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EXTIRPATION BY INTROGRESSION?: GENETIC EVIDENCE REVEALS HYBRIDIZATION BETWEEN INTRODUCED *CHRYSEMYS PICTA* AND ENDANGERED WESTERN PAINTED TURTLES (*C. P. BELLII*) IN BRITISH COLUMBIA

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***Abstract.*—Western Painted Turtles (*Chrysemys picta bellii*) are the only native species of freshwater turtle extant in Western Canada and are a species-at-risk in British Columbia (BC). Individuals with morphology consistent with non-native eastern North American subspecies have been recently detected in Burnaby Lake, an important stronghold for this listed species within BC. Here, we used mitochondrial and nuclear DNA sequence (CR and *c-mos*, respectively) and genotypic data (nine microsatellites) collected for Painted Turtles sampled in Burnaby Lake (n=39) and from across the range of *C. picta* (n = 73) to investigate whether individuals with atypical *C. p. bellii* morphology are non-native, and to reconstruct the source(s) and number of introduction events. We found 14 adult, morphologically atypical individuals are likely non-native based on the genetic evidence, exhibiting CR haplotypes, and *c-mos* and private microsatellite alleles that have not been otherwise detected in the western range of *C. p. bellii*. We are unable, however, to infer the subspecies or locality of origin based on the genetic data, but low levels of relatedness among non-native Painted Turtles suggest multiple sources of introduction. There was also evidence for introgression, as nine sampled juveniles and two morphologically native adults possessed varying combinations of non-native CR haplotypes, and non-*bellii* nuclear alleles. As the source of the non-native individuals has not been identified, monitoring of lakes across the BC Lower Mainland, including Burnaby Lake, should be on-going to readily identify introduction events and prevent future degradation of the genetic integrity of this listed species of high conservation value.**

Key Words.—*Chrysemys picta bellii*; hybridization; introgression; non-native; Painted Turtle

INTRODUCTION

The introduction and establishment of non-native species and individuals can impose severe impacts to native fauna, including niche displacement, competitive exclusion, interruption of mutualisms, and in extreme cases, extinction or extirpation (Mooney and Cleland 2001). If interbreeding with a resident form occurs, hybridization may lead to genetic introgression, potentially compromising a population from a conservation standpoint (Rymer and Simberloff 1996; Huxel 1999). Consequently, the detection of non-native and hybrid individuals is of the utmost importance for informing management action (Allendorf et al. 2001). For amphibians and reptiles, a common source of introduction of non-natives is through release of unwanted pets, particularly turtles (Krysko et al. 2011).

The Western Painted Turtle (*Chrysemys picta bellii*) is one of four overlapping subspecies of Painted Turtle distributed throughout North America (Ernst and Lovich 2009). At its northwestern range margin in British Columbia (BC), Canada, the Western Painted Turtle is listed as Endangered (COSEWIC 2006). It is the only extant native pond turtle found in Western Canada and for that reason, holds special ecological and cultural significance (COSEWIC 2006). In the Lower Mainland of BC, Burnaby Lake is an important stronghold of the species and is one of only two sites in the region where recruitment has been documented (Aimee Mitchell, pers. comm.). In 2010, volunteers observing nesting on a newly created artificial beach identified six females that displayed color patterns on their plastrons that are atypical of the native *C. p. bellii* (Fig. 1a and 1b) and more similar to non-native conspecifics (Aimee Mitchell, pers. comm.). As hybridization is common among subspecies of Painted Turtles (Weller et al. 2010), the presence of putative, non-native conspecifics in Burnaby Lake has led to concerns regarding genetic contamination within this

