyethylene bottles. All tools that came directly or indirectly into contact with the otoliths were non metallic and acid washed with 2% ultrapure HNO_3. To remove the remaining adhering tissue, otoliths were sonicated in ultrapure water for 30 minutes, triple rinsed in ultrapure water, and then dried in a laminar flow hood. For determination of elemental composition, otoliths were transferred to acid washed polyethylene bottles, dissolved in 200 µL of high purity nitric acid, and filled with ultrapure water resulting in a 10 mL 2% $HN0_3$ solution.

Water Collection

Water samples were obtained in duplicate from seven sites; four sampling sites were located in the Bow, two from the Cave and Basin Marsh, and one from Wolverine Creek, a tributary to the Bow River. The Bow River samples were taken upstream and downstream of Wolverine Creek near where the Bow River longnose dace were collected, and upstream and

downstream of the Cave and Basin Marsh. From the Marsh, one water sample was taken at the largest inlet stream to the Cave and Basin Marsh and another at the Marsh outlet stream where it enters the Bow River. Samples were collected according to the remote location recommendations of Shiller (2003) with modifications according to Clarke et al. (2007). Fifty millilitre high-density polyethylene bottles (Fischer Scientific, Ottawa, ON) and 50 mL syringes (Sigma Aldrich, Oakville, ON) were cleaned with ultrapure water and filled with 2% high purity nitric acid. After two weeks, the acid was removed and the bottles and syringes rinsed five times with ultra-pure water. At the field sites, a 40 mL sample of water was drawn into the syringe. Ten millilitres of this sample was expelled through a nylon filter (25 mm by 0.45 μm, Fischer Scientific) to condition the filter and the remaining 30 mL filtered into a cleaned polyethylene bottle and acidified with 600 μL of high purity nitric acid resulting in a 2% $HN0_3$ solution.

Analytical Procedures

Water and dissolved otolith analyses were completed with a PS 1000-UV inductively coupled plasma–optical emission spectrometer (ICP–OES) (Teledyne Instruments Leeman Laboratories, Hudson, NH) at the University of Northern British Columbia. The elements measured included Ba, Ca, Sr, Li, Zn, Mg and Mn. Four calibration standards prepared from traceable (NIST) standards were run for every 10 samples analyzed. Laboratory blanks and field procedural blanks were also included in the analysis.

Calculations

The relationship between Strontium concentrations in dace otoliths to water samples was calculated to develop an incorporation coefficient comparing the molar ratios of Strontium to Calcium modified from Morse and Bender (1990):

$$D_{Sr} = (Sr:Ca)_{otolith} / 0.400432) / (Sr:Ca)_{water}$$

The value 0.400432 represents the portion of Calcium in the aragonite ($CaCO_3$) otolith. Strontium was examined because of high detection levels and frequency of use (Martin et al. 2004; Clarke et al. 2007). Other trace elements including Barium and Manganese were also measured but not considered for analysis due to low detection levels (Appendix II).

To determine a water elemental signature that would be characteristic of fish caught in the Cave and Basin Marsh, the incorporation coefficient was determined for Bow River longnose dace. Water chemistry from the four sample sites on the Bow River showed little difference and it was assumed that Bow River longnose dace did not move beyond the areas sampled. By re-arranging the equation above, this relationship could be used to determine a "projected" water chemistry elemental ratio for water from which the Marsh fish were captured.

$$\text{Projected } Sr:Ca_{water} = (Sr:Ca_{otolith} / 0.400432) / D_{Sr}$$

This formula was also used to calculate the projected $Sr:Ca_{water}$ of the Cave and Basin Marsh based on the otolith elemental signature. However, this does not take into account the higher average annual water temperature for the hotspring fed Marsh. Hence, I calculated projected $Sr:Ca_{water}$ ratios of the Cave and Basin Marsh based on an estimated higher annual temperature difference of 15°C. Martin et al. (2004) found a significant linear relationship between temperature and Sr:Ca ratios; a 1°C increase in temperature increased incorporation coefficient

by 5%. Thus, I multiplied D_{Sr}, the incorporation coefficient, by 1.75 to correct for a putative 15°C difference in temperature of the Marsh compared to the Bow River.

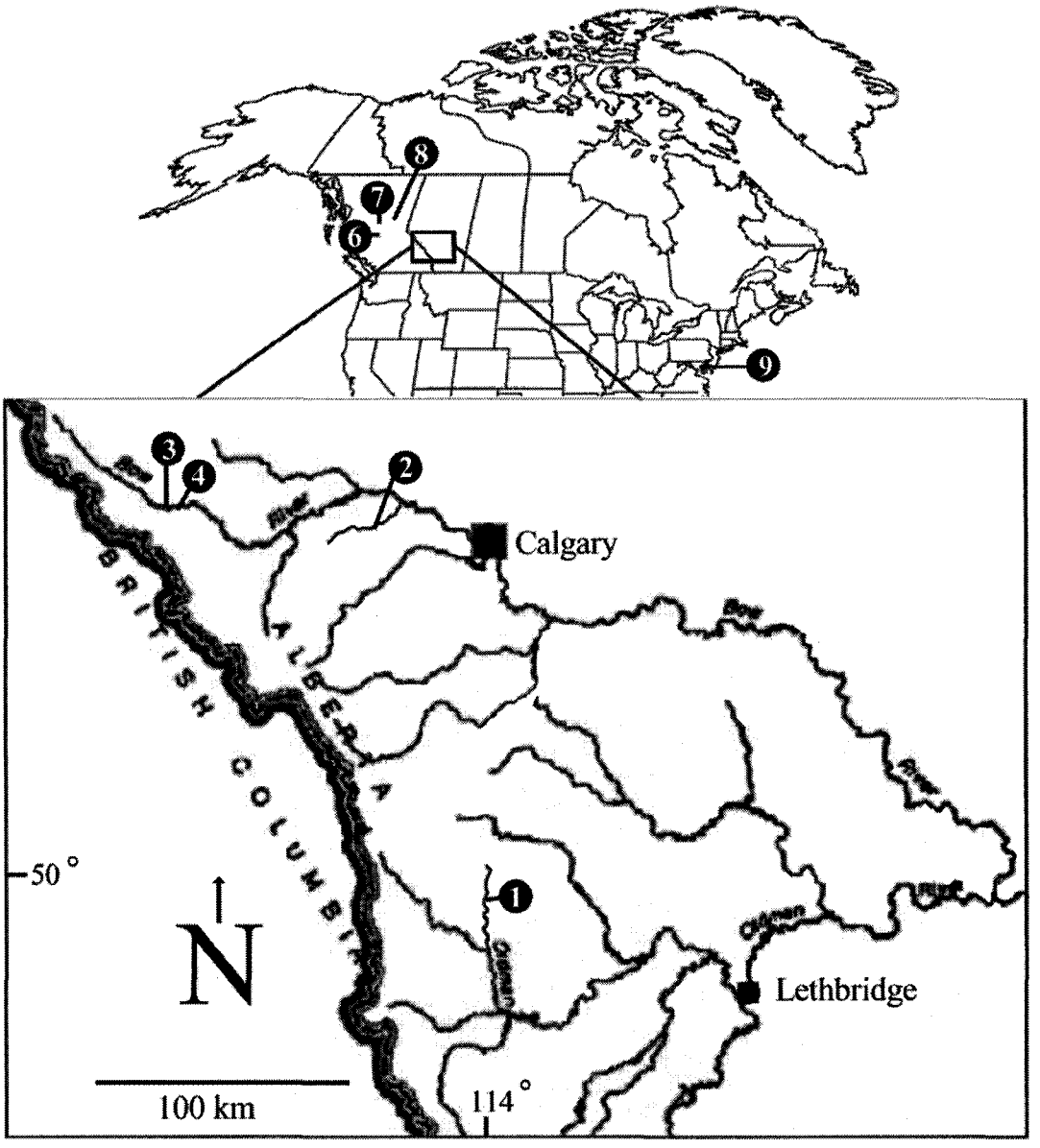

Figure 1. Sampling sites for *Rhinichthys cataractae* and *Rhinichthys atratulus.* 1. Callum Creek (CMC; Oldman drainage), 2. Jumpingpound Creek (JPC; Bow drainage), 3. Bow River (BOR; Bow drainage), 4. Cave & Basin Marsh (CBM; Bow drainage), 5. Archived museum samples collected from the Marsh (BLD; Bow drainage) 6. Blackwater River (BWR; Fraser drainage), 7. Cale Creek (CLC; Fraser drainage), 8. Parsnip River (PSR; Peace drainage), 9. Herring Run (Back River watershed, MD).

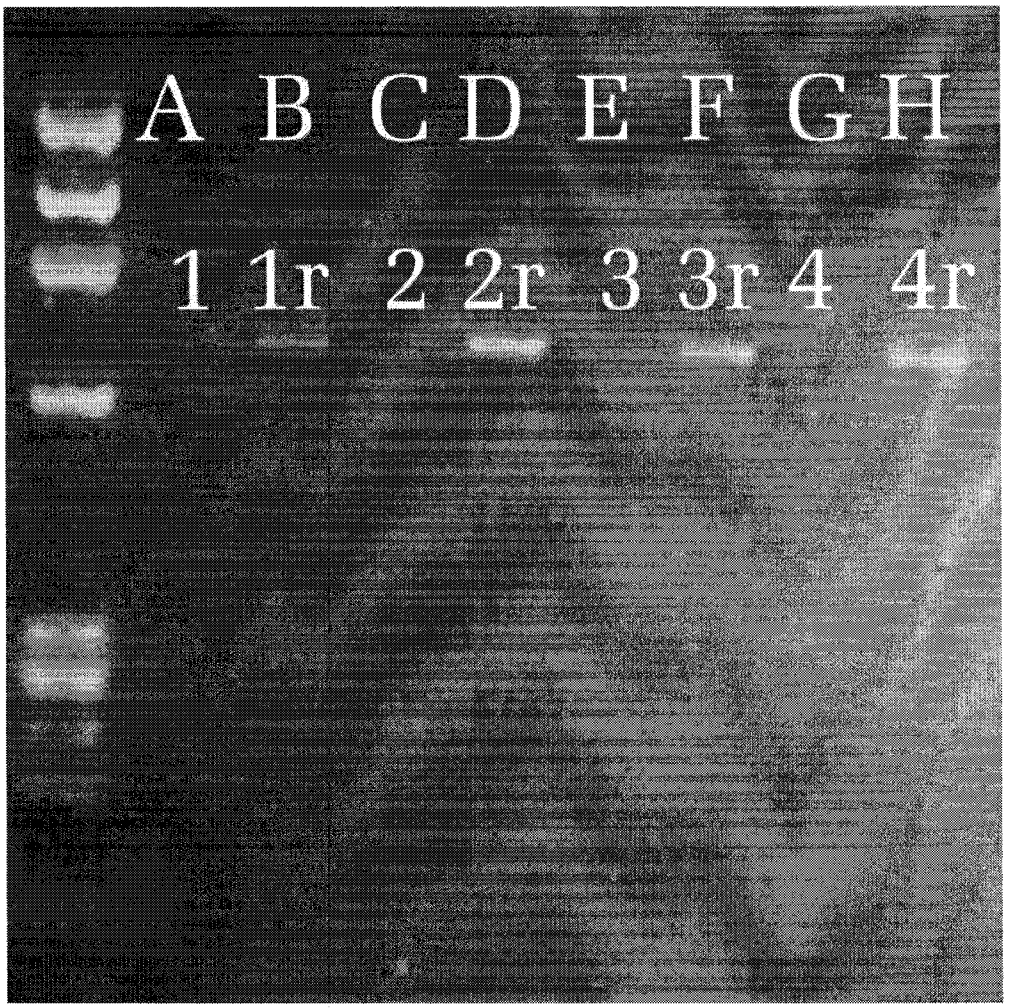

Figure 2. Polymerase chain reaction amplification and re-amplification (r) of archived samples (USNM 44045-1,-2,-3, and -4). Lanes B, D, F, and H show the re-amplification of the PCR product from lanes A, C, E, and G.

Table 1. Longnose dace specific cytochrome *b* primers.

Name	Start	T_a	Forward Primer	Direction
Rc1F	45	58	CGGTGCACTAGTTGACCTTCC	Forward
Rc2F	86	58	CGCTATGGAACTTCGGATCC	Forward
Rc3F	136	58	CTGACAGGACTATTTCTGGCCA	Forward
Rc4F	167	55	CCTCCGACATCTCAACTGC	Forward
Rc5F	222	57	CTATGGCTGACTCATCCGGA	Forward
Rc6F	300	59	CGGCCTGTACTACGGGTCAT	Forward
Rc7F	331	62	GAGACCTGGAATATTGGCGTTGTC	Forward
Rc8F	393	57	TGTGCTCCCATGAGGACAA	Forward
Rc9F	454	57	GCAGTACCTTATATAGGTGACGCC	Forward
Rc10F	525	58	AACACGATTCTTCGCCTTCC	Forward
Rc1R	156	60	GGCCAGAAATAGTCCTGTCAGGA	Reverse
Rc2R	196	58	CGGACGAAAATGCAGTTGAG	Reverse
Rc3R	232	58	GTCAGCCATAGTTAACGTCTCGAC	Reverse
Rc4R	299	56	CGGGCAATGTGCATGTAA	Reverse
Rc5R	349	59	CGCCAATATTCCAGGTCTCCT	Reverse
Rc6R	410	57	TGTCCTCATGGGAGCACATAG	Reverse
Rc7R	448	62	GTAGATTCGTAATAACGGTGGCGC	Reverse
Rc8R	503	56	AAGCCACCTCAAATCCACTG	Reverse
Rc9R	570	56	GGCGATAACGAACGGAAA	Reverse
Rc10R	639	59	GGAATTTAATCCGGCAGGGT	Reverse

Table 2. Optimal primers pairs and annealing temperature (T_a) used.

Primer pairs		Amplicon Size (bp)	Ta
Rc1F	Rc10R	573	48
Rc1F	Rc8R	438	48
Rc4F	Rc8R	316	48
Rc6F	Rc10R	319	48
Glud	Rc8r	483	48
Glud	Rc10r	619	48
Glud	Rc3r	208	52
Rc2F	Rc10R	533	48

Table 3. Screened Polymerase Chain Reaction (PCR) primers not selected for microsatellite fragment analysis.

Primer	Source	Reason for Exclusion
*Ca*1	Dimsoski et al 2000	failed amplification
*Ca*2	Dimsoski et al 2000	failed amplification
*Ca*3	Dimsoski et al 2000	failed amplification
*Ca*7	Dimsoski et al 2000	very poor amplification
*Ca*8	Dimsoski et al 2000	failed amplification
*Ca*11	Dimsoski et al 2000	samples either failed to amplify or amplified well
*Ca*14	Dimsoski et al 2000	poor amplification, low variability
Lco2	Turner et al. 2003	very poor amplification
*Lco*7	Turner et al. 2004	very poor amplification
*Lco*8	Turner et al. 2004	excellent amplification but low variability
*Rhca*15b	Girard an Angers 2006b	poor amplification
*Rhca*34	Girard an Angers 2006b	very poor amplification
*Rhca*52	Girard an Angers 2006b	stutter-difficult to score

Table 4. PCR and thermal cycler parameter modifications of previously published microsatellite amplification conditions (T_a = annealing temperature).

Primer	T_a (°C)	$MgCl_2$ (mM)	BSA (μg/μL)
*Lco*1	57	2	0.36
*Lco*3	57	2	0.36
*Lco*4	57	2	0.36
*Lco*5	57	2	0
*Ca*12	57	2	0
*Rhca*16	48	2	0.9
*Rhca*20	50	1.75	0.9
*Rhca*24	50	1.75	0.9
*Rhca*31	50	2	0.9

Table 5. Loci successfully amplified in archived longnose dace.

Sample	Successfully amplified loci
USNM 44045-1	*Rhca*31, *Lco*4
USNM 44045-2	*Rhca*31, *Lco*4
USNM 44045-3	*Lco*4
USNM 44045-4	*Rhca*20, *Rhca*31, *Lco*3, *Lco*1, *Lco*4
UMMZ 213828-5	*Rhca*16, *Rhca*20, *Rhca*31, *Lco*3, *Lco*4 *Lco*5, *Ca*12
UMMZ 213828-7	*Rhca*20, *Rhca*31, *Lco*3, *Lco*4, *Lco*5
UMMZ 213828-P1	*Rhca*31

RESULTS

MITOCHONDRIAL DNA – Cytochrome *b*

Extant Haplotypes

Eleven different cytochrome *b* haplotypes (457 bp) were found for *R. cataractae*. Pairwise sequence divergence ranged from 0.2 % (a single substitution) between several haplotype pairs (C1 and C2, C1 and C3, C1 and C5, C7 and C8, and C9 and C10) to 8.3% (28 substitutions) between 2 haplotype pairs (C4 and C10 and C4 and C11; Tables 6 and 7). Interspecific cytochrome *b* pairwise divergence between *R. cataractae* and *R. atratulus* ranged from 16.0% (47substitutions) between haplotypes C7 and BND2 to 19.4% (53 substitutions) between haplotypes C4 and BND2.

Many haplotypes (C2, C3, C4, C6, C8, and C11) were unique and were only found in a single fish in a specific population (Table 8). Haplotype C5 was also unique to a single river, the Parsnip, but occurred in both samples taken at that location. Three haplotypes (C1, C7, and C9) were shared in several populations. Haplotypes C1 through C4 were found in populations on the west slope of the continental divide. Haplotypes C7 through C11 were only found on the east slope of the continental divide in Alberta.

