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ARTICLE

Genomic Analysis Reveals Genetic Distinctiveness of the Paiute Cutthroat Trout *Oncorhynchus clarkii seleniris*

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Abstract

The Paiute Cutthroat Trout (PCT) *Oncorhynchus clarkii seleniris* is classified as a subspecies within the greater Cutthroat Trout *O. clarkii* ssp. complex and is federally listed as threatened under the Endangered Species Act. However, genetic studies to date have revealed very little genetic differentiation between the PCT and its closest relative, the Lahontan Cutthroat Trout (LCT) *O. clarkii henshawi*. These results casted doubt on whether the PCT is a genetically distinct subspecies or merely a phenotypic variant of the LCT. Here, we present a genomic analysis

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of Cutthroat Trout subspecies and populations to resolve the genetic and phylogenetic relationship between PCT and LCT. Our results demonstrate substantial genetic structure and differentiation between PCT and LCT populations. In contrast to current thinking, our phylogenetic reconstructions show the PCT to be a distinct evolutionary lineage that diverged from LCT before the LCT differentiated into its current populations (i.e., rather than PCT divergence due to geographic isolation from an LCT population in the Carson River). We conclude that the PCT is genetically distinct from the LCT.

The Cutthroat Trout *Oncorhynchus clarkii* subspecies complex represents an iconic and often heavily managed game species. The subspecies complex displays broad diversity in occupied habitat types, morphology, and life history strategies (Behnke 2002; Trotter 2008). Although well studied, Cutthroat Trout have a complicated phylogeographic history due to historical (multiple glacial retreats and re-invasions) and modern (anthropogenic movement, hybridization with nonnative species, and extirpation) events. This phylogeographic history presents a challenge for understanding relationships between Cutthroat Trout populations and subspecies designations throughout the American West. Genetic methods allow for an understanding of these relationships and have management implications beyond designating conservation units. For example, the threatened Greenback Cutthroat Trout *O. clarkii stomias* was thought to be on a recovery trajectory until a genetic analysis determined that many populations of conservation focus were actually Colorado River Cutthroat Trout *O. clarkii pleuriticus*, which had been translocated across the Continental Divide in the late-19th and early 20th centuries (Metcalf et al. 2007). These findings meant that there were fewer Greenback Cutthroat Trout populations than assumed by managers and thus a higher risk of extinction than previously thought.

One case in which a better understanding of the relationship between subspecies could benefit management involves two closely related subspecies: the Paiute Cutthroat Trout (PCT) *O. clarkii seleniris* and the Lahontan Cutthroat Trout (LCT) *O. clarkii henshawi*, both of which are federally listed as threatened under the Endangered Species Act (ESA) of 1973, as amended. The entire native range of the PCT lies within a 17.8-km stretch of Silver King Creek (SKC), which empties into the East Fork Carson River (USFWS 2013), the latter of which comprises a portion of the LCT's native range (Figure 1). Conversely, populations of LCT occupy a substantial portion of the Lahontan hydrographic basin, which covers northern Nevada, northeastern California, and southeastern Oregon (Truckee, Carson, Walker, Reese, Quinn, Humboldt, and Willow–Whitehorse River drainages). This area encompasses a diverse array of habitats (lakes, large rivers, and small streams), and the U.S. Fish and Wildlife Service (USFWS) has designated three geographic management units for LCT: (1) Western Lahontan Basin (Truckee, Carson, and Walker River watersheds); (2) Northwestern Lahontan Basin (Quinn River, Black Rock Desert, and Coyote Lake watersheds); and (3) Eastern Lahontan Basin (Humboldt River and tributaries; Figure 1; USFWS 2009).