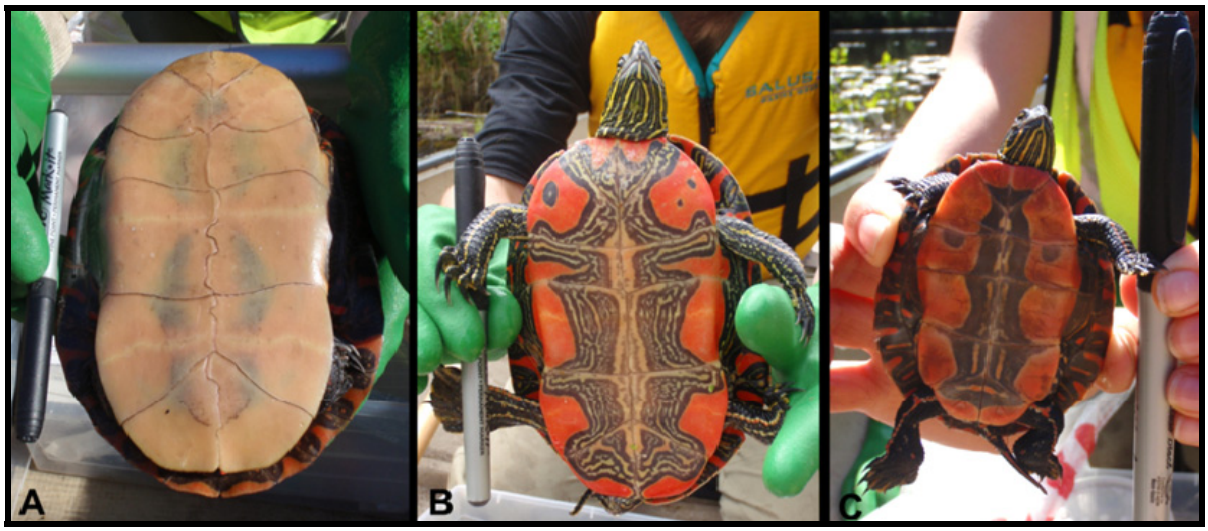

FIGURE 1. Morphology of sampled painted turtles. A. Turtle with atypical Western Painted Turtle (*Chrysemys picta bellii*) morphology sampled at Burnaby Lake, B. Example of a typical Western Painted Turtle, *C.* Juvenile sampled at Burnaby Lake with Western Painted Turtle morphology and a non-native control region haplotype. (Photographed by Anna Hall).

population of high conservation priority. In addition to diminishing the conservation value of the Burnaby Lake population, the introduction of non-native alleles may have fitness consequences if they are maladaptive to local conditions (Allendorf et al. 2001).

Genetic tools can be effective for investigating the presence and direction of hybridization, and degree of introgression, even in wide-ranging species (Gonzalez-Trujillo et al. 2012; Mikulicek et al. 2012; Vilaca et al. 2012). Here, we used mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) sequence data collected from across the range of *C. picta* to investigate whether individuals with atypical *C. p. bellii* morphology in Burnaby Lake are non-native, and to reconstruct their likely origin. Furthermore, we used nuclear microsatellite genotypic data to estimate relatedness among non-native individuals to infer whether introduction occurred from a single or multiple sources.

MATERIALS AND METHODS

Site and sampling.—Burnaby Lake is a large post-glacial lake in an urban setting within the city of Burnaby in the Lower Mainland of BC (Fig. 2). It is a popular lake for recreational activities and is easily accessible on all sides via a walking trail that circles its perimeter. From 2010 through 2013, we collected blood samples (~100µL) from the dorsal coccygeal vein from 30 adults (plastron length > 10 cm), and one juvenile turtle (individual 2–25, plastron length < 7 cm; Table 1). We stored blood in tubes containing a buffer solution (100 mM Tris-HCl, pH 8.0; 100 mM EDTA, pH 8.0; 10 mM NaCl; 0.5% SDS; Longmire et al. 1997) and the tubes were refrigerated until analysis. Of the adult turtles, 16 individuals qualitatively displayed typical *C. p. bellii* morphology whereas the other 14 displayed morphology that, to varying degrees, more closely resembled other subspecies of *C. picta* (Fig. 1). We also collected tissue samples from eight hatchling Painted Turtles from two nests (individuals H1-H8, Table 1) in 2013. We determined the sex of adults based on the length of the foreleg claws (Ernst and Lovich 2009) or by observing nesting (Table 1).

We used samples previously collected from turtles (n = 24) from 12 other lakes distributed across BC (Jensen et al. 2014) to represent province-wide genetic diversity of *C. p. bellii* (Fig. 2; Table 1). We obtained tissue samples from across North America from the archives of Dr. Bradley Shaffer (University of California, Los Angeles; n = 41) and the Queen's University Biological Station (n = 8) in Ontario which were used as range-wide references for the other subspecies (Fig. 2; Appendix Table 1). We extracted DNA from blood samples using the Nucleospin QuickBlood kit (Macherey-Nagel, Düren, Germany) following manufacturer's protocols. We then extracted DNA from tissue samples using the Nucleospin Tissue kit (Macherey-Nagel) following manufacturer's protocols.

Mitochondrial sequences.—We amplified a 668 base pair segment of mtDNA, including part of the control region (CR), as a single fragment using DES1 (Starkey et al. 2003) and Cp_CRExt (Jensen et al. 2014). Polymerase chain reactions (PCR) were carried out on a Veriti thermal cycler (Applied Biosystems, Foster City, California, USA) in 25 µL reactions

TABLE 1. Summary of putative status of sampled Burnaby Lake Western Painted Turtles (*Chrysemys picta bellii*) based on morphology, sex, and age class (where known), as well as mtDNA CR haplotype and nDNA alleles at specific positions on the *c-mos* oncogene and microsatellite loci. Shaded cells indicate *c-mos* alleles that are not found in BC Western Painted Turtles outside of Burnaby Lake. Alleles follow IUPAC conventions for nucleotides. Abbreviations are UK = unknown, F = female, M = male, § denotes CR haplotypes not native to BC, R = G and A, S = C and G, K = T and G, Y = C and T.