Archived longnose dace Haplotypes

Haplotypes C1, C7, and C9 were found in both extant populations and archived dace samples. C1 was the most abundant haplotype and found in three archived specimens (UMMZ 213828-5, UMMZ 213828-P1, and USNM 44045-4), whereas haplotypes C7 was found in two (USNM 44045-2 and USNM 44045-3), and C9 in a single fish (USNM 44045-1). Haplotype C6 (UMMZ 213828-7) from the University of Michigan Museum of Zoology was the only unique

haplotype among the archived samples but differed by only two substitutions which is a common level of differentiation among extant intraclade haplotypes within the same population (Table 6).

Phylogeny of Haplotypes

Minimum evolution analysis revealed that longnose dace branched into three highly distinct clades well supported by bootstrap values (Figure 3). Neighbour joining, maximum parsimony, and alternative schemes without corrected gamma values recovered identical tree topologies with only minor differences in bootstrap values (data not shown). Haplotypes from both the west and east slope of the continental divide (Fraser drainage and the Parsnip River) grouped within clade A, whereas extant sample haplotypes from the east slope of the continental divide split into two clades (B and C). Diversity within Clades A, B, and C, is 0.5%, 0.2%, and 0.3% respectively, whereas diversity among the clades ranges from 3.1% to 6.6% (Table 9). Three haplotypes (C1, C7, and C9) were found in both extant populations and archived dace samples and were representative of Clade A, B, and C. A minimum spanning tree resolved the same three Clades. Clades B and C were more closely related to one another than they were to Clade A. Haplotypes C1, C7, and C9 were designated as the inferred ancestral haplotypes (Figure 4).

Haplotype and Nucleotide Diversity

Haplotype diversity ranged from zero to 0.8095 for the populations with the fewest (Parsnip River) and highest (archived longnose dace) number of haplotypes respectively. Nucleotide diversity ranged from zero for the Parsnip River to 0.039871 for the archived longnose dace samples (Table 10).

Cytochrome *b* F_{ST} values based on haplotype frequency ranged from zero between the archived Banff longnose dace and Cale Creek to 0.6817 between the Parsnip River and Callum Creek populations (Table 11). Fish collected from areas in close proximity to one another did not differ significantly including the populations within the Bow and Fraser watersheds. However, two values between populations from separate watersheds did not differ significantly. These populations were the Bow River and Callum Creek and the Blackwater and Parsnip rivers. In addition, the archived longnose dace did not differ significantly from the Parsnip or Blackwater River populations.

Cytochrome *b* pairwise F_{ST} based on Kimura-2 distance revealed a similar pattern (Table 12). The single difference was that the archived longnose dace were significantly different from the Blackwater River population.

An analysis of molecular variance (AMOVA) based on haplotype frequency revealed 41.05% of the genetic variation between and 58.95% within populations (Tables 13, 14). All AMOVA variations were found to be highly significant.

MITOCHONDRIAL DNA – Combined Genes

Extant Haplotypes

A substantial level of intraspecific mtDNA diversity was detected between eleven *R. cataractae* haplotypes. Pairwise divergence ranged from 0.2 % (a single substitution) between several haplotype pairs (M1 and M2, M1 and M3, M1 and M5, and M9 and M10) to 7.9% (38 substitutions) between haplotype pairs M4 and M11. Interspecific mtDNA pairwise divergence between *R. cataractae* and *R. atratulus* ranged from 14.8% (63 substitutions) between

haplotypes M1 and BND1 to 18.2% (72 substitutions) between haplotypes M10 and BND2 (Table 15).

Many haplotypes (M3, M4, M6, M8, M10, and M11) were unique and were only found in a single fish in a specific population (Table 7). Haplotype M5 was also unique to a single river, the Parsnip, but occurred in both samples taken at that location. Three haplotypes (M1, M7, and M9) were shared in several populations. Haplotypes M1 through M4 were found in populations on the west slope of the continental divide and whereas M7 through M11 were only found on the east slope of the Continental Divide in Alberta.

Archived longnose dace Haplotypes

Haplotype M1 was shared among archived (UMMZ 213828-P1 and USNM 44045-4) and extant specimens of longnose dace. Unfortunately, resolution beyond this was not possible for archived longnose dace as specimens UMMZ 213828-5, UMMZ 213828-7, and all east slope cytochrome *b* haplotypes were unsuccessfully sequenced for the control region (Appendix IV).

Of note, when the tissue piece from the cheesecloth of UMMZ 213828-P2 was sequenced, the results demonstrated the signal of two separate fish for both cytochrome *b* and the control region suggesting that this piece of tissue was in fact two pieces of adherent tissue. Upon further examination, both the cytochrome *b* and control region sequences were typical of both Clades B and C: where the nucleotides of the inferred ancestral haplotypes concurred, the appropriate nucleotide signal was very strong and where the two haplotypes had variable sites, two nucleotide signals, one of the inferred ancestral Clade B haplotype (M7) and the other of the inferred ancestral Clade C (M9) haplotype occurred. Unfortunately, I was not given permission

to extract DNA from further specimens from UMMZ collection 213828 to determine whether or not the Clade C haplotype occurred in their Banff longnose dace samples.

Phylogeny of Haplotypes

Minimum evolution analysis of the combined sequence data revealed identical tree topologies to those of cytochrome *b* but with higher bootstrap values (Figure 3). Longnose dace branched into three highly distinct Clades, highly supported by bootstrap values. Neighbour joining, maximum parsimony, and alternative schemes without corrected gamma values also resulted in identical tree topologies with minor differences in bootstrap values (data not shown). Haplotypes from both the west and east slope of the continental divide (Fraser drainage and the Parsnip River) grouped within Clade A, whereas extant sample haplotypes from the east slope of the continental divide split into two clades (B and C). Diversity within clades A, B, and C, was 0.4%, 0.6%, and 0.3% respectively, whereas diversity among the clades ranged from 2.4% to 5.4% (Table 16). The minimum spanning tree was consistent with the neighbour joining, maximum parsimony, and minimum evolution analyses. Clades B and C were more closely related to one another than they were to Clade A. Haplotypes M1, M7, and M9 were designated as the inferred ancestral haplotypes (Figure 4).

Haplotype and Nucleotide Diversity

Haplotype diversity ranged from zero to 0.6667 for the populations with the fewest (Parsnip River) and highest (Blackwater River) number of haplotypes respectively. Nucleotide diversity ranged from zero for the Parsnip River to 0.01334 for the Bow River longnose dace (Table 17).

The combined mtDNA (cytochrome *b* and control region) F_{ST} values based on haplotype frequency ranged from 0.05832 between the Bow River and the Cave and Basin Marsh to 0.68165 between Callum Creek and both the archived dace and the Parsnip River (Table 18). All populations on the west slope of the continental divide (Blackwater River and Cale Creek), the Parsnip River, and the archived longnose dace did not significantly differ from one another. Bow River and Cave and Basin Marsh populations had a very low pairwise F_{ST} value and did not differ significantly. Population pairwise differences based on Kimura-2 distance also revealed a similar pattern (Table 19).

An analysis of molecular variance (AMOVA) based on haplotype frequency revealed 58.25% of the genetic variation among and 41.75% within populations (Table 13). An AMOVA based on Kimura-2 distance revealed 80.46% of the genetic variation among and 19.54% within populations (Table 14).

Comparison with other longnose dace cytochrome b sequences

The inclusion of previously published and unpublished longnose dace sequences provided additional support for my previous analyses and a comparison of longnose dace sequences from other regions. The 236 bp cytochrome *b* phylogenetic tree allowed my samples to be compared with longnose dace of Atlantic origin. The 457 bp cytochrome *b* phylogenetic trees allowed further resolution of longnose dace from Clades A, B, and C. Longnose dace branched into several lineages well supported by moderate to very high bootstrap values (Figure 4). Substantial geographic patterning revealed Atlantic, Pacific, and Great Plains phylogroups. All haplotypes within each phylogroup were greater than two percent divergent from all haplotypes within the other two phylogroups (Appendix III; Figure 3). Haplotype divergence

within the Pacific and Atlantic phylogroups were both less than two percent. All haplotypes from Clade A and the Columbia River system (J.D. McPhail, unpublished data) diverged less than two percent from one another (Appendix III, Table 20) and combined to form the Pacific phylogroup. Haplotype C6 (UMMZ 213828-7) branched off separately supported by a high level of bootstrap support based on 236 bp of cytochrome *b*, however, this same haplotype did not branch off separately when a larger sequence of 457 bp was examined (Figures 5, 6).

Most haplotypes within the Great Plains phylogroup were less than two percent divergent including haplotypes of Girard and Anger's (2006a) Mississippi lineage (haplotypes I – XII), Clade B including Ruby Creek, Montana, and QUEB and MANI sequences (J.D. McPhail, unpublished data). Conversely, Clade C haplotypes which grouped with LTSH sequence (J.D. McPhail, unpublished data) from the Red Deer River system in Alberta were greater than two percent divergent from all other Great Plains phylogroup haplotypes.

These additional trees allowed me to rule out an Atlantic origin of the longnose dace in my study. A higher degree of phylogenetic resolution within Clades A, B, and C was also gained in addition to and an indication of the broad geographic range of each longnose dace Clade.

MICROSATELLITE DNA – Extant Populations

Fragment Analysis

Microsatellite polymorphism in longnose dace was variable across loci and populations with expected heterozygosities ranging between 0.033 in *Lco*3 of Cave and Basin dace and 0.956 in *Lco*1 Jumpingpound Creek dace (Table 21). Observed heterozygosities ranged between 0.033 in *Lco*3 of Cave and Basin dace and 0.929 in *Ca*12 of Jumpingpound Creek. The loci *Lco*1 and *Ca*12 exhibited the highest level of variability ranging from 16 to 24 and 14 to 18 alleles

respectively. Most samples were in Hardy-Weinberg equilibrium, however, 1 out of 36 (9 loci from 4 populations) tests demonstrated a statistically significant heterozygote deficit (*Rhca* 24 from Callum Creek). This heterozygote deficit was examined and failed to show any evidence of null alleles, large allele drop out, or scoring error due to stuttering. Additionally, there were no significant departures from linkage disequilibrium between loci within populations.

There was significant variation in allele frequencies (Table 22) among populations. Most pairwise differences in both F_{ST} and R_{ST} were substantial and statistically significant with two exceptions. The pairwise comparison between dace of the Bow River and the Cave and Basin Marsh was neither substantial nor significant for either F_{ST} or R_{ST}. Additionally, the pairwise R_{ST} between Callum and Jumpingpound Creeks (Table 22) was also not significant. The overall value of the fixation index among the four populations was $F_{ST} = 0.02941$. Analysis of molecular variance among the 4 populations indicated that most of the total variance (97.06%) was attributed to the differences among populations compared to within populations (2.94%).

Population Assignment

One hundred and sixteen out of a possible 121 extant individuals were assigned to the population from where they were sampled (Table 23). Five fish were assigned as first generation migrants from other populations. Two Callum Creek fish were assigned to the Bow-Cave&Basin population, one Bow-Cave& Basin fish was assigned to Callum Creek, and one Jumpingpound Creek fish was assigned to Callum Creek. One Jumpingpound Creek fish was assigned to an unknown population which was not sampled.

MICROSATELLITE DNA – Archived longnose dace

Population Assignment

Archived samples had significant yet relatively low assignment values. Therefore, the archived fish could be assigned to at least one of the extant populations. The probability of these archived multilocus genotypes belonging to one of the extant populations ranged from 1.1 to 20.8 percent (Table 24). UMMZ 213828-5 was excluded from both the Jumpingpound Creek and Callum Creek populations but considered possible to exist in the Bow River-Cave and Basin population. UMMZ 213828-7 was excluded from both the Bow River-Cave and Basin population and Callum Creek populations but considered possible to exist in the Jumpingpound Creek population. USNM 44045-4 was not excluded from any of the three populations but had the highest probability of belonging to the Bow River-Cave and Basin Marsh population. Of note, samples UMMZ 213828-5 and UMMZ 213828-7, which were identified as Banff longnose dace, had low probabilities of belonging to extant populations, whereas 44045-4, that did not show the morphology of Banff longnose dace but had a Pacific lineage mtDNA haplotype, had a much greater possibility of belonging to the extant populations.

TRACE ELEMENT ANALYSIS

Water Chemistry

Elemental concentrations were generally higher from water samples collected from the Marsh compared to the Bow River (Appendix II). Substantial differences were also seen in the calculated elemental ratios for Marsh and Bow River samples. Strontium to calcium ratios for water samples collected from the Cave and Basin Marsh differed significantly from water samples collected from the Bow River (Figure 8) ($t_{10} = -22.05$, $p < 0.0001$). Average Sr:Ca ratios

were 4.34 and 7.21 mmol/mol for the Bow River and Cave and Basin Marsh water samples, respectively. The variation in elemental signatures values was small for both the Marsh and River, although the range in values was approximately 2-fold greater for the Bow River.

Otolith Chemistry

Elemental ratios for Sr:Ca from otoliths of fish caught in the Marsh were also higher than ratios from otoliths of Bow River fish (Figure 9). Strontium:Calcium ratios for longnose dace collected in the Marsh differed significantly from otolith samples collected from the Bow River ($t_{10} = -10.99$, $p < 0.001$). However, unlike the water samples, a much greater variation in elemental ratios existed for fish caught in the Marsh than in the Bow River. Additionally, there was no overlap in values of Sr:Ca ratios for Marsh and Bow River fish.

Projected Water Chemistry Based on Otolith Microchemistry

The calculated Sr incorporation coefficient of Bow River longnose dace was found to be 0.47 ± 0.16 (standard deviation). This number was used to calculate water signature values for the Marsh based on the elemental signatures for Sr in the Marsh otoliths; projected values ranged from 8.77 to 22.79 mmol/mol with a mean of 15.90 mmol/mol, more than 2 times greater than the mean measured Marsh $Sr:Ca_{water}$ ratio of 7.21 mmol/mol. Using the temperature compensation ratio developed by Martin et al. (2004), I calculated projected water chemistry values for a 15°C difference between the Marsh and the Bow River. The projected water elemental signatures for the Cave and Basin Marsh ranged from 5.01 to 13.02 mmol/mol and were higher than the measured Marsh Sr:Ca ratios, however the temperature factor reduced the difference (Figure 10). This temperature compensated calculation demonstrated an overlap

between the signature from the fish caught in the Marsh and fish caught in the Bow River – suggesting movement by at least some of the fish between the two environments.

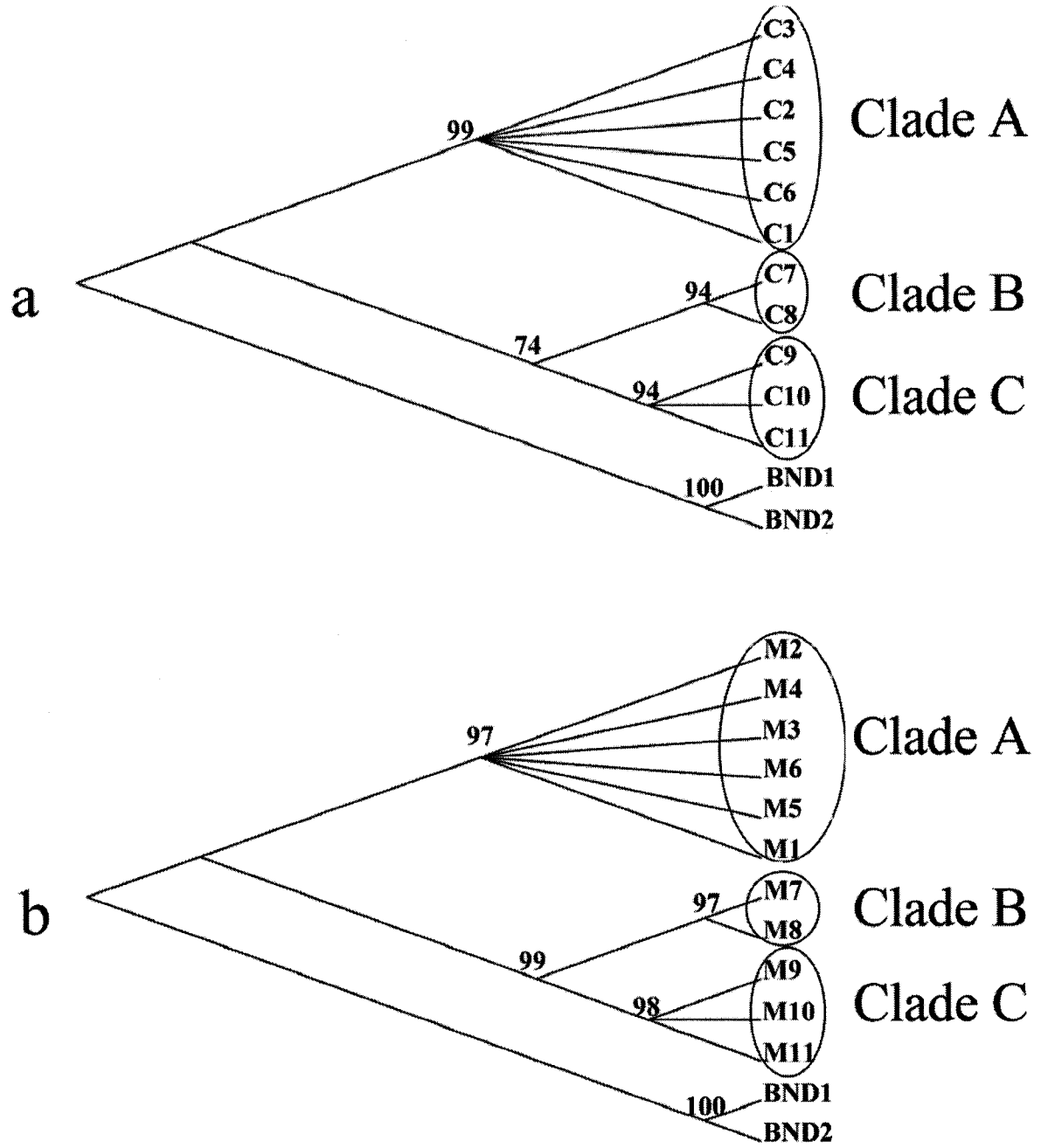

Figure 3. Minimum Evolution phenograms of the relationships among *Rhinichthys cataractae* and *R. atratulus* haplotypes. The numbers at the nodes represent bootstrap proportions based on 1000 replications. The two trees represent the analyses of (a) cytochrome *b* and (b) combined cytochrome *b* and control region.