Unlike all other Cutthroat Trout subspecies, PCT have few or no body spots (Snyder 1933, 1934); they were designated as a distinct subspecies based on this unique phenotype. Subsequently, using gill raker counts, Behnke and Zarn (1976) estimated that PCT diverged from the East Fork Carson River population of LCT roughly 5–8 thousand years ago (kya). Indeed, recent genetic studies revealed relatively little genetic differentiation between LCT and PCT (Peacock and Kirchoff 2007; Finger et al. 2009; Loxterman and Keeley 2012; Pritchard et al. 2012). However, there are few to no published studies exploring both the genetic structure and the phylogenetic relationship between LCT (rangewide) and PCT in any detail.

There are two major reasons for the lack of a robust phylogenetic analysis of PCT and LCT. First, both groups have an extensive history of anthropogenic manipulation. Sheepherders introduced PCT into fishless waters upstream of historical habitat in 1912 (Ryan and Nicola 1976). Beginning in the 1920s, nonnative trout were stocked into SKC, leading to the extirpation of the PCT from its historical range (Ryan and Nicola 1976). Since then, a series of translocations and chemical treatments has led to the present configuration of nine PCT refuge populations (four out-of-basin populations and five populations within the SKC watershed above fish barriers; Table 1). These translocated populations now represent all that remains of the genetic variation from the historical population, which may itself have had little genetic diversity. The LCT has also been widely translocated, has suffered substantial reduction in occupied habitat, and has been impacted by the stocking of nonnative fish throughout its range over the past 120 years (USFWS 1995, 2009). Second, molecular studies to date have not been designed specifically to examine the phylogenetic relationship between LCT and PCT, and most have used genetic markers representing only a small portion of the genome. The previous genetic studies suffered from ascertainment bias (Pritchard et al. 2012), lacked comprehensive sampling (Loxterman and Keeley 2012), focused specifically on introgression of PCT or LCT with Rainbow Trout *O. mykiss* or Golden Trout *O. clarkii aguabonita* sp. (Busack and Gall 1981; Cordes et al. 2004; Finger et al. 2009), or evaluated differentiation between LCT groups and only included the PCT as an outgroup (Nielsen and Sage 2002; Peacock and Kirchoff 2007).

Here, we present a genomic analysis of Cutthroat Trout subspecies and populations based on a next-generation sequencing approach (restriction-site-associated DNA sequencing [RAD sequencing]), with the goal of determining overall patterns of genetic structure between PCT and LCT and at the same time reconstructing a phylogeny to clarify evolutionary relationships between PCT and LCT.