Location	ID	Morphology	Sex	Age Class	CR Haplotype	*c-mos* 127	*c-mos* 205	*c-mos* 250	*c-mos* 255	*c-mos* 281	*c-mos* 358	*c-mos* 436	MSat Alleles
Burnaby	2-01	Atypical	F	Adult	S.3§	T	R	C	G	T	T	Y	Non-BC
Burnaby	2-04	Atypical	F	Adult	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	2-09	Atypical	F	Adult	S.3§	Y	G	S	R	K	Y	C	Non-BC
Burnaby	2-12	Atypical	UK	Adult	S.3§	Y	R	S	G	T	Y	Y	Non-BC
Burnaby	2-13	Atypical	UK	Adult	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	2-14	Atypical	UK	Adult	S.3§	Y	R	S	G	T	Y	Y	Non-BC
Burnaby	2-15	Atypical	F	Adult	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	2-17	Atypical	F	Adult	S.3§	T	R	C	G	T	T	Y	Non-BC
Burnaby	2-18	Atypical	M	Adult	S.3§	T	R	C	G	T	T	Y	Non-BC
Burnaby	2-23	Atypical	F	Adult	S.4§	T	R	C	G	T	T	Y	Non-BC
Burnaby	2-24	Atypical	F	Adult	S.3§	T	R	C	G	T	T	Y	Non-BC
Burnaby	2-26	Atypical	UK	Adult	S.3§	Y	R	S	G	T	Y	Y	Non-BC
Burnaby	2-36	Atypical	UK	Adult	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	2-37	Atypical	UK	Adult	S.3§	T	A	C	G	T	T	T	Non-BC
Burnaby	2-02	*bellii*	F	Adult	B.2	T	G	C	G	T	T	C	
Burnaby	2-03	*bellii*	UK	Adult	B.2	T	G	C	A	G	T	C	
Burnaby	2-05	*bellii*	F	Adult	B.2	T	G	C	G	T	T	C	
Burnaby	2-06	*bellii*	F	Adult	B.2	T	G	C	G	T	T	C	
Burnaby	2-07	*bellii*	F	Adult	B.1	T	G	C	G	T	T	C	
Burnaby	2-08	*bellii*	F	Adult	B.1	T	G	C	G	T	T	C	
Burnaby	2-140	*bellii*	M	Adult	B.1	T	G	C	G	T	T	C	
Burnaby	2-19	*bellii*	M	Adult	B.1	T	G	C	G	T	T	C	
Burnaby	2-25	*bellii*	UK	Juvenile	S.3§	T	R	C	G	T	T	Y	Non-BC
Burnaby	2-27	*bellii*	M	Adult	B.1	Y	G	S	G	T	Y	C	Non-BC
Burnaby	2-28	*bellii*	UK	Adult	B.1	T	G	C	G	T	T	C	
Burnaby	2-29	*bellii*	F	Adult	B.2	T	G	C	G	T	T	C	
Burnaby	2-34	*bellii*	F	Adult	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	2-35	*bellii*	F	Adult	B.2	-	-	-	-	-	-	-	-
Burnaby	3-02	*bellii*	F	Adult	B.1	T	G	C	G	T	T	C	
Burnaby	3-05	*bellii*	M	Adult	B.1	T	G	C	G	T	T	C	
Burnaby	5-06	*bellii*	M	Adult	B.1	T	G	C	G	T	T	C	
Burnaby	H1	*bellii*	UK	Hatchling	S.3§	T	G	C	G	T	T	C	
Burnaby	H2	*bellii*	UK	Hatchling	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	H3	*bellii*	UK	Hatchling	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	H4	*bellii*	UK	Hatchling	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	H5	*bellii*	UK	Hatchling	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	H6	*bellii*	UK	Hatchling	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	H7	*bellii*	UK	Hatchling	S.3§	T	G	C	G	T	T	C	Non-BC
Burnaby	H8	*bellii*	UK	Hatchling	S.3§	T	G	C	G	T	T	C	Non-BC
BC Other		*bellii* (n = 24)	-	-	B.1, B.2, B.3, S.1	T	G	C	G	T	T	C	
USA		*bellii* (n = 20)	-	-	-	T	G	C	G	T	T	C	
USA		*dorsalis* (n = 6)	-	-	-	Y	R	S	R	T	Y	Y	
USA and Canada		*marginata* (n = 14)	-	-	-	Y	R	S	R	K	Y	Y	
USA		*picta* (n = 9)	-	-	-	Y	G	S	G	K	Y	C	

FIGURE 2. Map of *Chrysemys* sampling for which the *c-mos* oncogene was sequenced, including Burnaby Lake (star). Subspecies distributions are according to Conant and Collins (1991) and are indicated as follows: *C. p. bellii* (blue circles); *C. p. dorsalis* (red squares); *C. p. marginata* (green diamonds); and *C. p. picta* (purple triangles). A complete list of samples sequenced at *c-mos* is provided in Appendix Table 1.

containing ~20–40 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 7.5µg bovine serum albumin, 0.4µM each primer and 0.5 U Kapa *Taq* DNA Polymerase (Kapa Biosystems Inc., Woburn, Massachusetts, USA). Cycling conditions consisted of 95° C for two min, 35 cycles of 95° C for 30 s, 55° C for one min, 72° C for two min, and a final extension at 72° C for 10 min. We performed the sequencing reactions using BigDye v3.1 Terminator chemistry (Applied Biosystems) and these were run on an Applied Biosystems 3130XL DNA automated sequencer. We visualized sequences and edited them using Sequencher 5.0 (Gene Codes Corp., Ann Arbor, Michigan, USA). We downloaded exemplar CR sequences (n = 47) representing all known haplotypes from across North America and British Columbia from GenBank, including popsets from Starkey et al. (2003, accession number JQ963656) and Jensen et al. (2014, accession numbers KF554012 and KF554064), respectively. All the CR sequences were aligned in Geneious (Biomatters Ltd., Auckland, New Zealand) using Geneious Aligner (default settings). We generated a haplotype network using statistical parsimony as implemented in TCS (Clement et al. 2000).