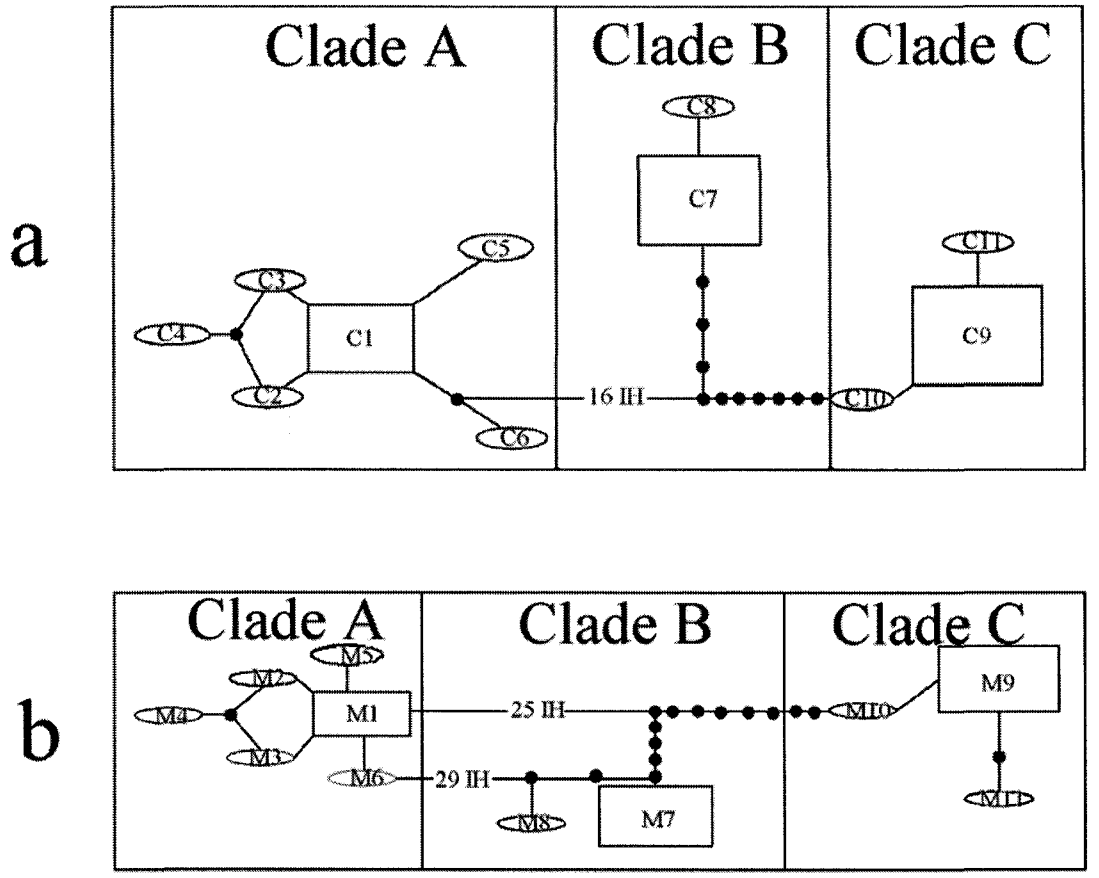

Figure 4. Minimum spanning trees for a. 11 haplotypes (C1 – C11) of a 457 bp section of cytochrome *b* and b. 11 haplotypes (M1-M11) of a 645 bp segment of mitochondrial DNA (cytochrome *b* and control region) among longnose dace specimens. Ovals represent haplotypes, rectangles represent the inferred ancestral haplotypes, and the size of these shapes corresponds to haplotype frequency. Black filled circles between *connections* represent inferred haplotypes (IH).

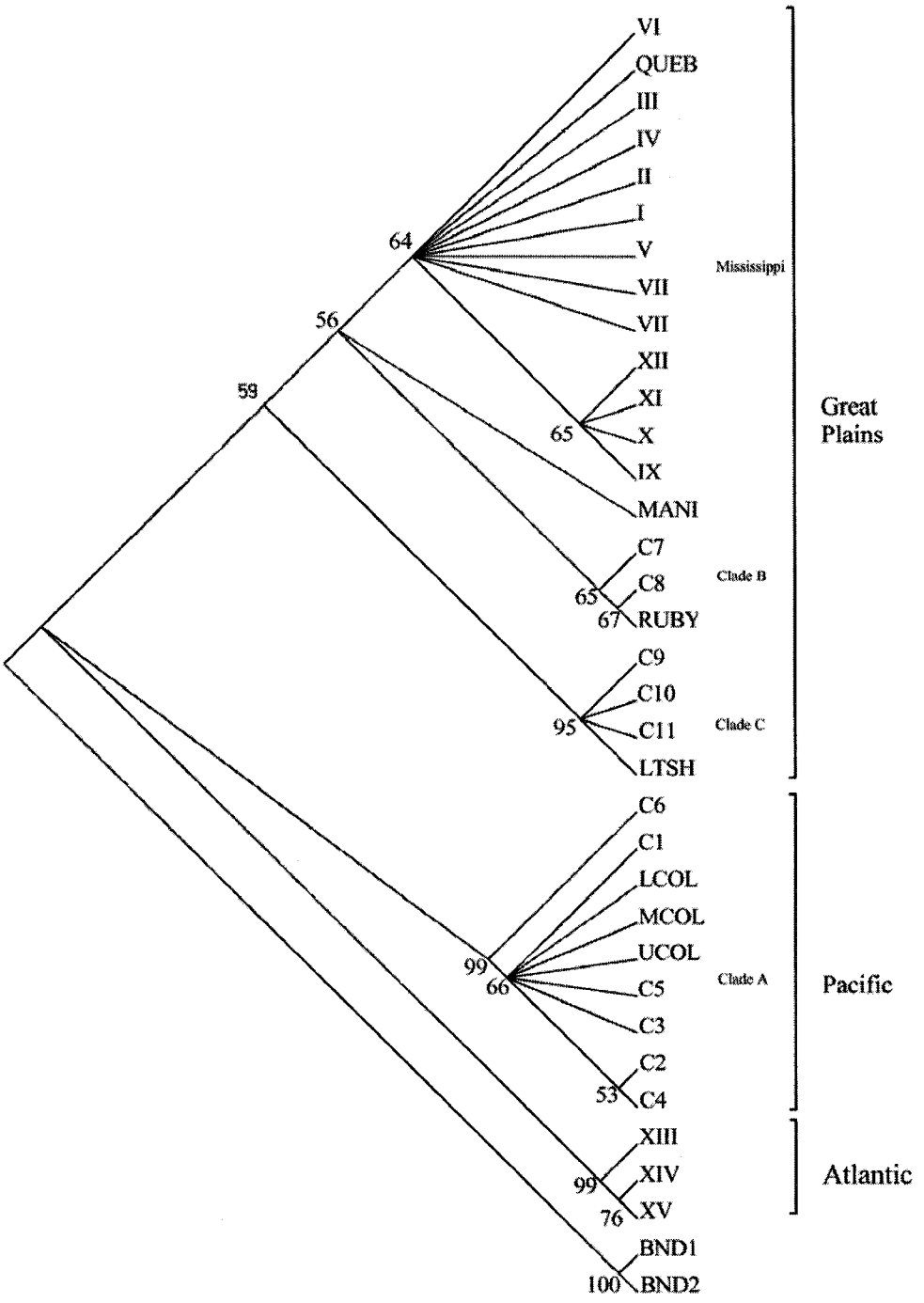

Figure 5. Minimum Evolution phenograms of the relationships among *Rhinichthys cataractae* and *R. atratulus* cytochrome *b* haplotypes (236 bp). The numbers at the nodes represent bootstrap proportions based on 1000 replications.

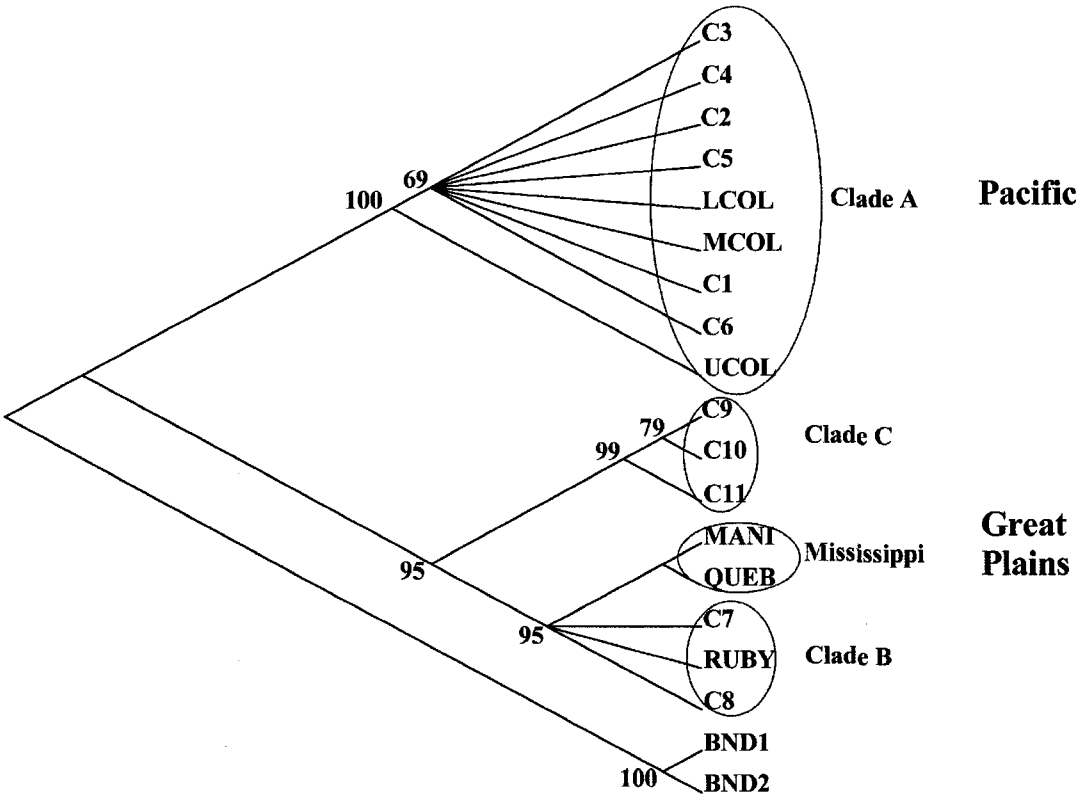

Figure 6. Minimum Evolution phenograms of the relationships among *Rhinichthys cataractae* and *R. atratulus* cytochrome *b* haplotypes (457 bp). The numbers at the nodes represent bootstrap proportions based on 1000 replications.

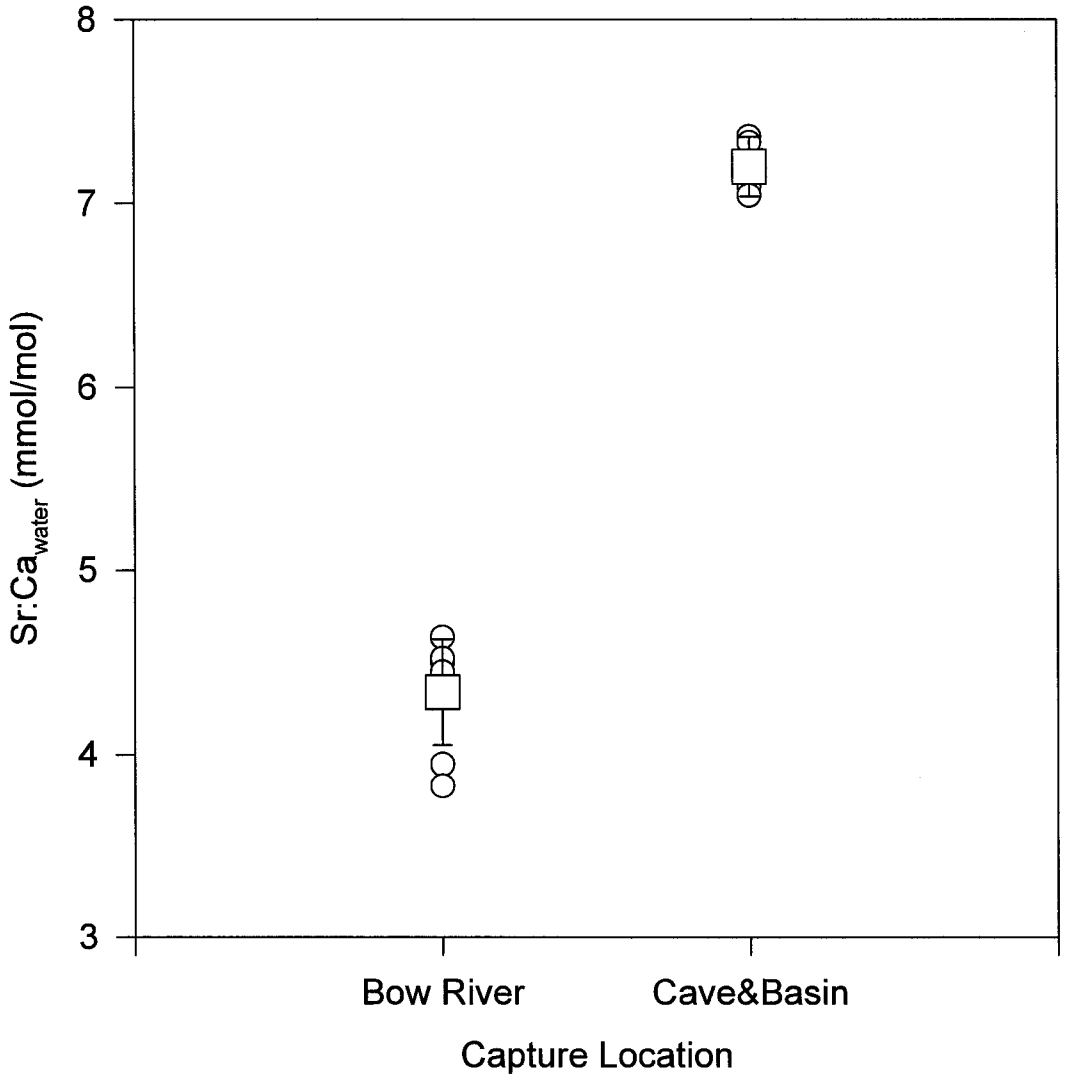

Figure 7. Strontium:Calcium ratios in the Bow River and Cave and Basin Marsh water samples. Circles represent individual water measurements and the squares represent the average value with standard deviation bars.