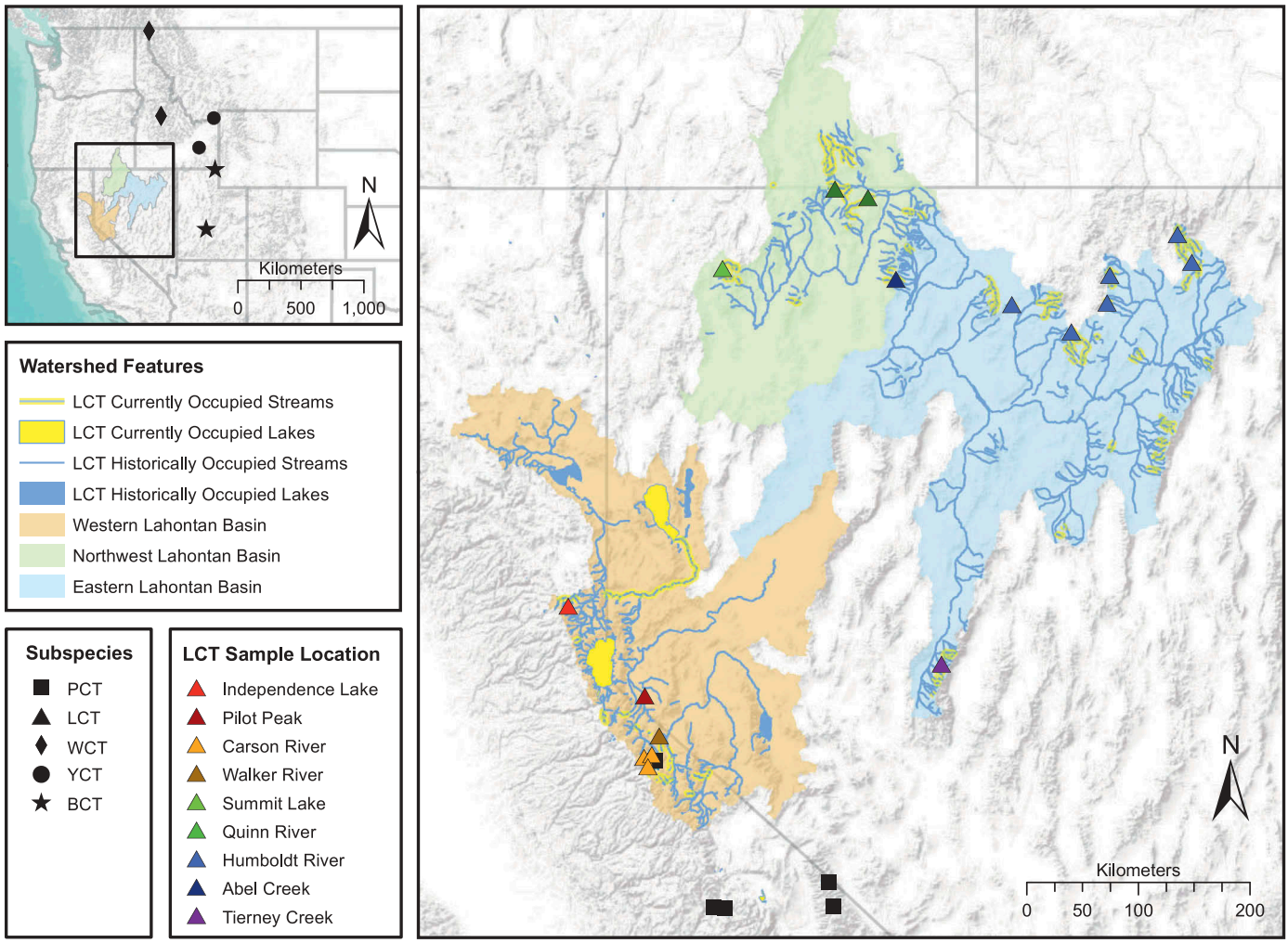

FIGURE 1. Map of the study area (California, Nevada, Utah, Idaho, and Oregon), where Cutthroat Trout samples were taken (PCT = Paiute Cutthroat Trout; LCT = Lahontan Cutthroat Trout; WCT = Westslope Cutthroat Trout; YCT = Yellowstone Cutthroat Trout; BCT = Bonneville Cutthroat Trout).

Our results provide evidence for substantial genetic structure and differentiation between PCT and LCT populations, and independent phylogenetic reconstructions show the PCT to be a distinct evolutionary lineage within the LCT and that the PCT is basal to all other groups of LCT. Therefore, in contrast to previous results, we conclude that the PCT is genetically distinct from the LCT.

METHODS

Sample Collection and Restriction-Site-Associated DNA Sequencing

The California Department of Fish and Wildlife collected individual fin clip samples from each of the nine existing PCT refuge populations. The Idaho Department of Fish and Game provided samples from three additional Cutthroat Trout groups: four individuals each from Yellowstone Cutthroat Trout (YCT) *O. clarkii bouvieri*, Westslope Cutthroat Trout (WCT) *O. clarkii lewisi*, and Bonneville Cutthroat Trout (BCT) *O. clarkii utah*. Finally, we added a number of LCT samples (collected by M. Peacock) from throughout the LCT's range (Figure 1; Table 1). The DNA was extracted using the Qiagen DNeasy extraction kit via the manufacturer's protocol. After extraction, genomic data were generated by single-end sequencing using the *Sbf*I restriction enzyme in accordance with the protocols given by Miller et al. (2007).