Nuclear sequences.—We sequenced a 519 base pair segment of the nuclear oncogene *c-mos* (Saint et al. 1998) for all Burnaby Lake individuals (n = 39), as well as for the representative samples from across BC (n = 24) and throughout North America (n = 49) (Table 1; Appendix Table 1). We used the CMOSG77 primer from Saint et al. (1998) in conjunction with Cp_CMOSG78 (5'-AGGGTGATGTCAAAGGAGTA GATGTC-3'; this study) in 25 µL reactions containing ~20–40 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 7.5µg bovine serum albumin, 0.4µM each primer and 0.5 U KAPA Taq DNA Polymerase. We ran polymerase chain reactions on a Veriti thermal cycler; conditions consisted of 94° C for two min, 35 cycles of 95° C for 30 s, 55° C for 30 s, 72° C for 45 s, and a final extension at 72° C for seven min. We then performed sequencing reactions using BigDye v3.1 Terminator chemistry and run on an Applied Biosystems 3130XL DNA automated sequencer. Sequences were visualized and edited using Sequencher 5.0. The *c-mos* sequences were aligned using Geneious Aligner (default settings) and examined for polymorphisms.

Microsatellites.—We collected nuclear genotypic data for all Burnaby Lake individuals at nine microsatellite loci (Appendix Table 2; Pearse et al. 2001; Hauswaldt and Glenn 2003; King and Julian 2004; Gonçalves da Silva et al. 2009). All forward primers were 5'- tailed with a M13 sequence and used in combination with an M13 primer of the same sequence 5'-labeled with one of four fluorescent dyes

(6-FAM, VIC, NED, PET) to facilitate automated genotyping (Schuelke 2000). We carried out polymerase chain reactions on a Veriti thermal cycler in 12.5 µL reactions containing ~20–40 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 7.5µg bovine serum albumin, 0.04 µM of the M13-tailed forward primer, 0.4 µM each of the reverse primer and the M13 fluorescent dye-labeled primer, and 0.5 U of Ampli*Taq* Gold DNA polymerase (Applied Biosystems). Reaction conditions for all primer sets were optimized using a 'touchdown' cycling program that consisted of: 95° C for 10 min; 35 cycles of 95° C for 30 s, annealing for 30 s, and 72° C for 45 s; and a final step of 72° C for 7 min. We used two versions of the annealing step in the 'touchdown' program. The annealing temperature decreased 1° C each cycle from 59° C or 55° C until it reached 51° C or 45° C, respectively, at which point the remaining cycles continued with a 51° C or 45° C annealing temperature. Loci were co-loaded and run on an Applied Biosystems 3130XL DNA automated sequencer. We scored all alleles using bins in the software Genemapper 4.0 (Applied Biosystems). Bins were evaluated with the program Tandem (Matschiner and Salzburger 2009), and all allele calls were manually verified.

Previous research across a BC range-wide sampling of Western Painted Turtles indicated no evidence of linkage disequilibrium or null alleles at the sampled loci (Jensen et al. 2014), and consequently, were not tested again here. We assessed deviation from Hardy-Weinberg equilibrium (HWE) using exact tests, as implemented in GENEPOP 3.3 (Raymond and Rousset 1995; Rousset 2008). We corrected type I error rates for multiple comparisons using the sequential Bonferroni (Rice 1989). We calculated allelic diversity, observed and expected heterozygosities at each locus using ARLEQUIN (Excoffier et al. 2005). A list of private alleles was tabulated using GenAlEx (Peakall and Smouse 2006). We estimated pairwise relatedness (Queller and Goodnight 1989) among Burnaby Lake individuals in GenAlEx (Peakall and Smouse 2006).

RESULTS

Mitochondrial DNA haplotype variation and network analysis.—We detected four mtDNA CR haplotypes across BC, each different by a single base pair change (Fig. 3; haplotypes B.1, B.2, B.3, and S.1). All individuals identified as putatively non-native based on morphology had CR haplotypes common to eastern North America, differing from BC native haplotypes by at least four character state changes. Of those 14 individuals, 13 shared a single haplotype (haplotype S.3; Fig.3). The other morphologically atypical individual had a different haplotype not otherwise sampled in BC (haplotype S.4; Fig. 3). Haplotype S.4 is found in *C. p. bellii* individuals; however, it has previously only been detected within the eastern-most portion of the range in Wisconsin, Illinois and Minnesota (Jensen 2013). An adult that was morphologically identified as *C. p. bellii* (2-34) was also found to possess a non-native CR haplotype (S.3). Moreover, a juvenile turtle (2-25), morphologically identified as native (Fig. 1c) and all eight of the hatchling turtles exhibited the more common of the non-native CR haplotypes, S.3.