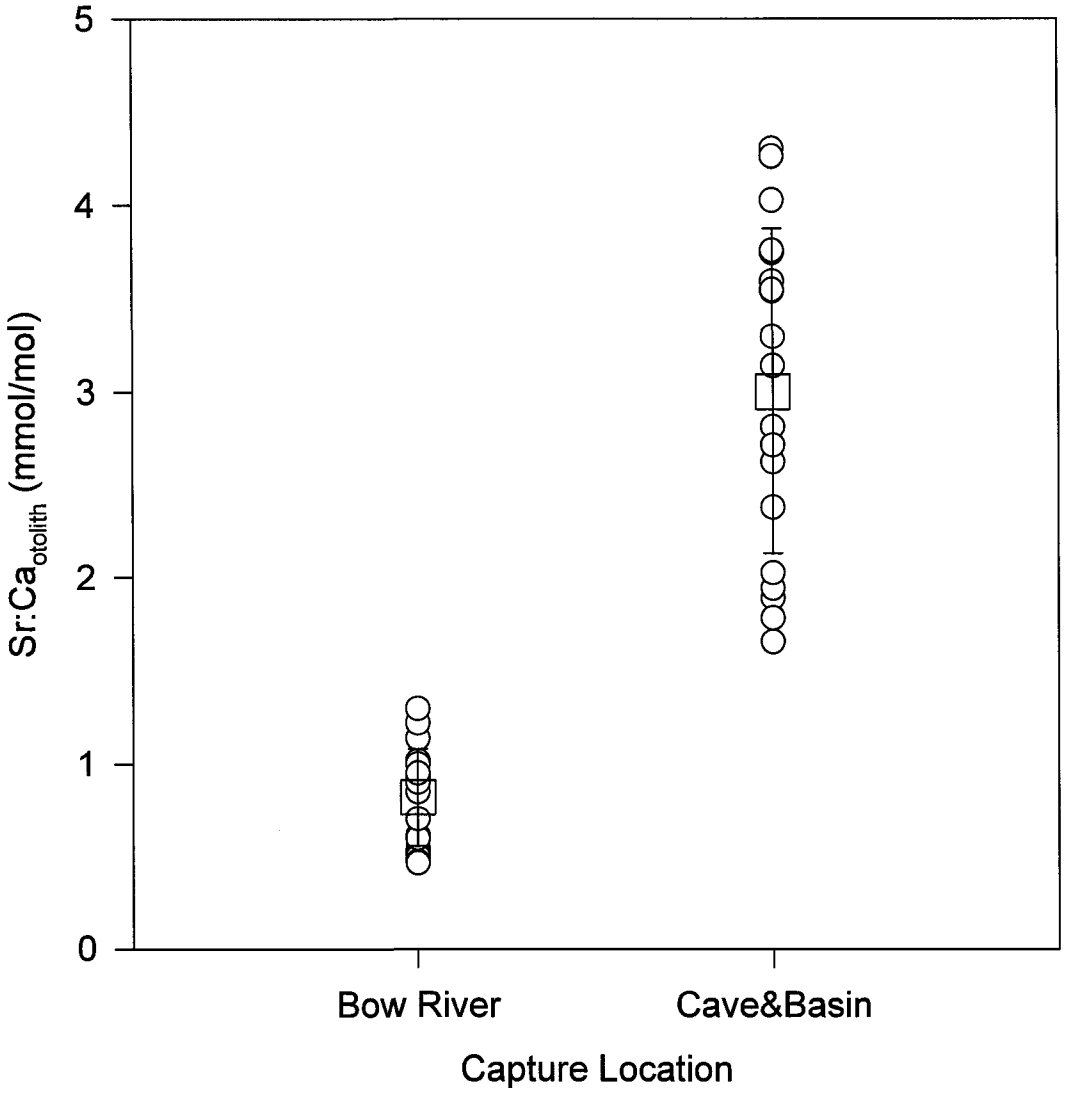

Figure 8. Otolith Strontium:Calcium ratios of Bow River and Cave and Basin Marsh longnose dace. Circles represent individual longnose dace samples and the squares represent the average value with standard deviation bars.

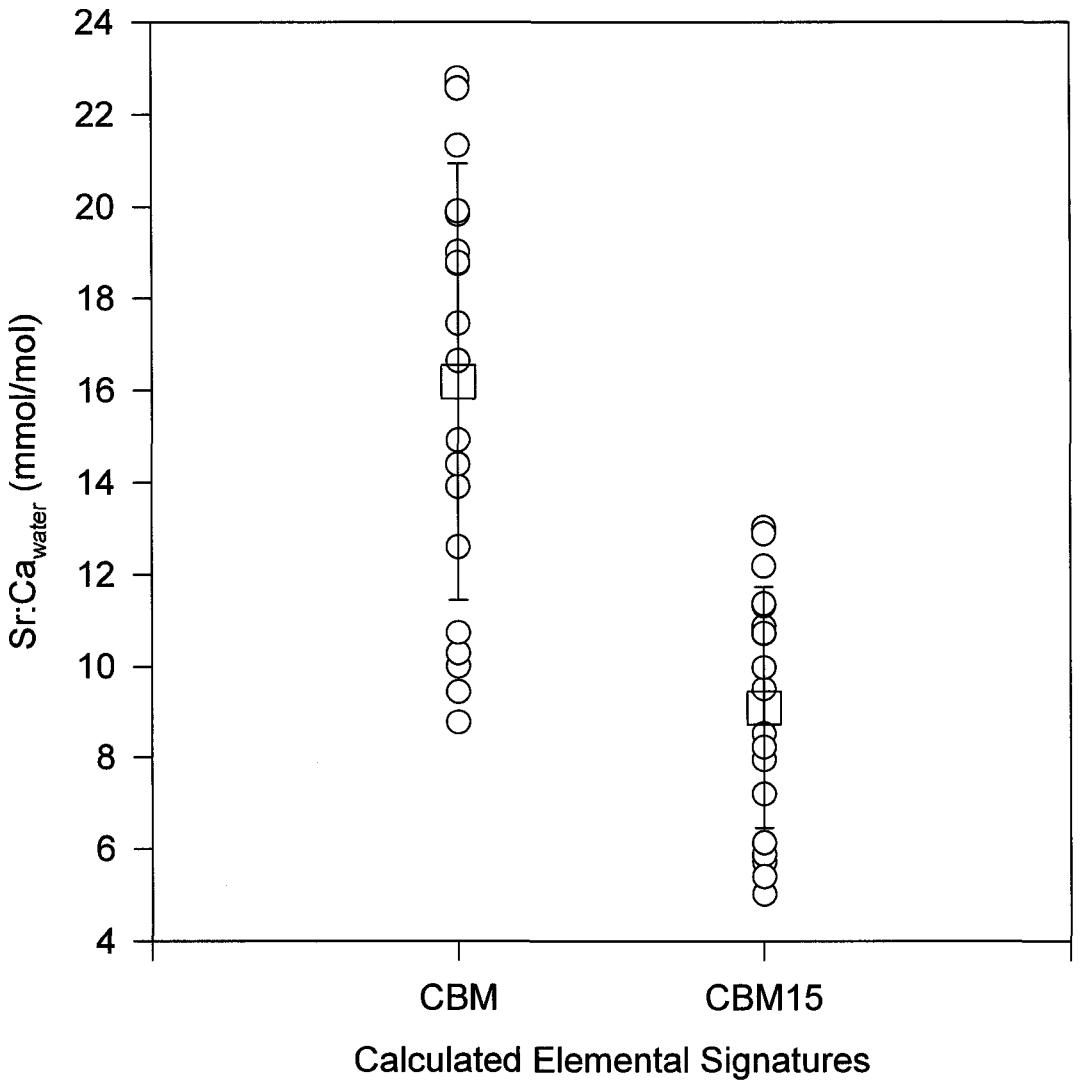

Figure 9. Projected Strontium:Calcium$_{water}$ ratios of the Cave and Basin Marsh (CBM) capture sites. CBM15 represents the putative 15 °C increase in mean annual Marsh temperature compared to the Bow River. Circles represent individual predictions and squares represent the means with standard deviations.

Table 6. Number of nucleotide substitutions and estimated percentage sequence divergence (Kimura-2, in parentheses) among *R. cataractae* (C1 – C11) *and R. atratulus* (BND1 and BND2) cytochrome *b* haplotypes (457 bp).

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	BND1	BND2
C1	-												
C2	1(0.2)	-											
C3	1(0.2)	2(0.4)	-										
C4	3(0.7)	2(0.4)	2(0.4)	-									
C5	1(0.2)	2(0.4)	2(0.4)	4(0.9)	-								
C6	2(0.4)	3(0.7)	3(0.7)	5(1.1)	3 (0.7)	-							
C7	22(5.0)	23(5.3)	23(6.4)	25(5.7)	23(5.2)	22(5.0)	-						
C8	23(5.2)	24(5.5)	24(6.8)	26(6.0)	24(5.5)	23(5.2)	1(0.2)	-					
C9	24(5.5)	25(5.7)	25(7.1)	27(6.2)	25(5.7)	24(5.5)	12(2.7)	13(2.9)	-				
C10	25(5.7)	26(6.0)	26(7.5)	28(6.4)	26(5.9)	25(5.7)	11(2.5)	12(2.7)	1(0.2)	-			
C11	25(5.7)	26(6.0)	26(7.5)	28(6.4)	26(5.9)	25(5.7)	13(2.9)	14(3.1)	1(0.2)	2(0.4)	-		
BND1	50(12.4)	51(12.7)	51(19.0)	53(13.2)	51(12.6)	52(12.9)	48(11.9)	49(12.2)	48(11.8)	49(12.1)	49(12.1)	-	
BND2	50(12.4)	51(12.7)	51(18.2)	53(13.2)	51(12.6)	52(12.9)	47(11.6)	48(11.9)	49(12.1)	50(12.4)	50(12.4)	28(6.5)	-

Table 7. Polymorphic sites within cytochrome *b* sequences for each haplotype. Note: site position one is equivalent number to *Rhinichthys cataractae* site position 214 (Girard and Angers 2006a).

```
                    111 1111111111 1122222222 2222223333 3333333333 3333333333 3334444444 44
       1122334 5567889000 1223467779 9900123466 7889990000 0112222334 5566677888 9990112223 34
     1251736257 3654362147 0584670361 5706279209 2473692568 9170369251 0924837039 2582362594 76
-------------------------------------------------------------------------------------------------
C1   ATACTCGTTA CAACTAGACA CCTAAACTCC ATATGTCAAG GGGATCCTGT TACACAAACG GGAAATACTT TGCCGTGTGC CT
C2   .......... .......... .......... .......... .......... .........A .......... .......... ..
C3   .......... .......... .......... .......... .......... .......... ...C...... .......... ..
C4   .......... .......... .......... .......... .......... .........A ...C...... .......... T.
C5   .......... .......... .......... G......... .......... .......... .......... .......... ..
C6   .......... .......... .......... .......... .........C .......... .....C.... .......... ..
C7   GC.T..A..G T......GT. .......... ....A..GG. A....T...C ..T.TGG.T. .A........ ..T.A..... ..
C8   GC.T..A..G T......GT. .......... ....A..GG. A....T..AC ..T.TGG.T. .A........ ..T.A..... ..
C9   G..T..A..G ........TG ..C.G..... ....A..GG. A..G.T...C .GT.T.G... .A......C. ..T.A.A... ..
C10  GC.T..A..G ........TG ..C.G..... ....A..GG. A..G.T...C .GT.T.G... .A......C. ..T.A.A... ..
C11  G..T..A..G ........TG ..CGG..... ....A..GG. A..G.T...C .GT.T.G... .A......C. ..T.A.A... ..
BND1 G...CAAC.G .GG..GA... TT...TTATT .CGCC..GCC A.A.CT.A.. C.TGA.G... .AT.G.GGCA CATTCC.AAT ..
BND2 G.G.CAA.CG .CGTC.A... TT...TTATT ....CCTGCC AAA..TTA.. C.T.TGGG.. A.C.G...CA .AT.CC.CAT .G
```

Table 8. Distribution frequency of the 11 cytochrome *b*, 6 control region, and 11 mtDNA haplotypes in the longnose dace populations. Callum Creek (CMC), Bow River (BOR), Cave & Basin Marsh (CBM), Smithsonian (MNH), University of Michigan Museum of Zoology (UMM), Blackwater River (BWR), Cale Creek (CLC), Parsnip River (PSR).

	Population							
	CMC	BOR	CBM	MNH	UMM	BWR	CLC	PSR
Haplotype								
				Cytochrome *b*				
C1				1	2	2	5	
C2							1	
C3						1		
C4							1	
C5								2
C6					1			
C7		5	8	2				
C8	1							
C9	8	5	2	1				
C10	1							
C11			1					
				Control Region				
D1				1	2	3	6	2
D2							1	
D3	1							
D4		5	8					
D5	9	5	2					
D6			1					
				Combined Genes				
M1 (C1xD1)				1	1	2	4	
M2 (C3XD1)						1		
M3 (C2XD1)							1	
M4 (C4xD1)							1	
M5 (C5xD1)								2
M6 (C1xD2)							1	
M7 (C7xD4)		5	8					
M8 (C8xD3)	1							
M9 (C9xD5)	8	5	2					
M10 (C10xD5)	1							
M11 (C11xD6)			1					

Tissue pieces UMMZ 213828-P1 and P2 are not included.

Table 9. Percent cytochrome *b* divergence within and between suggested longnose dace clades and blacknose dace. Intraclade divergence in italics.

	BND	Clade A	Clade B	Clade C
BND	-	12.4	11.5	11.8
Clade A		*0.5*	5.4	6.0
Clade B			*0.2*	2.8
Clade C				*0.3*

Table 10. Sample locations (Fig.1) of longnose dace populations, sample size (n) and number of cytochrome *b* haplotypes (nh) detected for each population and genetic diversity indices of the population (haplotypic diversity (h, Nei and Tajima 1981) and nucleotide diversity (π, Nei 1987).

Sample Location (Drainage)	n	nh	h	π
CMC (Oldman)	10	3	0.3778 ± 0.1813	0.006864 ± 0.004389
BOR (Bow)	11	2	0.5556 ± 0.0745	0.016570 ± 0.009559
CBM (Bow)	10	3	0.4727 ± 0.1617	0.013509 ± 0.007848
BWR (Fraser)	3	2	0.6667 ± 0.3143	0.001470 ± 0.001829
CLC (Fraser)	7	3	0.5238 ± 0.2086	0.002337 ± 0.001995
PSR (Peace)	2	1	0	0
BLD (Bow)	6	4	0.8667 ± 0.1291	0.033844 ± 0.020405

Tissue piece UMMZ 213828-P1 was not included as it matched and could have been a piece of UMMZ 213828-5

Table 11. Population pairwise F_{ST} of cytochrome *b* (based on haplotype frequency).

	BOR	CBM	CMC	CLC	BWR	PSR	BLD
BOR	-						
CBM	**0.05832**	-					
CMC	**0.22222**	0.50063	-				
CLC	0.45847	0.50566	0.55884	-			
BWR	0.41176	0.47435	0.54458	**0**	-		
PSR	0.54545	0.60497	0.68165	0.58435	**0.57143**	-	
BLD	0.32973	0.38066	0.43056	0.33333	**0.24167**	0.38262	-

Bold values are not significantly different from one another ($P > 0.05$).

Table 12. Population pairwise F_{ST} of longnose dace cytochrome *b* (based on Kimura-2 distance)

	BOR	CBM	CMC	CLC	BWR	PSR	BLD
BOR	-						
CBM	**0.00873**	-					
CMC	**0.22733**	0.50365	-				
CLC	0.82112	0.83619	0.91429	-			
BWR	0.79443	0.82436	0.91360	**0.03535**	-		
PSR	0.76822	0.80519	0.90557	0.45461	**0.52941**	-	
BLD	0.35996	0.38941	0.54246	0.24674	0.17091	**0.06741**	-

Bold values are not significantly different from one another ($P > 0.05$).

Table 13. Analysis of molecular variance (haplotype frequency) results for hierarchal genetic subdivision of longnose dace populations.

	Cytochrome *b* % of total variance	Combined genes % variance
Among Populations	41.05	41.75
Within Populations	58.95	58.75

All values were significantly differentiated ($P<0.01$)

Table 14. Analysis of molecular variance (Kimura-2) results for hierarchal genetic subdivision of longnose dace populations.

	Cytochrome *b* % variance	Combined genes % variance
Among Populations	64.25	80.46
Within Populations	35.75	19.54

All values were significantly differentiated ($P<0.01$)

Table 15. Number of nucleotide substitutions and percentage sequence divergence (Kimura-2, in parentheses) among *R. cataractae* and *R. atratulus* mtDNA (457 bp cytochrome *b* and 190 bp control region) haplotypes.

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	BND1
M2	1(0.2)											
M3	1(0.2)	2(0.3)										
M4	3(0.5)	2(0.3)	2(0.3)									
M5	1(0.2)	2(0.3)	2(0.3)	4(0.6)								
M6	2(0.3)	3(0.5)	3(0.5)	5(0.8)	3(0.5)							
M7	31(5.1)	32(5.2)	32(5.2)	34(5.6)	32(5.2)	33(5.4)						
M8	33(5.4)	34(5.6)	34(5.6)	36(5.9)	34(5.6)	31(5.1)	4(0.6)					
M9	33(5.4)	34(5.6)	34(5.6)	36(5.9)	34(5.6)	35(5.7)	14(2.2)	16(2.5)				
M10	34(5.6)	35(5.8)	35(5.8)	37(6.1)	35(5.8)	36(5.9)	13(2.1)	15(2.4)	1(0.2)			
M11	35(5.8)	36(5.9)	36(5.9)	38(6.3)	36(5.9)	37(6.1)	16(2.6)	18(2.9)	2(0.3)	3(0.5)		
BND1	63(10.7)	64(10.8)	64(10.8)	66(11.2)	64(10.8)	65(11.0)	68(11.6)	70(12.0)	68(11.6)	69(11.8)	68(11.6)	
BND2	65(11.0)	66(11.2)	66(11.2)	68(11.6)	66(11.2)	67(11.4)	69(11.8)	71(12.1)	71(12.2)	72(12.4)	71(12.2)	33(5.4)

Table 16. Percent mtDNA 647 bp (190 bp control region and 457 bp cytochrome *b*) divergence within and between suggested longnose dace clades and blacknose dace. Intraclade divergence in italics.

	BND	Clade A	Clade B	Clade C
BND	-	11.1	11.9	11.9
Clade A		*0.4*	5.4	5.8
Clade B			*0.6*	2.4
Clade C				*0.3*

Table 17. Sample locations (Fig.1) of longnose dace populations, sample size and number of mtDNA haplotypes detected for each population and genetic diversity indices with standard error of the population (haplotypic diversity (h, Nei and Tajima 1981) and nucleotide diversity (π, Nei 1987).