Bioinformatics Pipeline and Alignment to the Rainbow Trout Genome

The RAD sequences were sorted into individuals based on unique, 8-bp barcodes (using custom Perl scripts; available from the authors upon request). Sequences were aligned to the Rainbow Trout reference genome (Berthelot et al. 2014) by using the Burrows–Wheeler aligner (BWA)–

TABLE 1. Cutthroat Trout samples included in restriction-site-associated DNA sequencing libraries. Information includes subspecies, analysis group code, creek or lake, the general area or drainage of sample locations, year sampled, and number (*N*). Western, Eastern, and Northwestern refer to the three Lahontan Basin management units for LCT (see Figure 1).

Subspecies	Analysis group code	Creek or lake	General area/drainage/basin	Year sampled	*N*
Paiute Cutthroat Trout	PCT	Cabin Creek	Mono County, California (CA)	2000	2
	PCT	Corral Valley Creek	Silver King Creek/Western	2000	2
	PCT	Coyote Valley Creek	Silver King Creek/Western	2000	2
	PCT	Stairway Creek	Madera County, CA	2000	2
	PCT	Sharktooth Creek	Fresno County, CA	2000	2
	PCT	North Fork Cottonwood Creek	Mono County, CA	2000	2
	PCT	Four Mile Canyon Creek	Silver King Creek/Western	2000	2
	PCT	Fly Valley Creek	Silver King Creek/Western	2000	2
	PCT	Upper Silver King Creek	Silver King Creek/Western	2000	2
Lahontan Cutthroat Trout	INDL	Independence Lake	Truckee River/Western	2001	23
	CARS	East Fork Carson River	Carson River/Western	2001	2
	CARS	Poison Flat Creek	Carson River/Western	2001	2
	CARS	Murray Canyon Creek	Carson River/Western	2001	2
	PPKS	Pilot Peak Strain	Truckee River/Western	2008	4
	WALK	Slinkard Creek	Walker River/Western	2001	2
	SUML	Summit Lake	Northwestern	2001	22
	QUIN	Line Canyon Creek	Quinn River/Northwestern	2001	2
	QUIN	Washburn Creek	Quinn River/Northwestern	2001	2
	HBCT	Gance Creek	Humboldt River/Eastern	2000	21
	HBCT	Foreman Creek	Humboldt River/Eastern	2001	2
	HBCT	Frazer Creek	Humboldt River/Eastern	2000	2
	HBCT	Tierney Creek	Reese River/Eastern	2000	2
	HBCT	Beaver Creek (Maggie)	Humboldt River/Eastern	2001	2
	HBCT	West Marys River	Humboldt River/Eastern	2001	13
	HBCT	Main-stem Mary's River	Humboldt River/Eastern	2000	11
	HBCT	Abel Creek	Little Humboldt River/Eastern	2001	2
Yellowstone Cutthroat Trout	YCT	Henrys Lake	Upper Snake River/Henry's Fork, Idaho (ID)	1998	2
	YCT	Blackfoot River	Upper Snake River/Blackfoot, ID	2002	2
Westslope Cutthroat Trout	WCT	Cannuck Creek	Moyle River/Kootenai drainage, ID	2005	2
	WCT	Garden Creek	Main Fork Salmon River drainage, ID	2002	2
Bonneville Cutthroat Trout	BCT	Glenwood Fish Hatchery	Sevier River, Utah (UT)	2004	2
	BCT	Bear Lake	Bear Lake, ID/UT	2003/2004	2
Total					162

minimal exact match (MEM) algorithm in BWA software (Li and Durbin 2009) and were outputted as Sequence Alignment/Map (SAM) files. Alignment success was around 70%, and the raw read counts, number of aligned reads, and alignment success of each individual are provided in Supplementary Table S.A.1 (available in Supplement A in the online version of this manuscript). Sorted and indexed Binary Alignment/Map (BAM) files used in all downstream analyses were created from these SAM files by using SAMtools (Li and Durbin 2009; Li 2011). All subsequent analyses were conducted on sites that passed a minimum phred quality score of 20 (Q20). We used the program ngsParalogs (https://github.com/tplinderoth/ngsParalog) to find and tag paralogous and duplicate sites. Any RAD

locus containing a paralogous or duplicate site was removed before further analysis.