Nuclear c-mos variation.—We were not able to recover *c-mos* sequence from adult individual 2-35, which was subsequently left out of the nuclear DNA-based analyses. The *c-mos* oncogene was highly variable in Painted Turtles, with 20 variable sites distributed along the length of the 519 base pair sequence. Most of the *c-mos* alleles found in Western Painted Turtles were common across all of the subspecies of Painted Turtle. Consequently, these common alleles were not informative for assigning individuals to subspecies. However, there were some alleles that were not found in Western Painted Turtles that were found only in morphologically non-native Burnaby Lake individuals, and in morphologically native individual 2-27 (Table 1). There was one individual, Burnaby adult 2-03, that had alleles at two loci that were not found in any other individual in the North American dataset (Table 1). The hatchling turtles possessed alleles that were common across subspecies (Table 1).

Microsatellite genotypic variation and relatedness.—We were not able to recover genotypic data from adult individual 2-35, which was subsequently left out of the microsatellite analyses. Two loci were found to significantly deviate from HWE (TerpSH2 and TerpSH7); however this was not surprising given the hypothesis of mixed origin of individuals. Consequently, all nine microsatellite loci were retained in downstream analyses, with the final dataset including 5.0% missing data. Levels of observed and expected heterozygosities were 0.768 and 0.752, respectively, with an average of 9.6 alleles per locus.

We detected one or more private alleles not otherwise found across BC (Jensen et al. 2014) in the following samples: (1) all 15 adults and the single juvenile (2-25) with a non-native CR haplotype; (2) seven of the eight hatchlings; and (3) one morphologically native adult (2-27). The presence of these non-BC alleles likely accounts for the very high allelic diversity (9.6