Location (drainage)	N	nh	h	π
CMC (Oldman)	10	3	0.3778 ± 0.1813	0.005733 ± 0.003576
BOR (Bow)	11	2	0.5556 ± 0.0745	0.013340 ± 0.007620
CBM (Bow)	10	3	0.4727 ± 0.1617	0.011152 ± 0.006389
BWR (Fraser)	3	2	0.6667 ± 0.3143	0.001038 ± 0.001292
CLC (Fraser)	7	4	0.7143 ± 0.1809	0.002543 ± 0.001941
PSR (Peace)	2	1	0	0
BLD (Bow)	2	1	0	0
Total	45	11		

Archived samples USNM 44045-4 and tissue piece UMMZ 213828-P1.

Table 18. Population pairwise F_{ST} of combined cytochrome *b* and control region (based on haplotype frequency).

	BOR	CBM	CMC	CLC	BWR	PSR	BLD
BOR	-						
CBM	**0.05832**	-					
CMC	**0.22222**	0.50063	-				
CLC	0.37328	0.42312	0.47396	-			
BWR	0.41176	0.47435	0.54458	**0**	-		
PSR	0.54545	0.60497	0.68165	0.44809	**0.57143**	-	
BLD	0.54545	0.60497	0.68165	**0**	**0**	**1.00000**	-

Bold values are not significantly different from one another ($P > 0.05$).

Table 19. Population pairwise F_{ST} of control cytochrome *b* and control region (based on Kimura-2 distance).

	BOR	CBM	CMC	CLC	BWR	PSR	BLD
BOR	-						
CBM	**0.00718**	-					
CMC	**0.23582**	0.50204	-				
CLC	0.86347	0.87886	0.93451	-			
BWR	0.83254	0.85510	0.92773	**0**	-		
PSR	0.82395	0.84911	0.92578	**0.34091**	**0.67666**	-	
BLD	0.81720	0.84326	0.92295	**0**	**0**	**1.0000**	-

Bold values are not significantly different from one another ($P > 0.05$).

Table 20. Percentage haplotype divergence (kimura-2) among *R. cataractae* and *R. atratulus* cytochrome *b* haplotypes (457 bp). Sequences of additional longnose dace were provided by Dr. J.D. McPhail, University of British Columbia. Abbreviations for sample locations are: LCOL, Willamette River, OR; MCOL, Similkameen River, BC; UCOL, Upper Columbia River, near Cranbrook, BC; RUBY, Ruby Creek, Upper Missouri system, MT; MANI, Wilson Creek, MB; and QUEB, Quebec.

	LCOL	MCOL	UCOL	RUBY	MANI	QUEB	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	BND1	BND2
LCOL	-																		
MCOL	0.000	-																	
UCOL	0.004	0.004	-																
RUBY	0.055	0.055	0.050	-															
MANI	0.053	0.053	0.048	0.013	-														
QUEB	0.050	0.050	0.045	0.011	0.007	-													
C1	0.000	0.000	0.004	0.055	0.053	0.050	-												
C2	0.002	0.002	0.007	0.057	0.055	0.052	0.002	-											
C3	0.002	0.002	0.007	0.058	0.055	0.053	0.002	0.004	-										
C4	0.007	0.007	0.011	0.062	0.060	0.057	0.007	0.004	0.004	-									
C5	0.002	0.002	0.007	0.057	0.055	0.052	0.002	0.004	0.004	0.009	-								
C6	0.004	0.004	0.009	0.055	0.053	0.050	0.004	0.007	0.007	0.011	0.007	-							
C7	0.050	0.050	0.045	0.007	0.007	0.004	0.050	0.052	0.053	0.057	0.052	0.050	-						
C8	0.052	0.052	0.048	0.004	0.009	0.007	0.052	0.055	0.055	0.060	0.055	0.052	0.002	-					
C9	0.055	0.055	0.055	0.029	0.029	0.027	0.055	0.057	0.057	0.062	0.057	0.055	0.027	0.029	-				
C10	0.057	0.057	0.052	0.027	0.027	0.025	0.057	0.060	0.060	0.065	0.060	0.057	0.025	0.027	0.002	-			
C11	0.057	0.057	0.057	0.031	0.031	0.029	0.057	0.060	0.060	0.065	0.060	0.057	0.029	0.031	0.002	0.004	-		
BND1	0.124	0.124	0.125	0.128	0.117	0.119	0.124	0.127	0.127	0.133	0.127	0.130	0.119	0.122	0.119	0.122	0.122	-	
BND2	0.124	0.124	0.124	0.125	0.122	0.122	0.124	0.127	0.127	0.133	0.127	0.130	0.117	0.119	0.121	0.124	0.124	0.065	-

Table 21. Population genetic statistics summarizing variation at 9 microsatellite loci in longnose dace (*Rhinichthys cataractae*) sampled from Western Alberta.

Population	Locus								
	*Lco*1	*Lco* 3	*Lco*4	*Lco*5	*Ca*12	*Rhca*16	*Rhca*20	*Rhca*24	*Rhca*31
Bow River									
N	24	27	32	30	25	32	32	26	32
H_O	0.917	0.074	0.438	0.733	0.840	0.625	0.844	0.769	0.625
H_E	0.916	0.073	0.381	0.693	0.886	0.687	0.731	0.778	0.517
N_A	16	2	2	6	14	5	9	10	3
Cave and Basin									
N	29	30	32	33	24	33	33	26	33
H_O	0.897	0.033	0.563	0.606	0.833	0.697	0.848	0.654	0.667
H_E	0.941	0.033	0.458	0.622	0.885	0.671	0.793	0.763	0.507
N_A	23	2	2	6	17	5	10	14	2
Jumpingpound Creek									
N	29	29	29	29	28	29	29	29	29
H_O	0.897	0.310	0.690	0.552	0.929	0.862	0.671	0.724	0.345
H_E	0.956	0.272	0.639	0.696	0.925	0.755	0.617	0.858	0.407
N_A	24	3	3	7	18	5	7	15	2
Callum Creek									
N	27	29	29	29	29	29	28	27	28
H_O	0.889	0.276	0.483	0.517	0.793	0.724	0.679	**0.519**	0.321
H_E	0.946	0.251	0.424	0.604	0.936	0.691	0.775	0.871	0.275
N_A	24	3	3	7	18	5	7	15	2

N = sample size, H_O = observed heterozygosity, H_E = expected heterozygosity, N_A = number of alleles. Values of H_O that are in bold represent significant deviations from H_E.

Table 22. Pairwise R_{ST} (above diagonal) and F_{ST} (below diagonal) values between four extant longnose dace (*Rhinichthys cataractae*) populations sampled from Western Alberta.

	BOR	CBM	JPC	CMC
BOR	-	**0**	0.01589	0.04563
CBM	**0.00177**	-	0.05386	0.04033
JPC	0.02943	0.03621	-	**0.00256**
CMC	0.04118	0.05301	0.01384	-

F_{ST} values are based on variation in allele frequency at nine microsatellite loci. R_{ST} values are based on a distance method (Sum of squared size difference). Bold values do not significantly differ (P>0.05).

Table 23. Population Assignment of extant longnose dace and detection of first generation migrants.

	Assigned Population			
	BOR/CBM	JPC	CMC	Other
Source of Individuals				
BOR/CBM	62	0	1	0
JPC	0	27	1	1
CMC	2	0	27	0

Table 24. Assignment of archived longnose dace to extant populations. Values indicate probability of occurrence in population. Bold values indicate sample could occur in population.

	Assigned Population		
	BOR/CBM	JPC	CMC
Sample			
USNM 44045-4	**0.2083**	**0.1428**	**0.1992**
UMMZ 213828-7	0.0077	**0.0152**	0.0013
UMMZ 213828-5	**0.0110**	0.0068	0.0009

DISCUSSION

The results presented in this thesis use molecular approaches to re-evaluate a sub-species listed as extinct based on the disappearance of diagnostic morphological characteristics (lower dorsal fin ray and lateral line scale counts). Banff longnose dace were listed by COSEWIC in 1987 and reconfirmed in 2000 as an extinct sub-species based on the gradual loss of these unique morphological features specific to a small population of longnose dace found exclusively within the Marsh below the Cave & Basin Hotsprings (COSEWIC 2003). In contrast, my mtDNA data did not support the sub-species status of the Banff longnose dace. Nevertheless, the population contributed to a unique assemblage of animals found within the Marsh that deserved protection. The data gathered in this study has allowed me to answer several questions pertaining to the sub-species status and origin of longnose dace within the Cave & Basin Marsh.

What are the phylogenetic relationships among R. c. smithi and extant longnose dace?

Mitochondrial DNA sequences may not provide indisputable evidence for the taxonomical classification of a sub-species, however, it is commonly chosen to identify intraspecific evolutionary lineages (reviewed in Avise 2000). Haplotypes identified in longnose dace from this study fit into three major clades of distinct lineage with intraclade divergences less than 0.7%, a value typical of a species re-colonizing formerly glaciated areas which tends to have a few widely dispersed haplotypes (Bernatchez and Wilson 1998). Assuming a mtDNA divergence rate of 1-2% per million years (Brown et al. 1979; Wilson et al. 1985), a separation time from 350 000 to 700 000 years is expected between the most diverse clades, indicating divergence within each clade occurred within the Pleistocene. Divergence among these clades was much greater, ranging from 3.1% to 7.4%, indicating separation times from 1.55 to 7.4

million years ago (mya). This timeline largely predates the Pleistocene and suggests that the three clades occupied separate glacial refugia. The existence of several lineages of longnose dace has been previously reported (McPhail and Lindsey 1970), but their subspecific status remains largely unclear. Girard and Angers (2006a) identified two lineages of longnose dace from Quebec, one of Atlantic origin and the other hypothesized to be of Mississippian origin. Additionally, I identified three Clades from sites in British Columbia and Alberta.

From archived samples of longnose dace collected in the Marsh more than 100 years ago, mtDNA sequences revealed haplotypes belonging to three distinct Clades. Although, only two of the Clades are presently found in longnose dace collected from the Marsh, a comparison with haplotypes from other extant populations reveals phylogenetic relationships for putative sub-species of longnose dace. Such relationships will provide insight into the dispersal routes for this species post glacially. To gain an understanding of the phylogenetic relationship for the archived samples of Banff longnose dace, I will first examine the phylogenetic relationships for extant populations of longnose dace collected in British Columbia and Alberta. An examination of changes in haplotype frequency over time for fish collected in the Cave & Basin Marsh will then be used to reveal competitive interactions among different forms of this species.

Extant haplotypes of Clade A were found in the two Upper Fraser River tributaries and the one Upper Peace tributary. McPhail and Lindsay (1986) indicated that the Upper Fraser River system contains only the Columbian (Pacific) form of longnose dace (*R. cataractae dulcis)*. This was supported by the inclusion of the longnose dace sequences from the Columbia Watershed in the Pacific Clade. Geographic patterning combined with the low level of intra-clade mtDNA divergence suggests a Pacific refuge of origin for Clade A longnose dace. The contribution of longnose dace of Pacific origin to fish captured in the Cave & Basin Marsh in

1892 is not surprising considering the high vagility of this species and the fact that the Pacific refugium has contributed to the re-colonization of Alberta by no less than nine species. Three of these species, the mountain whitefish (*Prosopium williamsoni* Girard), the westslope cutthroat trout (*Oncorhynchus clarki lewisi* Girard) and the bull trout (*Salvelinus confluentus* Suckley), are widely accepted to be of Pacific origin (Nelson and Paetz 1992). Furthermore, bull trout in the South Saskatchewan River system have been demonstrated to be of Pacific origin with the use of mitochondrial DNA (Taylor et al. 1999). Morphological variation of longnose dace in the Upper Peace suggests invasion from two different origins, the Pacific and likely an eastern population (Lindsey and McPhail 1986). Only one haplotype, however, was found in the two samples collected in the Parsnip system. Verification of multiple haplotypes within the Upper Peace watershed, therefore, requires further investigation.

Populations on the east slope of the Continental divide from Alberta branched into two Clades (B and C) more closely related to one another than to either the Pacific or Atlantic clades. Clades B and C are not likely to have originated from either the Pacific or Atlantic refugia, suggesting another refugium for fish fauna during the last glaciation. McPhail (2007) described the Great Plains refugium which is the dominant source of fish throughout Alberta for watersheds that flow into the Hudson Bay. Additionally, there is evidence that this refugium contained at least two semi-isolated refugia: the Mississippi and the Missouri which were separated from each other by a sheet of ice until 12 800 years ago (Cross et al. 1986; Crossman and McAllister 1986). My genetic analysis is consistent with fish found in southern Alberta dispersing from two refugia. Pairwise sequence divergence among cytochrome *b* haplotypes from Clade B in my study, Girard and Angers (2006a) proposed Mississippian lineage, and sequences collected from Quebec, Manitoba, Alberta, and Montana (J.D. McPhail, University of

British Columbia, unpublished data) were all less than two percent. Sequence divergence between 0.5 and two percent is typical of northern species occupying the same glacial refugium (Bernatchez and Wilson 1998) suggesting that fish from clade B are most likely from the proposed Mississippi refugium. The differentiation observed within this lineage is likely attributable to physical barriers that may have led to isolation and divergence causing the differentiation among clade B dace collected for my study and specimens from Quebec and Manitoba.

Clades B and C branched off from all other clades demonstrating their moderately close relationship, however, a greater than 2% sequence divergence between these clades suggests that ancestral populations occupied separate refugia during the Pleistocene. The Missourian refuge is highly likely for clade C because of its occurrence throughout Alberta and moderate divergence from the proposed Mississippian lineage. Historically, these two Clades may have evolved separately but the secondary contact between the two lineages has undoubtedly been extensive and Clades B and C are likely better to be considered together as the Great Plains lineage.

A less likely origin for Clade C is a refuge within Alberta. Nevertheless, there is evidence supporting the existence of an Albertan refugium provided by genetic differentiation in populations of lake trout (*Salvelinus namaycush* Walbaum; Wilson and Hebert 1988), Arctic grayling (*Thymallus arcticus* Pallas) fossils (Burns 1991), and endemic cold water fish and invertebrate taxa (Crossman and McAllister 1986).

My results demonstrate that two different evolutionary lineages (Pacific and Great Plans) of longnose dace came into secondary contact in the Cave & Basin Marsh. None of the mtDNA lineages proved to be unique or restricted to the Cave & Basin Marsh. Instead haplotypes from the Marsh were found in several other locations in North America. Avise (2000) describes this

phylogeographic pattern as a "deep gene tree, major lineages broadly sympatric". This pattern is typical of species exhibiting high levels of vagility. Although the longnose dace is a small species, it appears to have excellent ability to disperse based on the fact that it is ubiquitous throughout North America, thus can be described as a highly vagile species. Consequently, in the late 1800s, the Cave & Basin Marsh was a zone of secondary admixture between allopatrically evolved sub-species. Zones of secondary contact between distinct lineages of longnose dace likely has occurred elsewhere in Canada such as in Ste-Anne of the St. Lawrence River drainage, however, introduction may have obscured the signal as suggested by Girard and Angers (2006a). Additionally, the Peace system is known to have both *R. c. dulcis* and *R. c. cataractae* based on morphology (Lindsey and McPhail 1986). Other examples of secondary contact between intraspecific lineages in North American fish species have been demonstrated for lake whitefish, *Coregonus clupeaformis* Mitchill, (Bernatchez and Dodson 1990; Bernatchez and Dodson 1991), brown bullhead, *Ameiurus nebulosus* Lesueur, (Murdoch and Hebert 1994) and lake cisco, *Coregonus artedi* Lesueur (Turgeon and Bernatchez 2001).

Was the Banff longnose dace a distinct subspecies endemic to the Cave & Basin Marsh?

The Banff longnose dace was designated a distinct sub-species based on geographical isolation and morphological uniqueness. It was proposed that this form of longnose dace could have survived the last ice age within a refugium along the east slope of the continental divide near present day Banff and Jasper (Crossman and McAllister 1986). My examination of mtDNA sequences from cytochrome *b* and the control region, however, does not support this sub-species designation. Archived and extant longnose dace were found to share common mtDNA haplotypes from each of three different evolutionary lineages. This finding indicates that the

Banff longnose dace was a post glacial immigrant and not a pre-glacial relict endemic to the Cave & Basin Marsh.

The lack of concordance between morphological data and genetic data, however, is not without precedence in the literature. My finding is similar to that reported for another fish, the Athabasca rainbow trout (*Onchorynchus mykiss*). The Athabasca rainbow trout was thought to be a unique sub-species originating from the Banff-Jasper refugium, but molecular genetic analysis revealed similar mtDNA haplotypes to nearby populations on the western side of the continental divide (McCusker et al. 2000). Later, Taylor et al. (2006) used microsatellites to reveal a lack of genetic distinctiveness for the Athabasca rainbow demonstrating a high likelihood of postglacial immigration from adjacent populations of the Fraser River. Endemic taxa to the Banff-Jasper refugium include isopods, amphipods, and plants (reviewed in Crossman and McAllister 1986), however, to date, there is no evidence of any fish species utilizing this proposed refugium.

If the Banff longnose dace was indeed a distinct sub-species, sub-speciation would have occurred postglacially in the Marsh, likely as a result of the occupation of the novel hot springs fed habitat. Speciation in novel habitats has been previously identified in the threespine stickleback (*Gasterosteus aculeatus*) complex with nuclear DNA (reviewed in McKinnon and Rundle 2002). Mitochondrial DNA, however, is believed to be particularly susceptible to biases in this complex of fish. For example, mtDNA results for threespine stickleback in Japan are inconsistent with other markers and geological data (reviewed in McKinnon and Rundle 2002). My examination of nuclear DNA (microsatellites) was limited and further analysis of nuclear DNA of Banff longnose dace would help to resolve this issue. Unfortunately, the large sample size required for this analysis combined with the low number of Banff longnose dace appropriate and available for genetic analysis make this research problematic.