Genetic structure among and between Cutthroat Trout subspecies: principal components analysis.—To determine overall genetic structure, we conducted two principal components analyses (PCAs): one that included all Cutthroat Trout subspecies and one that examined only PCT and LCT populations. To reduce bias created by differences in coverage between individuals, we subsampled all individuals to 500,000 reads (the lowest number of reads for any individual included in the PCA) by using SAMtools. Per-site minor allele frequencies and genotype probabilities were estimated in ANGSD (Analysis of Next-Generation Sequencing Data; Korneliussen et al. 2014). Minimum minor allele frequency was set to 0.05, genotypes were called at 99% posterior probability, and only sites that were genotyped in at least half of the individuals were used. Genetic covariance matrices between individuals were calculated by using the ngsCovar module of ngsTools (Fumagalli et al. 2014); principal component (PC) axes summarizing population structure were derived from the matrices by classic eigenvalue decomposition and were visualized using the ggplot2 package in R (R Development Core Team 2013). The PCA results, population history, and geographic information were used to group individuals for further downstream analysis.

Genetic structure among and between Cutthroat Trout subspecies: phylogenetic analysis.—For each individual, we created a separate FASTA file containing consensus sequences of the reference genome using individual alignment files (i.e., BAM files). Consensus sequences were built by using individual read depths to call for the most common base at a site using ANGSD, which accounts for uncertainties presented by low-coverage data. Bases with a quality score below 13 on the Phred 33 scale were disregarded. In case of ties, a random base was chosen from among the bases with the highest counts. To obtain homologous genomic regions suitable for phylogenetic studies, RAD sequences mapped to the same scaffold in each individual were concatenated according to genomic position. However, we did not further concatenate the RAD loci into one super matrix (i.e., one single sequence per individual) because empirical studies have shown that concatenation methods for multilocus sequence data can result in misleading phylogenies since gene tree heterogeneity is not considered (Song et al. 2012). This problem can be especially important for shallow phylogenies containing multiple closely related species or populations in which incomplete lineage sorting will undoubtedly result in major gene tree heterogeneity (Carstens and Knowles 2007; Knowles and Carstens 2007).

Therefore, in this study, instead of employing the more widely used concatenation (i.e., super-matrix) methods, we used a coalescence-based method to estimate the species phylogeny from a collection of gene trees by treating each concatenated set of RAD sequences within scaffolds as independent genomic regions (Song et al. 2012; Saglam et al. 2016). This method not only allowed different genomic regions to have different topologies (enabling us to effectively model gene tree heterogeneity) but also resulted in a species/population tree where each branching event describes divergence between species/populations as opposed to divergence of genes or haplotypes. Focusing on species trees is also important when phylogenies are used to estimate divergence times because the timing of gene divergence usually predates speciation or divergence of populations, resulting in overestimation of divergence times (Edwards and Beerli 2000). Moreover, external information used to calibrate phylogenies (e.g., fossil data or geological events) reflects the timing of species or population splits; therefore, the appropriate medium for applying these calibrations is the species tree as opposed to the gene tree (McCormack et al. 2011).

To generate unbiased estimates of species history, we conducted independent phylogenetic reconstructions from two genomic subsets by using the multispecies coalescent. Each genomic subset was made up of 50 randomly chosen scaffolds containing concatenated RAD sequences. Individual sequences of each scaffold were aligned to one another via the Clustal X algorithm (Larkin et al. 2007), and the resulting alignments for each scaffold were sorted into separate FASTA-formatted files. We also subsampled the number of individual sequences in each scaffold down to four sequences per population to remove any bias that might arise from different numbers of sequences per species/population. As advised by Huang and Knowles (2016), we did not truncate our data set to exclude RAD loci with missing data (i.e., loci that were not present in all individuals) because doing so has been shown to bias phylogenetic results, as it disproportionately favors loci with low mutation rates and filters out highly divergent regions. Such missing data will appear as alignment gaps in our FASTA files.