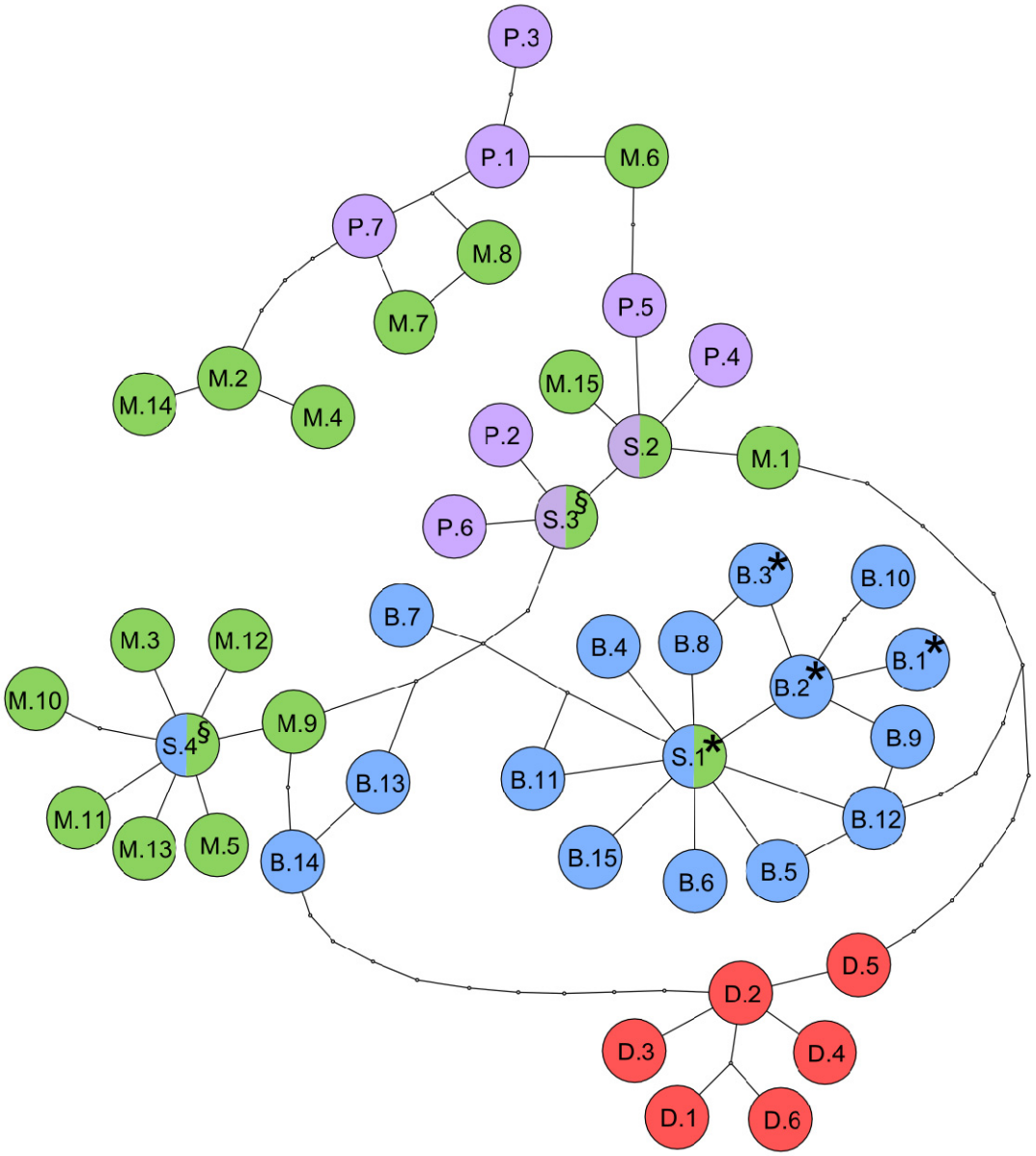

FIGURE 3. Haplotype network reconstructed based on the mtDNA control region data. Circles indicate detected haplotypes. Single colored circles are those found in only one subspecies. Dual colored nodes are those detected in more than one subspecies according to the following: *C. p. bellii* (blue); *C. p. dorsalis* (red); *C. p. marginata* (green); and *C. p. picta* (purple). Frequencies of the haplotypes are not indicated. Inferred but unsampled nodes are indicated as links in the network. Asterisks indicate haplotypes that were sampled in native individuals; § indicates haplotypes that were sampled in the morphologically non-native turtles in Burnaby Lake.

alleles per locus) detected in Burnaby Lake, relative to average levels (5.2 alleles per locus) previously found across the province for these loci (Jensen et al. 2014).

Average relatedness among morphologically atypical adult individuals was low (mean 0.03), ranging from -0.39 to 0.73. In contrast, average relatedness among hatchlings was high (mean 0.35) but variable, ranging from -0.03 to 0.86, and consistent with the hatchlings originating from two nests. Likewise, relatedness among morphologically native individuals ranged from -0.25 to 0.67 (mean 0.14). Average relatedness between morphologically atypical and native individuals was the lowest of all the comparisons (mean -0.164).

DISCUSSION

All 14 adult, morphologically atypical individuals are likely non-native in origin based on the genetic evidence, exhibiting a CR haplotype and, in most

cases, *c-mos* and private microsatellite alleles that have not been otherwise detected in the western range of *C. p. bellii*. We are unable, however, to infer the subspecies or locality of origin based on the genetic data. Although informative for the purposes of exclusion (i.e. BC native or non-native, Jensen 2013), there is not sufficient mtDNA CR differentiation to warrant continued recognition of *C. p. picta*, *C. p. marginata* and *C. p. bellii* as separate evolutionary lineages (Starkey et al. 2003), precluding identification to a specific taxonomic entity. Similarly, we did not find diagnostic variation within *c-mos* to enable inferences on origin, other than to exclude potential regional sources.

The putative non-native individuals have been observed nesting at Burnaby Lake, but it was previously unknown whether the offspring were progeny of two non-natives or whether they were hybrids. Here, we show genetic evidence of hybridization between the introduced non-natives and the local population of *C. p. bellii*. The juvenile turtle that was sampled is a hybrid exhibiting *C. p. bellii* morphology but a non-native mtDNA CR haplotype, non-*bellii c-mos* alleles, and non-BC microsatellite alleles. Moreover, the eight hatchlings sampled are also hybrids, as they display native morphology but all have non-native mtDNA CR haplotype S.3 and all but one (H1) have non-BC microsatellite alleles. These results indicate that genetic introgression is present in the younger cohorts of this population.

We also detected adult hybrids, as individual 2-34 and 2-27 are morphologically *C. p. bellii*, yet genetic evidence suggests mixed ancestry. Both individuals possess non-BC, private microsatellite alleles, with 2-34 having a non-native mtDNA CR haplotype and 2-27 exhibiting non-native *c-mos* alleles. As individual 2-27 has a native mtDNA CR haplotype but non-native nDNA alleles, we can conclude that there have been matings between non-native males and native females, in addition to the more commonly detected matings between non-native females and native males in the other hybrids. The majority of non-native individuals are genetically unrelated, likely indicating multiple introduction events from varied sources. This finding is consistent with a hypothesis of the release of unwanted pets as the mechanism of introduction, as has been reported for other turtle species in the BC Lower Mainland (Bunnell 2005) and around the world (Cadi and Joly 2004; Krysko et al. 2011), however, the source of introduced individuals at Burnaby Lake is currently unknown. It is also unknown when the first introductions occurred, but the presence of adult hybrids (plastron length > 10 cm) suggests it pre-dated the last five years at minimum.

Chrysemys subspecies do naturally hybridize where their ranges overlap (Weller et al. 2010), yet individuals involved in such crosses originate from the same geographical area and ecological conditions. As the non-native individuals at Burnaby Lake likely originated from eastern North America, they may possess gene variants maladapted to BC, increasing the probability of outbreeding depression (Allendorf et al. 2001). Frankham et al. (2011) provide a decision tree for predicting whether outbreeding depression is likely to occur when populations interbreed. In the case of Burnaby Lake, this decision tree recommends that non-native and native individuals be prevented from interbreeding due to the likelihood that populations from which the native and non-native turtles originated have been isolated without gene flow for at least 500 years (Starkey et al. 2003), the threshold advocated for expecting outbreeding depression (Frankham et al. 2011). As the Burnaby Lake population of Western Painted Turtles is one of only two in the region where recruitment has been documented, a decrease in fitness in that population could detrimentally affect the ability of the species to persist in the Lower Mainland of BC.