Is there utility in using multiple approaches to address conservation issues?

A comparison of the results of Renaud and McAllister (1988) with my own, demonstrates a lack of concordance among morphological and mitochondrial DNA characters. Dissimilarity among morphological and molecular characters suggests that phylogenetic history is not being consistently recovered and that re-evaluation of the characters is necessary (Larson 1998). Larson recommends the use of informative characters combined with a systematic method for identifying misleading information in order to elucidate patterns of common descent. The reasons for the unique morphology of the Banff longnose dace were not examined in this study however, the facts that the Pacific Clade appears to be extirpated and that the unique morphology is no longer observed suggest that the lost Clade may be correlated with the change in morphology. This does not mean that mitochondrial DNA is responsible for the morphological changes, but that the two factors may be correlated.

USNM 44045-4, UMMZ 213828-5, and UMMZ 213828-7 all exhibited Pacific mtDNA haplotypes of Clade A but only the latter two exhibited the unique Banff longnose dace morphology as assessed by Renaud and McAllister (1988). Additionally, USNM 44045-1 and many extant marsh longnose dace exhibited the inferred ancestral haplotype of Clade C but only the former exhibited seven dorsal fin rays, a trait restricted to Banff longnose dace. The same pattern may also be true for Clade B, however, USNM 44045-2 could be neither excluded nor confirmed as a Banff longnose dace based on morphology.

The above examples demonstrate that in the 1890s in the Banff region *R. c. cataractae* and *R. c. smithi* shared identical inferred ancestral haplotypes from each of Clades A, C, and possibly B. The collection location of UMMZ 213828 was the Cave & Basin Marsh, whereas all

samples of USNM 44045 were recorded as collected from hot and cold springs. Samples USNM 44045-1 through USNM 44045-4 may have been reared in the Marsh, collected in the Marsh as first generation migrants, or collected in a nearby 'cool springs'. The latter two possibilities would not have exposed dace to the higher temperatures during embryogenesis explaining the typical longnose dace morphology.

The lack of concordance among morphological and molecular characters reveals the need to determine if the unique morphological traits are heritable. Interestingly, the two features (number of fin rays and scales) used to classify the Banff longnose dace as a sub-species often decrease in number as egg incubation temperature increases (reviewed in Barlow 1961; Fahy 1980). The hot spring fed Cave & Basin Marsh provides an environment that exposes eggs to higher temperatures which may provide suitable conditions to cause such changes. It is believed that temperature in the Marsh has been consistent over the last 100 years (Renaud and McAllister 1988), suggesting that environmental determinants for the Banff longnose dace morphology may not be likely. Temperature has remained stable, yet the traits unique to the Banff longnose dace have been gradually lost over time. However, only the Great Plains lineage of longnose dace was found in the extant Cave & Basin Marsh population. Although speculative, it is possible that either the Pacific lineage of longnose dace may exhibit a phenotypic response to temperature resulting in the Banff longnose dace morphology or adaptive radiation occurred in the Marsh. Additionally, the disappearance of the Pacific clade in the Cave & Basin Marsh is also consistent with the introgression hypothesis of Renaud and McAllister (1988). Hence, genotype, temperature induced phenotype, or a combination of both factors may have been responsible for the unique morphology. When one combines my genetic results with Renaud and McAllister's

(1988) morphological results, genetic swamping of the Pacific lineage of longnose dace by the Great Plains lineage appears even more likely.

My mtDNA research indicates that historically, the Cave & Basin Marsh was habitat for two lineages of longnose dace. Presently, these same two lineages are found throughout Western Canada and the Northwestern United States, however, only the Great Plains lineage was discovered in extant longnose dace collected from the Marsh. The Pacific lineage appears to have been swamped out by longnose dace from the Great Plains lineage and has been extirpated from the region.

Although, my mtDNA results do not support the sub-species classification of the Banff longnose dace, the loss of Pacific Clade haplotypes indicates a loss of genetic diversity within this population of longnose dace. The fact that genetic loss within a species has occurred within Banff National Park should cause concern for our ability to effectively protect species. However, the Pacific lineage of longnose dace in the Marsh likely represented a remnant population which was vulnerable to extirpation through genetic swamping regardless of human presence and modification in the Marsh. My data indicates that introgression was occurring before 1892. The most parsimonious explanation is that genetic drift occurred until the Pacific haplotype became extirpated. The Banff longnose dace was likely a remnant population of Pacific lineage that was prone to genetic drift and swamping due to its small population size.

The fact that the Banff longnose dace was designated as extinct based solely on morphological differences raises questions regarding our past ability to assess the taxonomy of a species and use that information for status designation. Scientists always have differing opinions regarding research in their respective fields, but when assigning a distinct status to a population and later listing that population as extinct, substantial consideration of all factors should be

examined. Morphology was the preferred tool available to determine the taxonomic status of the Banff longnose dace and Renaud and McAllister (1988) used the best information available to make their conclusions. However, considering the controversy regarding the taxonomy of the Banff longnose dace, the decision to list the Banff longnose dace as extinct was questionable. Fortunately, Parks Canada maintained interest in the taxonomic status of this putative sub-species and provided the impetus for re-assessing the designation using molecular genetic techniques.

The importance of using multiple criteria to determine taxonomy cannot be overstated. Studies in which researchers used morphology, behaviour, and genetics have confirmed the taxonomy of several species (Gavin et al. 1999; Pasquet 1999; Haig et al. 2004). Conversely many studies using these same criteria have lacked concordance (Larson 1997; Ball and Avise 1992; Avise and Nelson 1989; Williams et al. 2004; Zink 2004; Zink et al. 2004). Confirmation among multiple characters validates taxonomy whereas dissimilarity demonstrates the need for further reassessment as technologies improve. My research used mitochondrial DNA to assess common descent among longnose dace. It is unknown whether phenotype, genotype, or a combination of both led to the unique Banff longnose dace morphology, however, future studies could use additional criteria to determine its cause(s). The lack of concordance between morphological and genetic data indicates that phylogeny is not being consistently revealed. An examination of the effects of temperature, Cave & Basin Marsh water, or hybridization on the morphology of the two lineages of longnose dace could be used to recreate the conditions that lead to the Banff longnose dace morphotype. Another interesting study would be to analyze microsatellite population structure of Pacific lineage longnose dace in regions where this lineage may have crossed into Alberta to determine the possible source population of the Banff longnose

dace. Unfortunately, obtaining a sufficient sample size of Banff longnose dace for nuclear genetic analysis would prove problematic.

Is there connectivity between the Cave & Basin Marsh and the Bow River?

The present study revealed that longnose dace populations from the Bow River and Cave & Basin Marsh are not significantly different from one another based on analysis of microsatellite DNA. High gene flow, therefore, occurs between the two adjacent water bodies. Further, the lack of significant differences in either pairwise F_{ST} or R_{ST} indicated that the temperature difference between the Marsh and the Bow River is not a barrier to gene flow.

There is a relationship between genetic differentiation and geographic distance for the populations examined: as the distance between populations increased from the Cave & Basin Marsh, the pairwise F_{ST} and R_{ST} values increased, providing possible evidence of isolation by distance. However, isolation by distance analysis is required to confirm this. Surprisingly, the Jumpingpound Creek and Callum Creek populations did not differ significantly based on the pairwise F_{ST} value, although the R_{ST} value did differ significantly. Calculating pairwise F_{ST} may be a more logical model because it tends to show better detection of intraspecific variation than R_{ST} (reviewed in Balloux and Lugon-Moulin 2002). Jumpingpound Creek is in the Bow River watershed and Callum Creek is in the Oldman River watershed. These watersheds join to form the South Saskatchewan watershed, however, the distance between these two populations is quite large, and they are presently separated by several dams. Obviously gene flow is not occurring between these two populations. I did not examine mitochondrial DNA in Jumpingpound Creek, however, it is possible that a greater percentage of these fish are of the same mtDNA lineage as those from Callum Creek which may explain the lower degree of divergence between these two

populations. Additionally Bow Falls may have provided somewhat of a historical fish barrier limiting gene flow and isolating longnose dace above the falls. Regardless of the model used and the significance of the pairwise comparisons between F_{ST} and R_{ST}, the values for both demonstrate a lower degree of differentiation than expected.

Strong population structure was also found based on the assignment test. The vast majority of dace were assigned to their population of origin. Interestingly, when the three archived samples were included in the analysis, probability estimates indicated that they could have been assigned to one or more of the extant populations sampled. Also, the sample with the highest assignment value had a Pacific lineage mtDNA haplotype but was larger than typical Banff longnose dace specimens.

Otolith microchemistry analysis of extant longnose dace also provided evidence for connectivity between the Bow River and the Cave & Basin Marsh. Although, there was considerable difference in the $Sr:Ca_{otolith}$ values, the range in values for fish caught in the Marsh was greater than for fish caught in the Bow River. The temperature compensated calculations of Cave & Basin Marsh longnose dace demonstrated a substantial amount of variation in Cave & Basin Marsh longnose dace $Sr:Ca_{otolith}$ values. This may indicate that different regions of the Marsh have different water chemistries or that the water chemistry varies seasonally. However, my trace element microchemistry concentrations of Calcium and Magnesium in the Marsh waters were within the range of values of Grasby and Lepitzki's (2002) winter Marsh values for these same two elements. This demonstrates that trace element concentrations in the Cave and Basin Marsh are stable both temporally and spatially. My water samples were taken at inflow and outflow Marsh sources indicating consistency throughout the Marsh. Elemental ratios from water samples collected in the Marsh, therefore, indicate a relatively homogeneous signal.

Using the temperature compensation estimates of Martin et al. (2004), it appears that there is considerable overlap in otolith Sr:Ca ratios for fish caught in the Marsh and fish caught in the Bow River. Some of the Marsh otoliths had $Sr:Ca_{otolith}$ values below the water chemistry values in the Marsh demonstrating that some Marsh fish likely migrated from the Bow River. The otolith microchemistry results, therefore, complement the genetic findings which indicate connectivity and movement of fish between the Marsh and the Bow River leading to high levels of gene flow.

Should COSEWIC reassess the status of the Banff longnose dace?

The effectiveness of protecting endangered species and populations of animals has been debated for many decades, and even the legal mechanisms by which we protect animals or their habitat has been questioned (Mooers et al. 2007). These same controversies are also evident in the listing of subspecific taxa (Haig et al. 2006). In Canada, the recognition and listing of populations below the species level is guided by the concept of "Designatable Units" (DUs) according to Green (2005). Initially, status is assigned by first examining the species as a whole, and then, by examining DUs below the species level when a single status designation is not sufficient to accurately reflect probabilities of extinction. Designatable Units may be recognized on the basis of the four following criteria: established taxonomy, genetic evidence, range disjuncture, and biogeographic distinction.

Designatable units recognized on the basis of established taxonomy. The established taxonomy of the Banff longnose dace (*R. c. smithi*) is that of a distinct sub-species based on lower numbers of dorsal fin rays and lateral line scales. However, my mtDNA evidence does not support the morphological evidence of a distinct sub-species. Nor does it support the *R. c. smithi*

classification. My data demonstrated that Banff longnose dace specimens shared haplotypes with different lineages of longnose dace. The most common haplotypes were of Pacific lineage. My mtDNA evidence suggests that the Banff longnose dace morphology was likely correlated with the Pacific lineage of DNA. Hence, the current classification for the Banff longnose dace of *Rhinichthys cataractae smithi* is not appropriate.

Designatable Units recognized on the basis of genetic evidence. My research demonstrated three mtDNA lineages of longnose dace which could each be considered DUs. The Banff longnose dace shared mtDNA haplotypes with extant populations demonstrating that it did not merit DU status with this genetic marker. Examining DUs below the species level in longnose dace has previously revealed that the Nooksack dace's cytochrome *b* sequence differs from that of the Pacific lineage of longnose dace by approximately 2.5% (McPhail 2007). This degree of divergence is greater than that of the difference between some other species in the Genus *Rhinichthys,* specifically Umatilla and leopard dace. Interestingly, the Nooksack dace has not been designated as a separate species due to dissimilarity between morphological and mtDNA signal. Much like the Banff longnose dace, the Nooksack dace has fewer lateral line scales than the Pacific lineage of longnose dace. The Banff longnose dace, however, was designated subspecies status based on morphology. My research has revealed Banff longnose dace shared haplotypes with extant longnose dace and exhibits dissimilarity between morphological and mtDNA signal. This raises questions on the merit of the subspecies designation.

Genetic evidence can also include heritable morphological traits. Renaud and McAllister (1988) believed that the Banff longnose dace merited subspecies status based on lower numbers of dorsal fin rays and lateral line scales. As previously stated, it is unknown whether these

morphological differences are heritable traits or environmentally induced due to the higher hot springs temperatures. This raises further questions as to the validity of the distinct subspecies status.

Designatable Units recognized on the basis of biogeographic distinction. My evidence suggests that the unique morphological traits of the Banff longnose dace may have been correlated with the Pacific lineage of longnose dace. The existence of a Pacific lineage of longnose dace in Alberta demonstrated biogeographic distinction. The past and present occurrence of this Pacific lineage is relatively unknown with the exception of my data for archived specimens from the Cave & Basin Marsh. Other fish from the Pacific refugium including mountain whitefish, westslope cutthroat trout, and bull trout colonized the Bow River watershed, however, they did not come into secondary contact with allopatrically evolved conspecifics. Longnose dace existed in several glacial refugia, are highly vagile, and are ubiquitous throughout North America providing more opportunities for secondary contact than the other Bow watershed species of Pacific origin which likely only evolved in a single Pacific refugium.

Regardless of the subspecies status, the Banff longnose dace population represented a unique assemblage of fish that no longer exists in the Cave & Basin Marsh. The biogeographic distinction demonstrates that it merited protection and designation but the designation of an extinct sub-species remains unresolved due to the unknown effects caused by the hot spring fed environment. Unless it can be proved that the morphological traits are heritable I would hesitate to exclusively use this evidence for designating subspecies status.

The correlation between the loss of the Banff longnose dace morphology and the disappearance of the Pacific lineage demonstrates that the Banff longnose dace does not merit

the *Rhinichthys cataractae smithi* classification. In order to properly name the Banff longnose dace, taxonomic clarity within longnose dace is first required. I recommend the use of *Rhinichthys cataractae cataractae* for the two Great Plains lineages of longnose dace lineages and *Rhinichthys cataractae dulcis* for the Pacific lineage of longnose dace. Then, I recommend the Banff longnose dace be reclassified as *Rhinichthys cataractae dulcis* and designated as extirpated from the Cave & Basin Marsh.

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APPENDICES

Appendix I: Records of Banff Longnose dace archived museum collections and information on the samples. Analyzed samples underlined.

Smithsonian Institute's National Museum of Natural History
Collection: USNM 4405 (8)
Accession #: 025440
Fixative: dried
Collected by: P. Macoun
Date: 1891
Location: cold and hot springs in Banff

University of Michigan Museum of Zoology
Collection: UMMZ 213828 (8)
Collected by: Eigenmann
Date: 1892
Fixative: 70% EtOH
Previous #: Indiana University (IU 4409)
30-34 mm SL

University of Michigan Museum of Zoology
Collection: UMMZ 219672
Fixative: Curator believes undoubtedly fixed in formalin and later transferred to 70% ETOH
Collected by:
Other notes: LD X BLD hybrid
Date: 1941
Previous # NMC 58-0226
Size: 25-38 mm
Field # Z219672

American Museum of Natural History
Collections: AMNH 5514 & 17368. Type and paratypes of R.c.s.
Size: (1) holotype 36.4 mm and paratypes (4) 23.4-37.1
Fixative: Curator believes formalin fixed b/c alcohol preserved fish have white eyes these do not but HI Smith initially preserved these specimens in 'alcohol'.
Collected by: HI Smith
Date: July 1915
Note: recording of a collection

Royal Ontario Museum
Collections: ROM 7113 or 1713
Size: 6 juvenile
Fixative: formalin

Collected by: E.H. Craigie
Date: June 1925
Other notes: All Alberta specimens in ROM formalin fixed.

National Museum of Natural Sciences, National Museum of Canada
Collections (number): NMC58-226 (84), NMC71-218 (16), NMC81-1159 (1), NMC81-1160 (1)
Collected by: various, JC Ward 1971, Lantienge & McAllister, Lantienge & McAllister
Date: 1920-1940, 1971, 1981, 1981

University of Alberta Museum
Collections: UAMZ 4613 (1), UAMZ 4614 (1), UAMZ 4615 (5)
Collected by: Nelson
Date: 1981

Canadian Museum of Nature
4 collections of *Rhinichthys cataractae smithi*
Fixative: initially fixed in 10% formalin, later transferred into 50% isopropanol. Since the late 1980's, transferred into 70% ethanol through graded series (30% ethanol, 50% ethanol and finally 70%).

The Natural History Museum
Collections: BMNH 1893.2.7.355-364, Banff longnose dace (10)
BMNH 1893.2.7.365-374 (10)
BMNH 1893.2.7.375-379 (10)
Fixative: 70% Industrial Methylated Spirit.
Other notes: curator indicated specimens are so old that preservation histories were not recorded, however, they most likely would have been previously fixed in formaldehyde.