The phylogenetic history of species/populations was estimated for both genomic subsets independently using the multispecies coalescent procedure (*BEAST; Heled and Drummond 2010) as implemented in the Markov chain–Monte Carlo (MCMC) program BEAST (Bouckaert et al. 2014). Prior to these analyses, we determined the best-fit model of evolution for each scaffold independently by using both Akaike's information criterion and the Bayesian information criterion implemented in Molecular Evolutionary Genetics Analysis version 6.0 (Tamura et al. 2013). All loci conformed to the Jukes–Cantor model of evolution (Jukes and Cantor 1969); therefore, XML files for analysis in BEAST were set up in BEAUTI version 2.2.0 (under a Jukes–Cantor model of nucleotide substitution, a strict molecular clock, a linear population size model for the multispecies coalescent, and a Yule model of divergence/speciation). The clock rate parameter was fixed to 1; therefore, branch lengths were estimated in units of substitutions per site. A uniform prior between 0 and 1,000 was set on the birth rate parameter (λ) of the Yule model, and a Jeffrey's prior was set for the population size model. Jeffrey's prior is a scale-invariant uninformative prior, which can be thought of as a uniform prior on the log scale.

For each subset, we conducted two different MCMC runs with chain lengths of 100 million, sampling every 10,000 generations. We checked for convergence of the two runs by using TRACER version 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). The effective sample size values of each run were over 200, so we combined the two runs by using LogCombiner version 2.2.0 (included in the BEAST package) for a final sample size of 18,000 states and 18,000 trees after removing the first 1,000 states and 1,000 trees of each run as burn-in. Resulting trees were summarized with TreeAnnotater version 2.2.0 (included in BEAST) to obtain a consensus tree and were visualized with FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The FASTA and XML files used in all analyses are provided in Supplements B and C (available in the online version of this paper).

Divergence time estimates.—Divergence times were estimated from the two species/population trees obtained from independent *BEAST analyses of the two genomic subsets. To estimate divergence times, we used the penalized likelihood method implemented in PATHd8 (Britton et al. 2007), which smooths substitution rates between sister groups by sequentially taking the mean path length from an internal node to all terminal branches attached to it. PATHd8 needs a fixed calibration node, and for this purpose we used prior estimates of the divergence between Rainbow Trout and Cutthroat Trout to calibrate the two species trees. However, since there is some uncertainty about the timing of divergence between Rainbow Trout and Cutthroat Trout, we conducted three separate estimates for each species tree by setting the calibration information at 2, 4, and 6 million years ago (mya; 2–4 mya: Wilson and Turner 2009; 6 mya: McKay et al. 1996, Smith et al. 2002).

RESULTS

We obtained a total of 1,967,735,864 reads from 162 individuals from three RAD sequencing libraries. After aligning with the Rainbow Trout genome, individual alignment counts ranged from 9,474 to 6,497,183, with an average of 2,192,850 reads per individual. Twelve individuals were discarded from further analysis because their alignment counts were less than 500,000.

Genetic Structure among and between Cutthroat Trout Subspecies: Principal Components Analysis

Genetic structure among Cutthroat Trout subspecies (LCT, PCT, BCT, YCT, and WCT) was generally high, with almost all subspecies forming distinct clusters on the first two PC axes (Figure 2). Principal component axis 1, which represented 28.7% of the variation, separated LCT and PCT from the other subspecies; PC2 explained 12.22% of the variation and clearly separated WCT from the other subspecies. However, close examination of the PCT and LCT clusters in Figure 2 suggested finer structuring between those two subspecies. To further investigate this structure, we conducted a separate PCA and only included PCT and LCT individuals. Those results showed a clear separation between PCT and the LCT populations along PC1, which explained 6.09% of the variation (Figure 3). Additional structure within LCT could also be observed along PC2, which amounted to 3.45% of the variation and separated Western and Eastern Lahontan Basin LCT populations, while Northwestern Lahontan Basin LCT populations were more spread out (Figure 3).