Some cases of apparent hybridization detected in this study resulted in individuals that have native morphology, indicating that genetic testing may be required to identify hybrids accurately. More than half of the individuals captured and sampled in this study were found to be non-native or hybrids, suggesting that they may constitute a high proportion of all Painted Turtles in Burnaby Lake. Further research is necessary in order to better quantify the extent of hybridization and introgression in this population, requiring increased sampling of individuals and demographic groups, especially juveniles and hatchlings.

Around the world, there is increasing documentation of native turtle populations becoming genetically polluted through hybridization with suspected released pet turtles (Fong and Chen 2010; Parham et al. 2013). In such cases, intensive management over a long period of time will be required to preserve the native gene pool. If applied to Burnaby Lake, the initial step would be to remove non-native and confirmed hybrid individuals. This would need to be followed by on-going genetic monitoring for the presence of non-native alleles for years or potentially decades afterwards given the long life span and potential for sperm storage in Painted Turtles (Pearse et al. 2001). Moreover, genetic monitoring should include both morphologically native and non-native individuals, given our findings of cryptic introgression. Additional introduction events could occur in the future, as the source of non-native individuals in Burnaby Lake has yet to be determined. Monitoring of lakes across the BC Lower Mainland, including Burnaby Lake, should be on-going to promptly identify introduction events

and prevent future degradation of the genetic integrity of this listed species of high conservation value.

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APPENDIX TABLE 1. Source and locality information for *Chrysemys* samples for which *c-mos* was sequenced.

Voucher	State/ Province	Region	Subspecies
BE.136	BC	Vancouver Island	*bellii*
BE.137	BC	Vancouver Island	*bellii*
BE.17	BC	Vancouver Island	*bellii*
Burn.21	BC	Lower Mainland	*bellii*
Burn.212	BC	Lower Mainland	*bellii*
Burn.213	BC	Lower Mainland	*bellii*
Burn.214	BC	Lower Mainland	*bellii*
Burn.2140	BC	Lower Mainland	*bellii*
Burn.215	BC	Lower Mainland	*bellii*
Burn.217	BC	Lower Mainland	*bellii*
Burn.218	BC	Lower Mainland	*bellii*
Burn.219	BC	Lower Mainland	*bellii*
Burn.22	BC	Lower Mainland	*bellii*
Burn.223	BC	Lower Mainland	*bellii*
Burn.224	BC	Lower Mainland	*bellii*
Burn.225	BC	Lower Mainland	*bellii*
Burn.226	BC	Lower Mainland	*bellii*
Burn.227	BC	Lower Mainland	*bellii*
Burn.228	BC	Lower Mainland	*bellii*
Burn.229	BC	Lower Mainland	*bellii*
Burn.23	BC	Lower Mainland	*bellii*
Burn.234	BC	Lower Mainland	*bellii*
Burn.235	BC	Lower Mainland	*bellii*
Burn.236	BC	Lower Mainland	*bellii*
Burn.237	BC	Lower Mainland	*bellii*
Burn.24	BC	Lower Mainland	*bellii*
Burn.25	BC	Lower Mainland	*bellii*
Burn.26	BC	Lower Mainland	*bellii*
Burn.27	BC	Lower Mainland	*bellii*
Burn.28	BC	Lower Mainland	*bellii*
Burn.29	BC	Lower Mainland	*bellii*
Burn.32	BC	Lower Mainland	*bellii*
Burn.35	BC	Lower Mainland	*bellii*
Burn.56	BC	Lower Mainland	*bellii*
Burn.H1	BC	Lower Mainland	*bellii*
Burn.H2	BC	Lower Mainland	*bellii*
Burn.H3	BC	Lower Mainland	*bellii*
Burn.H4	BC	Lower Mainland	*bellii*
Burn.H5	BC	Lower Mainland	*bellii*
Burn.H6	BC	Lower Mainland	*bellii*
Burn.H7	BC	Lower Mainland	*bellii*
Burn.H8	BC	Lower Mainland	*bellii*
Cran.51	BC	Sunshine Coast	*bellii*
GBHNR.1	BC	Lower Mainland	*bellii*
OF.26	BC	Okanagan	*bellii*
OF.27	BC	Okanagan	*bellii*
OF.29	BC	Okanagan	*bellii*
OF.30	BC	Okanagan	*bellii*
PA.24	BC	Vancouver Island	*bellii*
PA.28	BC	Vancouver Island	*bellii*
RE.1	BC	Okanagan	*bellii*
RE.2	BC	Okanagan	*bellii*
RE.3	BC	Okanagan	*bellii*
RE.4	BC	Okanagan	*bellii*
RV.32	BC	Thompson	*bellii*
RV.85	BC	Thompson	*bellii*
RV.98	BC	Thompson	*bellii*
SL.33	BC	Lower Mainland	*bellii*
SL.37	BC	Lower Mainland	*bellii*
TI.113	BC	Texada Island	*bellii*
TI.23	BC	Texada Island	*bellii*
WE.318	BC	Sunshine Coast	*bellii*
WE.34	BC	Sunshine Coast	*bellii*
HBS27533	CO	Douglass	*bellii*
HBS28620	CO	La Plata	*bellii*
HBS28303	ID	Boundary	*bellii*
HBS28134	KS	Sherman	*bellii*
HBS23616	MN	Houston	*bellii*
HBS23629	MN	Houston	*bellii*
HBS28277	MT	Lewis and Clark	*bellii*
HBS27748	ND	Rolette	*bellii*
HBS28625	NM	San Juan	*bellii*
HBS28644	NM	Apache	*bellii*
HBS28177	SD	Bennet	*bellii*
HBS27461	WA	Grant	*bellii*
HBS27466	WA	Grant	*bellii*
HBS27476	WA	Grant	*bellii*
HBS27486	WA	Okanogan	*bellii*
HBS27495	WA	Okanogan	*bellii*
HBS27502	WA	Spokane	*bellii*
HBS27513	WA	Spokane	*bellii*
HBS29249	WA	Clark	*bellii*
HBS23656	WI	Lincoln	*bellii*
HBS27284	AL	Marshall	*dorsalis*
HBS27288	AL	Marshall	*dorsalis*
HBS23303	AR	Lonoke	*dorsalis*
HBS23169	IL	Alexander	*dorsalis*
HBS23170	IL	Alexander	*dorsalis*
HBS31533	LA	St. Martin	*dorsalis*
HBS23365	IN	Kosciusko	*marginata*
HBS27248	KY	Franklin	*marginata*
HBS28026	NH	Sullivan	*marginata*
HBS28001	NY	Clinton	*marginata*
HBS27179	OH	Shelby	*marginata*
HBS27185	OH	Shelby	*marginata*
ONT.1	ON	Frontenac	*marginata*
ONT.3	ON	Frontenac	*marginata*
ONT.4	ON	Frontenac	*marginata*
ONT.5	ON	Frontenac	*marginata*
ONT.6	ON	Frontenac	*marginata*
ONT.7	ON	Frontenac	*marginata*
ONT.8	ON	Frontenac	*marginata*
ONT.9	ON	Frontenac	*marginata*
HBS26213	GA	Jackson	*picta*
HBS26058	MA	Middlesex	*picta*
HBS33050	MA	Worcester	*picta*
HBS28035	ME	Kennebec	*picta*
HBS28041	ME	Kennebec	*picta*
HBS28049	NH	Salem	*picta*
HBS31658	NJ	Sussex	*picta*
HBS27581	NY	Sufflolk	*picta*
HBS23658	RI	Kent	*picta*