Appendix II. Trace Element Microchemistry of Water Samples.

Element	Ca	Sr	Ba	Li	Mg	Zn	Mn
				ug/ml			
Location							
Bow (upstream Wolverine Ck.)	29.53	0.133	0.013	0.002	11.61	0.001	<0.001
Bow (upstream Wolverine Ck.)	29.49	0.130	0.011	0.004	11.97	0.003	<0.001
Bow (downstream WolverineCk)	31.99	0.126	0.012	0.003	13.54	0.001	<0.001
Bow (downstream WolverineCk)	33.00	0.126	0.013	0.002	13.52	0.001	<0.001
Bow (upstream C&B Marsh)	30.77	0.143	0.014	0.003	11.85	0.003	0.002
Bow (upstream C&B Marsh)	30.99	0.140	0.013	0.001	11.78	0.003	0.002
Bow (downstream C&B Marsh)	33.43	0.148	0.013	0.004	11.69	0.003	0.002
Bow (downstream C&B Marsh)	33.05	0.147	0.013	0.005	11.79	0.002	0.002
Wolverine Ck	50.85	0.113	0.017	0.005	17.48	0.003	<0.001
Wolverine Ck	51.03	0.118	0.016	0.003	17.42	0.003	<0.001
Cave&Basin outflow	288.21	2.044	0.031	0.040	53.47	0.001	0.012
Cave&Basin outflow	296.00	2.085	0.030	0.036	54.29	0.002	0.012
Cave&Basin inflow	341.09	2.512	0.027	0.042	60.92	0.004	<0.001
Cave&Basin inflow	345.70	2.534	0.027	0.041	61.75	0.004	<0.001

Appendix III. Pairwise haplotype divergence among *R. cataractae* and *R. atratulus* cytochrome *b* haplotypes (236 bp). Abbreviations for sample locations are: LCOL, Willamette River, OR; MCOL, Similkameen River, BC; UCOL, Upper Columbia River, near Cranbrook, BC; LTSH, Red Deer River system near Drumheller, AB; RUBY, Ruby Creek, Upper Missouri system, MT; MANI, Wilson Creek, MB; and QUEB, Quebec.

	C1	C2	C3	C4	C5	C6	LCOL	MCOL	UCOL
C1									
C2	0.004								
C3	0.004	0.009							
C4	0.009	0.004	0.004						
C5	0.004	0.009	0.009	0.013					
C6	0.009	0.013	0.013	0.017	0.013				
LCOL	0	0.004	0.004	0.009	0.004	0.009			
MCOL	0	0.004	0.004	0.009	0.004	0.009	0		
UCOL	0	0.004	0.004	0.009	0.004	0.009	0	0	
C7	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
C8	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
RUBY	0.058	0.063	0.063	0.067	0.063	0.058	0.058	0.058	0.058
C9	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
C10	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
C11	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
LTSH	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
I	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
II	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
III	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
IV	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
V	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
VI	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
VII	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
VIII	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
IX	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
X	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
XI	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
XII	0.058	0.063	0.063	0.068	0.063	0.058	0.058	0.058	0.058
MANI	0.053	0.058	0.058	0.063	0.058	0.053	0.053	0.053	0.053
QUEB	0.054	0.058	0.058	0.063	0.058	0.054	0.054	0.054	0.054
XIII	0.073	0.078	0.077	0.082	0.078	0.083	0.073	0.073	0.073
XIV	0.073	0.078	0.077	0.082	0.078	0.083	0.073	0.073	0.073
XV	0.068	0.073	0.073	0.077	0.073	0.078	0.068	0.068	0.068
BND1	0.141	0.147	0.146	0.152	0.147	0.152	0.141	0.141	0.141
BND2	0.131	0.137	0.136	0.142	0.137	0.142	0.131	0.131	0.131

Appendix III. Continued.

	C7	C8	RUBY	C9	C10	C11	LTSH	I	II
C1									
C2									
C3									
C4									
C5									
C6									
LCOL									
MCOL									
UCOL									
C7									
C8	0.004								
RUBY	0.009	0.004							
C9	0.022	0.026	0.03						
C10	0.022	0.026	0.03	0					
C11	0.022	0.026	0.03	0	0				
LTSH	0.022	0.026	0.03	0	0	0			
I	0.009	0.013	0.017	0.022	0.022	0.022	0.022		
II	0.009	0.013	0.017	0.022	0.022	0.022	0.022	0	
III	0.009	0.013	0.017	0.022	0.022	0.022	0.022	0	0
IV	0.009	0.013	0.017	0.022	0.022	0.022	0.022	0	0
V	0.009	0.013	0.017	0.022	0.022	0.022	0.022	0	0
VI	0.013	0.017	0.022	0.026	0.026	0.026	0.026	0.004	0.004
VII	0.009	0.013	0.017	0.022	0.022	0.022	0.022	0	0
VIII	0.009	0.013	0.017	0.022	0.022	0.022	0.022	0	0
IX	0.013	0.017	0.022	0.026	0.026	0.026	0.026	0.004	0.004
X	0.013	0.017	0.022	0.026	0.026	0.026	0.026	0.004	0.004
XI	0.013	0.017	0.022	0.026	0.026	0.026	0.026	0.004	0.004
XII	0.013	0.017	0.022	0.026	0.026	0.026	0.026	0.004	0.004
MANI	0.009	0.013	0.017	0.022	0.022	0.022	0.022	0.009	0.009
QUEB	0.009	0.013	0.017	0.022	0.022	0.022	0.022	0	0
XIII	0.044	0.049	0.053	0.058	0.058	0.058	0.058	0.054	0.054
XIV	0.044	0.049	0.053	0.058	0.058	0.058	0.058	0.054	0.054
XV	0.04	0.044	0.049	0.054	0.054	0.054	0.054	0.049	0.049
BND1	0.125	0.131	0.136	0.12	0.12	0.12	0.12	0.125	0.125
BND2	0.11	0.116	0.12	0.116	0.116	0.116	0.116	0.121	0.121

Appendix III. Continued.

	III	IV	V	VI	VII	VIII	IX	X	XI
C1									
C2									
C3									
C4									
C5									
C6									
LCOL									
MCOL									
UCOL									
C7									
C8									
RUBY									
C9									
C10									
C11									
LTSH									
I									
II									
III									
IV	0								
V	0	0							
VI	0.004	0.004	0.004						
VII	0	0	0	0.004					
VIII	0	0	0	0.004	0				
IX	0.004	0.004	0.004	0.009	0.004	0.004			
X	0.004	0.004	0.004	0.009	0.004	0.004	0		
XI	0.004	0.004	0.004	0.009	0.004	0.004	0	0	
XII	0.004	0.004	0.004	0.009	0.004	0.004	0	0	0
MANI	0.009	0.009	0.009	0.013	0.009	0.009	0.013	0.013	0.013
QUEB	0	0	0	0.004	0	0	0.004	0.004	0.004
XIII	0.054	0.054	0.054	0.058	0.054	0.054	0.049	0.049	0.049
XIV	0.054	0.054	0.054	0.058	0.054	0.054	0.049	0.049	0.049
XV	0.049	0.049	0.049	0.054	0.049	0.049	0.044	0.044	0.044
BND1	0.125	0.125	0.125	0.131	0.125	0.125	0.131	0.131	0.131
BND2	0.121	0.121	0.121	0.126	0.121	0.121	0.126	0.126	0.126

Appendix III. Continued.

	XII	MANI	QUEB	XIII	XIV	XV	BND1	BND2
C1								
C2								
C3								
C4								
C5								
C6								
LCOL								
MCOL								
UCOL								
C7								
C8								
RUBY								
C9								
C10								
C11								
LTSH								
I								
II								
III								
IV								
V								
VI								
VII								
VIII								
IX								
X								
XI								
XII								
MANI	0.013							
QUEB	0.004	0.009						
XIII	0.049	0.049	0.054					
XIV	0.049	0.049	0.054	0				
XV	0.044	0.044	0.049	0.004	0.004			
BND1	0.131	0.125	0.125	0.136	0.136	0.136		
BND2	0.126	0.12	0.121	0.131	0.131	0.131	0.082	

Appendix IV. Summary of morphological features and genetic data for archived longnose dace specimens from the Smithsonian Museum of Natural History (USNM) and the University of Michigan, Museum of Zoology (UMMZ). Haplotypes were based on mitochondrial DNA sequences and population assignments were determined from microsatellite loci.

Specimen	Classification	Rationale	Haplotype			Clade
			mtDNA	cyt*b*	CR	
USNM 44045-1	*R. c. smithi*	7 dorsal fin rays	---	C9	---	C
USNM 44045-2	*unknown*		---	C7	---	B
USNM 44045-3	*unknown*		---	C7	---	B
USNM 44045-4	*unknown*		M1	C1	D1	A
UMMZ 213828-5	*R. c. smithi*	Renaud & McAllister (1988)	---	C1	---	A
UMMZ 213828-7	*R. c. smithi*	Renaud & McAllister (1988)	---	C6	---	A
UMMZ 213828-P1	*unknown*	tissue piece	M1	C1	D1	A
UMMZ 213828-P2	*unknown*	tissue piece (2 fish)	---	C7 & C9	---	B / C

Appendix V. Longnose dace cytochrome *b* sequences.

C1

ATGCACTAGTCGACCTTCCAACCCCGTCTAATATTTCAGCGCTATGAAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTCCTAGCCATACATTATACCTCCGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGGGCATCATTCTTCTTTATCT
GTATTTACATACACATTGCCCGCGGCCTATACTACGGGTCGTACCTTTATAAGGAGACCTGAAATATCGGCGTTGTT
TTACTTCTCCTAGTCATAATAACAGCCTTCGTGGGCTATGTGCTCCCATGGGGACAAATATCTTTTTGAGGCGCTAC
CGTTATTACGAACCTACTATCAGCAGTGCCTTATATGGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C2

ATGCACTAGTCGACCTTCCAACCCCGTCTAATATTTCAGCGCTATGAAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTCCTAGCCATACATTATACCTCCGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGGGCATCATTCTTCTTTATCT
GTATTTACATACACATTGCCCGCGGCCTATACTACGGGTCGTACCTTTATAAGGAGACCTGAAATATCGGCGTTGTT
TTACTTCTCCTAGTCATAATAACAGCCTTCGTAGGCTATGTGCTCCCATGGGGACAAATATCTTTTTGAGGCGCTAC
CGTTATTACGAACCTACTATCAGCAGTGCCTTATATGGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C3

ATGCACTAGTCGACCTTCCAACCCCGTCTAATATTTCAGCGCTATGAAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTCCTAGCCATACATTATACCTCCGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGGGCATCATTCTTCTTTATCT
GTATTTACATACACATTGCCCGCGGCCTATACTACGGGTCGTACCTTTATAAGGAGACCTGAAATATCGGCGTTGTT
TTACTTCTCCTAGTCATAATAACAGCCTTCGTGGGCTATGTGCTCCCATGGGGACCAATATCTTTTTGAGGCGCTAC
CGTTATTACGAACCTACTATCAGCAGTGCCTTATATGGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C4

ATGCACTAGTCGACCTTCCAACCCCGTCTAATATTTCAGCGCTATGAAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTCCTAGCCATACATTATACCTCCGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGGGCATCATTCTTCTTTATCT
GTATTTACATACACATTGCCCGCGGCCTATACTACGGGTCGTACCTTTATAAGGAGACCTGAAATATCGGCGTTGTT
TTACTTCTCCTAGTCATAATAACAGCCTTCGTAGGCTATGTGCTCCCATGGGGACCAATATCTTTTTGAGGCGCTAC
CGTTATTACGAACCTACTATCAGCAGTGCCTTATATGGGTGACGCCCTCGTTCAGTGGATTTGAGGTGGCTT

C5

ATGCACTAGTCGACCTTCCAACCCCGTCTAATATTTCAGCGCTATGAAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTCCTAGCCATACATTATACCTCCGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGGATATACATGCTAACGGGGCATCATTCTTCTTTATCT
GTATTTACATACACATTGCCCGCGGCCTATACTACGGGTCGTACCTTTATAAGGAGACCTGAAATATCGGCGTTGTT
TTACTTCTCCTAGTCATAATAACAGCCTTCGTGGGCTATGTGCTCCCATGGGGACAAATATCTTTTTGAGGCGCTAC
CGTTATTACGAACCTACTATCAGCAGTGCCTTATATGGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C6

ATGCACTAGTCGACCTTCCAACCCCGTCTAATATTTCAGCGCTATGAAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTCCTAGCCATACATTATACCTCCGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGGGCATCATTCTTCTTTATCT
GTATTTACATACACATTGCCCGCGGCCTATACTACGGGTCGTACCTTTATAAGGAGACCTGAAATATCGGCGTTGTC
TTACTTCTCCTAGTCATAATAACAGCCTTCGTGGGCTATGTGCTCCCATGGGGACAAATATCTTCTTGAGGCGCTAC
CGTTATTACGAACCTACTATCAGCAGTGCCTTATATGGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C7

GCGCACTAGTTGACCTTCCAACCCCATCTAATATTTCAGCGCTATGGAACTTTGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTGTTTCTAGCCATACATTATACCTCCGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGAGCATCATTCTTCTTTATCT
GTATTTACATGCACATTGCCCGCGGCCTGTACTACGGGTCATACCTTTATAAGGAGACCTGAAATATTGGCGTTGTC
TTACTTCTTCTAGTTATGATGACAGCTTTCGTGGGCTATGTGCTCCCATGAGGACAAATATCTTTTTGAGGCGCTAC
CGTTATTACGAATCTACTATCAGCAGTACCTTATATGGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C8

GCGCACTAGTTGACCTTCCAACCCCATCTAATATTTCAGCGCTATGGAACTTTGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTGTTTCTAGCCATACATTATACCTCCGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGAGCATCATTCTTCTTTATCT
GTATTTACATGCACATTGCCCGCGGCCTGTACTACGGGTCATACCTTTATAAGGAGACCTGAAATATTGGCGTTATC
TTACTTCTTCTAGTTATGATGACAGCTTTCGTGGGCTATGTGCTCCCATGAGGACAAATATCTTTTTGAGGCGCTAC
CGTTATTACGAATCTACTATCAGCAGTACCTTATATGGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C9

GTGCACTAGTTGACCTTCCAACCCCATCTAATATTTCAGCGCTATGGAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTTCTGGCCATACATTATACCTCCGACATCTCAACTGCATTTTCGTCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGAGCATCATTCTTCTTTATCT
GTATTTACATGCACATTGCCCGCGGCCTGTACTACGGGTCATACCTTTATAAGGAGACCTGGAATATTGGCGTTGTC
TTGCTTCTTCTAGTTATAATGACAGCCTTCGTGGGCTATGTGCTCCCATGAGGACAAATATCTTTTTGAGGCGCCAC
CGTTATTACGAATCTACTATCAGCAGTACCTTATATAGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C10

GCGCACTAGTTGACCTTCCAACCCCATCTAATATTTCAGCGCTATGGAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTTCTGGCCATACATTATACCTCCGACATCTCAACTGCATTTTCGTCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGAGCATCATTCTTCTTTATCT
GTATTTACATGCACATTGCCCGCGGCCTGTACTACGGGTCATACCTTTATAAGGAGACCTGGAATATTGGCGTTGTC
TTGCTTCTTCTAGTTATAATGACAGCCTTCGTGGGCTATGTGCTCCCATGAGGACAAATATCTTTTTGAGGCGCCAC
CGTTATTACGAATCTACTATCAGCAGTACCTTATATAGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C11

GTGCACTAGTTGACCTTCCAACCCCATCTAATATTTCAGCGCTATGGAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTTCTGGCCATACATTATACCTCCGACATCTCGACTGCATTTTCGTCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGAGCATCATTCTTCTTTATCT
GTATTTACATGCACATTGCCCGCGGCCTGTACTACGGGTCATACCTTTATAAGGAGACCTGGAATATTGGCGTTGTC
TTGCTTCTTCTAGTTATAATGACAGCCTTCGTGGGCTATGTGCTCCCATGAGGACAAATATCTTTTTGAGGCGCCAC
CGTTATTACGAATCTACTATCAGCAGTACCTTATATAGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

C12

GTGCACTAGTTGACCTTCCAACCCCATCTAATATTTCAGCGCTATGGAACTTCGGATCCCTCCTAGGATTATGCTTA
ATTACTCAAATCCTGACAGGACTATTTCTGGCCATACATTATACCTCCGACATCTCGACTGCATTTTCGTCCGTAAC
ACACATCTGTCGAGACGTTAACTATGGCTGACTCATCCGGAATATACATGCTAACGGAGCATCATTCTTCTTTATCT
GTATTTACATGCACATTGCCCGCGGCCTGTACTACGGGTCATACCTTTATAAGGAGACCTGGAATATTGGCGTTGTC
TTGCTTCTTCTAGTTATAATGACAGCCTTCGTGGGCTATGTGCTCCCATGAGGACAAATATCTTTTTGAGGCGCCAC
CGTTATTACGAATCTACTATCAGCAGTACCTTATATAGGTGACGCCCTCGTCCAGTGGATTTGAGGTGGCTT