Genetic Structure among and between Cutthroat Trout Subspecies: Phylogenetic Analysis

Topography of phylogenetic trees obtained from the two independent sets of scaffolds was consistent, as both converged on very similar species/population trees with high nodal support values (Figures 4, 5). Individual gene trees used to calculate the species/population tree in both sets are provided in Supplements B and C. Posterior probabilities of nodes separating previously designated subspecies were all equal to 1, indicating very little gene tree heterogeneity between subspecies. Moreover, for these branches, the species trees from both scaffold sets showed identical topologies (Figures 4, 5). As expected, phylogenetic relationships between LCT populations were less certain, with higher degrees of gene tree heterogeneity indicated by lower nodal support values and discordant branching patterns of the two species/population trees for these lineages (Figures 4, 5).

Both phylogenetic trees gave high support for the genetic distinctiveness of PCT from LCT. According to both scaffold sets, PCT did not cluster with LCT populations but split off early on to form the basal node of Cutthroat Trout within the Lahontan basin (Figures 4, 5). Moreover, based on the variety of assumed divergence between Cutthroat Trout and Rainbow Trout (2, 4, or 6 mya), PCT may have diverged from LCT around 850–260 kya according to scaffold set 1 or 550–180 kya according to scaffold set 2 (Figures 4, 5).

DISCUSSION

In this study, we collected genomic data using RAD sequencing to characterize the population structure between two federally listed inland Cutthroat Trout subspecies, the LCT and PCT, as well as to reconstruct the phylogenetic relationships between these and other closely related Cutthroat Trout subspecies. Our results revealed a higher than expected level of genetic differentiation between PCT and LCT but also showed substantial genetic structuring between LCT subpopulations. Phylogenetic reconstructions gave further support for the distinctiveness of PCT from LCT and suggested that PCT diverged well before the LCT groups differentiated from each other.

Based on the inability to find unique genetic diversity, several previous studies (Nielsen and Sage 2002; Finger et al. 2009; Pritchard et al. 2012) placed PCT within the broader context of LCT diversity. In contrast, the present