APPENDIX TABLE 2. Characteristics of the nine microsatellite loci used in this study.

Locus	**Primer Sequences (5'-3')**	**Repeat Unit**	**Allele Size Range (bp)**	**PCR Method**	**M13 Label**	**Source**
CpGT108	CCTAGAAAGTAAGAACCAATTTCAG CCACCAACAGAAGGAAGTTAGTG	di-nucleotide (CA)4CT(CA)11	230–328	TD59-51	VIC	Goncalves da Silva et al. (2009)
CpGT124	TCGGGGAGCACACTATACC CTCAGCCCCAAAATGAAC	di-nucleotide (GT)31(GC)5	193–235	TD59-51	PET	Goncalves da Silva et al. (2009)
Cp3	ATCTTTAAGTCTGTGAACTTCAGGG CTGTCTCATGCAAAGCTGGTAG	di-nucleotide GT	150–182	TD55-45	NED	Pearse et al. (2001)
TerpSH2	TGGCCAGCAGGAGTAATG CTATTAGGGCAGAGACGAG	tetra-nucleotide AGAT	168–216	TD59-51	PET	Hauswaldt and Glenn (2003)
TerpSH3	TCCCCCAATGCACAC CTGCCCAATCCATTTAGA	tetra-nucleotide CAAA	287–320	TD55-45	FAM	Hauswaldt and Glenn (2003)
TerpSH7	CACACACACTGTATTTTGATA CTATGCCCTTTCTAGTTTG	tetra-nucleotide AGAT	121–168	TD59-51	VIC	Hauswaldt and Glenn (2003)
GmuD21	GCAGTTAGGCATTACTCAACATC AGGGTATGAATACAGGGGTGTC	tetra-nucleotide ATCT	166–222	TD55-45	VIC	King and Julian (2004)
GmuD28	AGCTGTTTGTCATCATACACTCTC TGGCCCTCATGTTTTATAAGTG	tetra-nucleotide ATCT	238–274	TD59-51	NED	King and Julian (2004)
GmuD62	GGTGGTATAGAAAATCCTAAAATGG GTGCAAACTGTCTGGAAATAGG	tetra-nucleotide ATCT	171–211	TD59-51	PET	King and Julian (2004)