BND1

GTGCACTAGTCGACCTCCCAACACCATCTAACATTTCAGCGCTATGGAACTTCGGGTCCCTCCTGGGATTATGCTTA
ATTACTCAGATCCTAACAGGACTATTCCTAGCTATACATTATACCTCTGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGTGATGTAAATTATGGCTGACTCATTCGGAACATGCATGCCAACGGCGCATCATTCTTCTTTATCT
GTATTTACATGCACATTGCCCGCGGCCTCTACTACGGCTCATACCTTTATAAGGAAACCTGAAACATTGGCGTAGTT

CTACTTCTTCTGGTAATAATGACAGCCTTCGTGGGCTATGTGCTCCCATGAGGTCAAATGTCTTTTTGGGGGGCCAC
CGTAATCACAAATCTATTATCAGCAGTCCCCTATATGGGAGACACCCTTGTCCAGTGGATTTGAGGTGGCTT

BND2

GTGCGCTAGTCGACCTCCCAACACCATCTAATATCTCAGCGCTATGGAACTTCGGCTCCCTCCTGGGATTATGTTTA
ATTACCCAAATCCTAACAGGACTATTCCTAGCTATACATTATACCTCTGATATCTCAACTGCATTTTCATCCGTAAC
ACACATCTGTCGTGATGTAAATTATGGCTGACTCATTCGGAATATACATGCTAACGGCGCATCATTCTTCTTCATCT
GTATTTATATGCACATTGCCCGCGGCCTCTACTACGGCTCATACCTTTATAAAGAAACCTGAAATATTGGTGTAGTT
CTACTTCTTCTAGTTATGATGACGGCCTTCGTGGGCTATGTACTCCCATGGGGCCAAATGTCTTTTTGAGGCGCCAC
CGTAATTACAAATCTACTATCAGCAGTCCCCTATATGGGCGACACCCTTGTCCAGTGGATGTGAGGTGGCTT

Appendix VI. Longnose dace control region sequences

D1

CTGATAGTAACCTATATGGTCCGGTGCCGTGTATAGCATTACATGTGCACAGTACATATATATGGTCTAACACACAC
ATGATATTATTCGTAATATTGTGTGTGTGTGTGTTAGGGCATATATATGTATTATCACCATTCATTTATCTTAACCT
AAAAGCAAGTACTAACATCTAAGACGTACATAGAC

D2

CTGATAGTAACCTATATGGTCCGGTGCCGTGTATAGCATTACATGTGCACAGTACATATATATGGTCTAACACACAC
ATGATATTATTCGTAATATTGTGTGTGTGTGTGTTAGGGCATATATATGTATTATCACCATACATATATCTTAACCT
AAAAGCAAGTACTAACATCTAAGACGTACATAGAC

D3

CTGATAGTAACCTATATGGTCCGGTGCCGTATGTAGCATTACATGCGTACAGTACACATATATGGTCTAGCACACAC
ACGATATTATCCGTAATATTGTGTGTGTGTGTGTTAGGGCATATATATGTATTATCACCATACATATATCTTAACCT
AAAAGCAAGTACTAACATCTAAGACGTACATAGAC

D4

CTGATGGTAACCTATATGGTCCGGTGCCGTATGTAGCATTACATGCGTACAGTACACATATATGGTCTAGCACACAC
ACGATATTATCCGTAATATTGTGTGTGTGTGTGTTAGGGCATATATATGTATTATCACCATTCATTTATCTTAACCT
AAAAGCAAGTACTAACATCTAAGACGTACATAGAC

D5

CTGGTAGTAACCTATATGGTCCGGTGCCGTATGTAGCATTACATGCGTACAGTACACATATATGGTCTAGCACACAC
ACGATATTATCCGTAATATTGTGTGTGTGTGTGTTAGGGCATATATATGTATTATCACCATTCATTTATCTTAACCT
AAAAGCAAGTACTAACATCTAAGACGTACATAGAC

D6

CTGGTAGTAACCTATATGGTCCGGTGCCGTATGTAGCATTACATGCGTACAGTACACATATATGGTCTAGCACACAC
ACGATATTATCCGTAATATTGTGTGTGTGTGTGTTAGGGCATATATATGTATTATCACCATTCATTTATCTTAACCT
AAAAGCAAGTACTAACGTCTAAGACGTACATAGAC

BND1

CTGATAGTAACCTATATGGTTCCGTACCGTGTATAGTATTACATGTGTACAGTACTTATATATGGTCTAACGCAACA
CATAATATTATTCGTAATATTGTGTGTTGTGTTAGTGCATATATATGTATTATCACCATTCATTTATCTTAACCCAA
AAGCAAGTACTAACGTCTAAGACGTACATAAGC

BND2

CTGATAGTAACCAATATGGTTCGGTACCGTGTATAGTATTACATGTGTACAGTACTTATATATGGTCTAACGCAACA
CATAATATTCTTTGTAATATTGTGTGTTGTGTTAGTGCATATATATGTATTATCACCATTCATTTATCTTAACCCAA
AAGCAAGTACTAACGTCTAAGACGTACATACGC

Appendix VII. Longnose dace microsatellite fragment length polymorphisms.

Bow River

	Loci																	
	Rhca20		Rhca31		Lco3		Lco5		Ca12		Rhca16		Lco1		Lco4		Rhca24	
11	107	107	170	200	245	245	143	147	197	253	115	115	323	351	228	228	283	387
12	115	121	150	170	245	247	147	149	185	213	115	117	247	287	228	228	283	323
13	107	121	150	150	245	245	147	147	197	205	115	123	323	351	228	228	?	?
14	107	121	170	170	245	245	147	147	213	217	115	123	327	331	228	228	283	283
15	113	121	150	150	245	245	143	147	205	225	123	123	351	359	228	228	283	323
16	107	107	150	150	245	245	147	149	197	205	115	115	231	335	224	228	317	323
17	107	109	150	170	245	245	143	147	253	253	115	115	231	323	228	228	283	323
18	107	113	170	170	245	245	143	147	185	213	115	121	231	359	224	228	313	323
19	107	121	150	170	245	245	143	149	205	213	115	121	299	323	224	228	313	387
20	117	123	150	170	245	245	143	149	213	213	115	121	247	343	224	228	283	331
110	107	117	170	170	245	245	147	147	205	229	115	121	299	323	228	228	283	327
111	107	107	150	150	245	245	145	145	213	265	115	121	231	303	224	228	283	387
112	107	127	150	150	245	245	143	147	201	225	121	121	231	323	228	228	283	323
113	107	121	150	170	245	245	141	145	185	201	115	121	323	323	228	228	283	303
114	107	121	150	170	?	?	?	?	?	?	115	121	?	?	224	228	283	303
115	107	121	150	170	245	245	143	147	205	229	115	115	231	231	224	228	283	283
116	107	123	150	150	245	245	145	147	253	253	117	121	?	?	228	228	?	?
117	115	121	150	170	245	245	143	147	?	?	115	119	351	363	224	228	?	?
118	107	121	150	150	245	245	147	147	181	193	117	121	307	379	224	228	283	283
119	107	117	150	170	245	245	147	147	205	229	121	121	?	?	224	228	?	?
210	107	121	150	170	245	245	141	147	?	?	121	121	?	?	228	228	387	389
211	107	127	150	170	245	245	97	147	?	?	115	121	?	?	224	228	231	231
212	107	107	150	170	245	245	97	143	213	229	115	115	327	335	224	228	231	283
213	107	107	170	170	?	?	143	147	?	?	115	115	?	?	224	228	?	?
214	107	121	150	170	?	?	143	147	?	?	115	121	299	315	228	228	323	327
215	107	121	150	170	245	245	143	147	185	205	115	121	299	315	228	228	323	323
216	113	121	150	170	?	?	143	143	?	?	115	123	231	247	228	228	283	283
217	117	121	150	170	?	?	145	145	205	205	117	117	?	?	228	228	?	?
218	117	121	150	170	245	245	145	147	181	205	115	119	?	?	224	224	283	331
219	119	121	150	170	245	245	143	147	181	205	115	123	323	327	224	228	323	387
311	115	121	150	170	245	247	?	?	185	209	123	123	247	287	228	228	317	323
312	113	121	150	150	245	245	143	147	205	221	115	117	351	359	228	228	283	323

Appendix VII. Continued.

Cave & Basin

	Loci																	
	Rhca20		Rhca31		Lco3		Lco5		Ca12		Rhca16		Lco1		Lco4		Rhca24	
0	119	123	150	150	245	245	143	143	197	213	121	121	231	379	228	228	283	283
1	107	117	150	170	245	245	143	147	197	233	115	121	231	299	224	228	283	283
2	107	107	150	170	245	245	147	149	189	205	115	123	231	279	224	228	283	331
3	107	121	150	170	245	245	143	147	213	249	115	123	231	299	228	228	283	289
4	107	127	150	150	245	245	141	145	213	265	115	121	231	231	224	228	243	283
5	111	119	150	150	245	245	147	147	205	237	115	115	315	379	224	228	287	323
6	117	117	150	170	245	247	147	147	189	213	117	121	359	383	224	228	283	327
7	121	121	170	170	245	245	143	147	189	213	115	115	315	323	224	224	283	315
8	111	127	150	150	245	245	147	147	193	205	121	123	231	323	224	228	321	323
9	117	117	150	170	245	245	147	149	213	273	115	121	323	335	228	228	283	315
10	121	121	150	170	245	245	143	149	205	225	115	115	251	347	224	228	283	323
100	121	125	150	170	245	245	147	147	193	193	115	119	299	379	228	228	283	291
101	107	117	150	170	245	245	143	143	?	?	119	119	231	291	224	224	283	283
102	117	121	170	170	245	245	143	149	181	193	121	121	307	331	224	228	313	323
103	107	121	150	170	245	245	143	149	181	213	115	119	303	343	224	228	283	283
104	107	121	150	170	245	245	147	147	?	?	115	115	235	327	224	228	283	313
105	111	117	150	170	245	245	143	149	213	213	115	119	351	351	224	228	283	293
106	117	121	150	170	245	245	147	147	?	?	115	121	291	299	228	228	291	291
107	107	117	150	170	245	245	147	147	?	?	115	121	303	343	228	228	?	?
108	107	117	150	170	245	245	147	147	?	?	115	115	307	379	228	228	283	283
109	107	127	170	170	245	245	97	143	213	213	115	121	291	303	224	228	283	327
200	107	115	150	150	?	?	143	147	193	197	115	119	?	?	228	228	?	?
201	107	121	150	170	245	245	143	147	?	?	115	117	323	335	224	228	287	287
202	107	121	150	170	245	245	143	143	213	213	115	119	?	?	?	?	?	?
203	107	121	170	170	?	?	143	147	?	?	115	117	311	315	224	228	283	283
204	117	123	150	170	245	245	143	147	193	205	115	115	231	231	228	228	323	323
205	107	121	170	170	245	245	147	147	?	?	115	121	299	379	224	228	283	387
206	107	121	150	170	245	245	143	147	181	193	115	115	279	307	224	228	323	323
207	107	121	150	170	245	245	143	147	?	?	115	121	295	379	224	228	291	317
208	107	127	150	150	245	245	143	147	253	257	119	121	223	299	228	228	243	243
209	107	121	150	170	245	245	143	143	205	209	115	123	243	311	228	228	?	?
300	107	133	150	170	245	245	143	147	229	253	121	123	?	?	228	228	?	?
302	121	127	150	170	?	?	143	147	185	205	115	121	?	?	224	228	291	331

Appendix VII. Continued.

Callum Creek

	Loci																	
	Rhca20		Rhca31		Lco3		Lco5		Ca12		Rhca16		Lco1		Lco4		Rhca24	
21	105	127	150	150	245	245	143	143	245	273	115	115	295	351	228	228	301	301
22	97	107	150	150	245	245	143	147	189	245	117	121	283	287	228	228	331	331
23	105	117	150	150	245	245	139	147	189	205	115	115	327	347	224	228	365	385
24	107	117	150	150	245	245	143	147	205	229	119	121	299	375	226	228	283	283
25	107	117	?	?	245	247	141	143	193	225	117	117	291	303	224	228	283	283
26	117	117	150	150	245	245	143	143	189	261	115	121	299	307	228	228	317	317
27	107	131	150	150	239	245	147	147	185	261	117	121	247	311	226	228	323	323
28	107	107	150	150	245	247	141	143	233	249	115	117	307	331	226	228	303	383
29	107	117	150	150	245	247	143	143	197	265	117	121	295	323	228	228	303	323
120	115	121	150	170	245	245	145	149	197	265	117	121	255	307	224	228	331	383
121	107	107	150	150	245	245	143	143	233	249	117	121	247	287	228	228	243	243
122	107	107	150	170	245	247	143	143	185	209	117	121	287	311	224	228	327	329
123	105	111	150	170	239	245	143	147	193	209	117	121	?	?	228	228	283	283
124	107	115	150	170	245	245	143	147	193	209	115	115	271	303	228	228	323	383
125	105	121	150	170	245	245	143	149	229	273	121	121	247	339	226	228	283	283
126	107	107	150	150	245	245	147	147	209	209	117	117	223	235	228	228	283	303
127	107	107	150	150	245	245	147	147	205	205	115	117	243	247	226	228	283	283
128	119	119	150	150	245	245	143	143	185	185	115	117	267	323	228	228	331	331
129	107	121	150	150	245	245	143	147	197	197	117	121	267	267	224	228	327	331
220	?	?	150	150	245	247	143	143	229	229	121	121	283	303	228	228	?	?
221	117	121	150	150	239	245	143	147	253	265	117	123	243	247	226	228	323	331
222	115	117	150	170	245	245	145	147	193	205	115	117	247	287	228	228	?	?
223	107	121	150	150	245	245	143	143	201	229	117	121	247	299	228	228	283	385
224	97	107	150	170	245	245	143	143	193	233	119	121	247	271	228	228	307	319
225	107	107	150	150	245	245	147	149	197	289	117	117	247	323	228	228	317	319
226	117	123	150	170	245	245	143	147	229	229	117	121	303	303	228	228	283	319
227	101	107	150	150	245	245	143	143	209	253	117	121	?	?	226	228	283	323
228	107	107	150	170	245	245	143	143	185	189	117	121	283	283	224	226	323	323
229	117	121	150	150	245	245	141	143	185	229	115	121	239	323	224	228	283	283

Appendix VII. Continued.

Jumpingpound Creek

	Loci																	
	Rhca20		Rhca31		Lco3		Lco5		Ca12		Rhca16		Lco1		Lco4		Rhca24	
70	107	121	150	150	245	245	141	141	245	265	115	121	223	235	224	224	283	303
71	107	121	150	170	245	245	143	143	229	257	115	115	279	311	224	224	327	379
72	107	115	150	170	245	245	143	147	205	257	115	121	291	303	224	228	327	381
73	107	117	150	150	245	247	143	143	177	193	117	121	327	327	226	226	283	335
74	107	107	150	150	245	247	143	147	185	229	121	121	299	327	226	228	283	283
75	107	121	150	150	245	245	141	147	229	233	117	121	247	335	226	228	283	283
76	107	117	150	170	245	245	143	145	213	229	115	123	295	323	228	228	283	327
77	107	107	150	150	245	245	143	147	185	205	117	123	231	311	228	228	331	333
78	115	117	150	150	245	247	145	147	185	185	117	121	255	315	224	228	331	333
79	115	117	150	150	245	247	143	155	213	233	121	123	299	303	224	228	283	333
170	107	115	150	170	245	245	143	147	233	249	121	123	303	323	224	228	283	283
171	107	107	170	170	245	247	143	143	205	229	117	117	311	323	224	228	331	387
172	117	117	150	170	239	245	145	145	185	201	117	121	251	315	224	226	335	335
173	107	107	150	170	245	245	143	147	185	261	117	119	239	303	224	228	283	299
174	107	107	150	170	245	245	143	149	197	233	121	123	291	315	226	228	283	323
175	107	117	170	170	245	247	143	149	177	193	115	123	295	327	228	228	283	327
176	107	107	150	170	245	245	143	143	?	?	121	121	279	307	224	228	327	331
177	107	121	150	150	245	245	141	151	225	229	117	121	247	247	228	228	323	323
178	107	117	150	170	245	245	147	151	197	273	119	121	315	339	226	228	335	387
270	107	117	150	150	245	247	143	143	233	245	115	123	287	287	226	228	335	341
271	107	113	150	150	245	245	147	147	185	185	117	119	279	311	224	228	323	331
272	121	121	150	150	245	245	145	147	173	229	117	121	299	315	228	228	283	283
273	107	109	170	170	245	245	143	143	197	229	115	121	279	287	224	228	283	283
274	107	117	150	150	245	245	143	147	185	193	117	121	299	331	224	226	387	391
275	107	121	150	150	245	245	143	143	185	193	119	121	267	331	224	228	323	387
276	107	107	150	170	245	245	147	147	201	213	115	121	287	299	226	228	283	387
277	107	107	150	150	245	247	143	143	209	265	119	121	283	303	224	224	283	331
278	107	107	150	150	245	245	143	143	245	257	121	123	251	279	224	226	319	323
279	107	129	150	150	245	245	147	149	193	233	117	121	275	287	224	228	315	315

Appendix VII. Continued.

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	Loci															
	Rhca20		Rhca31		Lco3		Lco5		Ca12		Rhca16		Lco1		Lco4		Rhca24	
UMMZ 213828-5	107	121	150	170	245	245	97	97	185	185	115	115	?	?	228	228	?	?
USNM 44045-4	107	129	150	170	245	245	?	?	?	?	?	?	307	315	228	228	?	?
UMMZ 213828-7	107	119	150	170	245	245	97	97	?	?	?	?	?	?	228	228	?	?