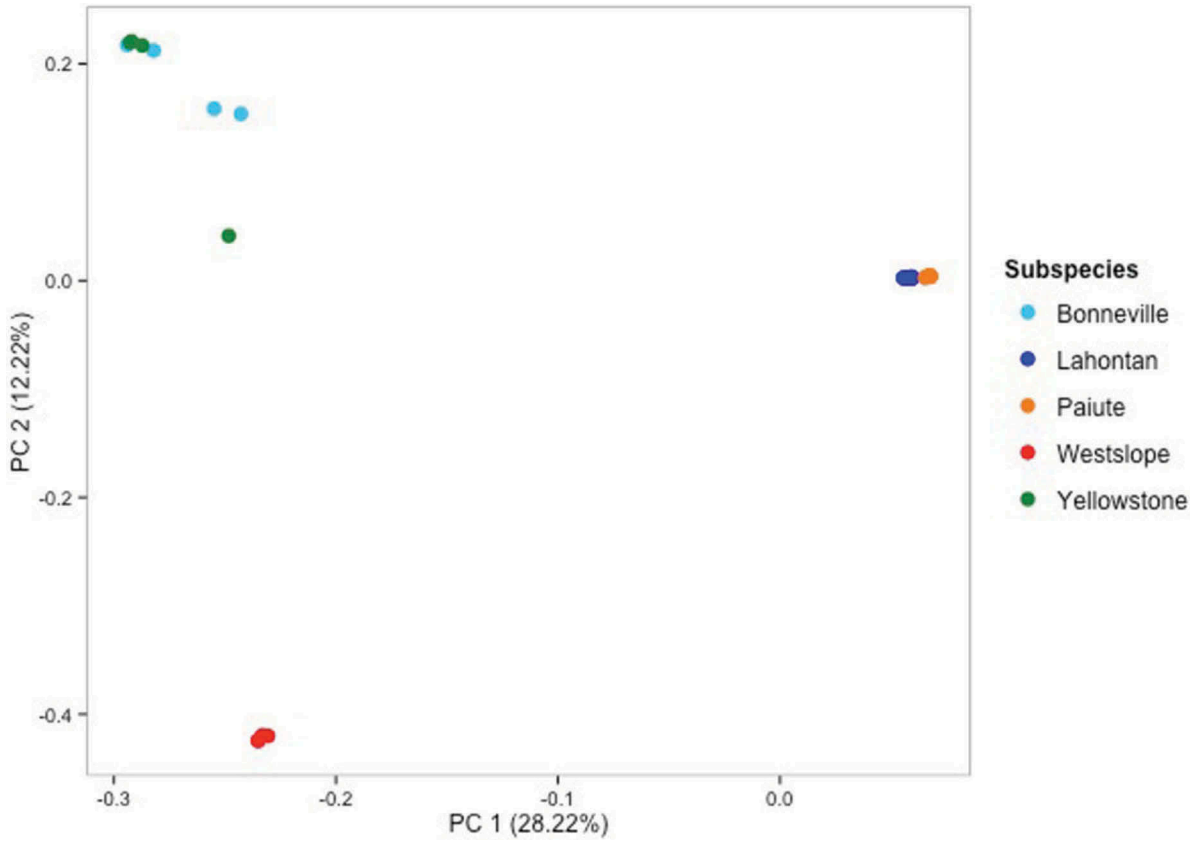

FIGURE 2. Principal components (PC) analysis plot created based on restriction-site-associated DNA sequencing data, depicting the relationships between Cutthroat Trout subspecies (Westslope, Yellowstone, Bonneville, Lahontan, and Paiute Cutthroat Trout) in and around the Lahontan basin.

analysis contradicts that assumption, as we show the presence of substantial genetic structure and differentiation between PCT and all LCT subpopulations (Table 2), even though this differentiation was not as pronounced as in other subspecies (YCT, BCT, and WCT). Phylogenetic reconstructions from two independent sets of 50 scaffolds (Figures 4, 5) also give high support to this scenario and—depending on the timing of divergence of Cutthroat Trout from Rainbow Trout—places this split somewhere between 850 and 180 kya. However, we would like to emphasize that our goal here was not to produce a definite answer of when these populations/subspecies diverged. We acknowledge that there is considerable uncertainty in the divergence times presented here, as we only had calibration information regarding a single node (divergence between Cutthroat Trout and Rainbow Trout), which itself is a topic of significant debate. In contrast, divergence times reported here should only be taken as a rough estimate of how long ago PCT could have diverged from LCT populations based on the available calibration information. Our dating analysis shows that even if we choose the closest possible reported divergence between Rainbow Trout and Cutthroat Trout (i.e., 2 mya), PCT can still be considered an independent lineage starting from 260 or 180 kya (Figures 4 and 5, respectively). This divergence time is still approximately 30–50 times greater than previous assumptions (5–8 kya: Behnke and Zarn 1976). However, apart from uncertainties introduced by calibration information, our analysis may overestimate divergence times because it does not fully take into account the extreme bottlenecks that may have taken place in these populations (Neville et al. 2006; Peacock and Kirchoff 2007; Peacock and Dochtermann 2012). When not accounted for, bottlenecks have the potential to artificially increase divergence times, as they can lead to greater than expected genetic distances (Gaggiotti and Excoffier 2000).

Although there are uncertainties regarding the timing of when PCT diverged from LCT, there is no ambiguity in the phylogenetic pattern showing that PCT diverged before the LCT populations differentiated from one another. Both of our phylogenetic reconstructions give unequivocal support for the basal position of PCT in relation to all LCT populations (Figures 4, 5). Indeed, there was no uncertainty in the two species/population trees for lineages giving rise to LCT populations, as nodal support values were all 1 and both trees had identical branching patterns, indicating that all

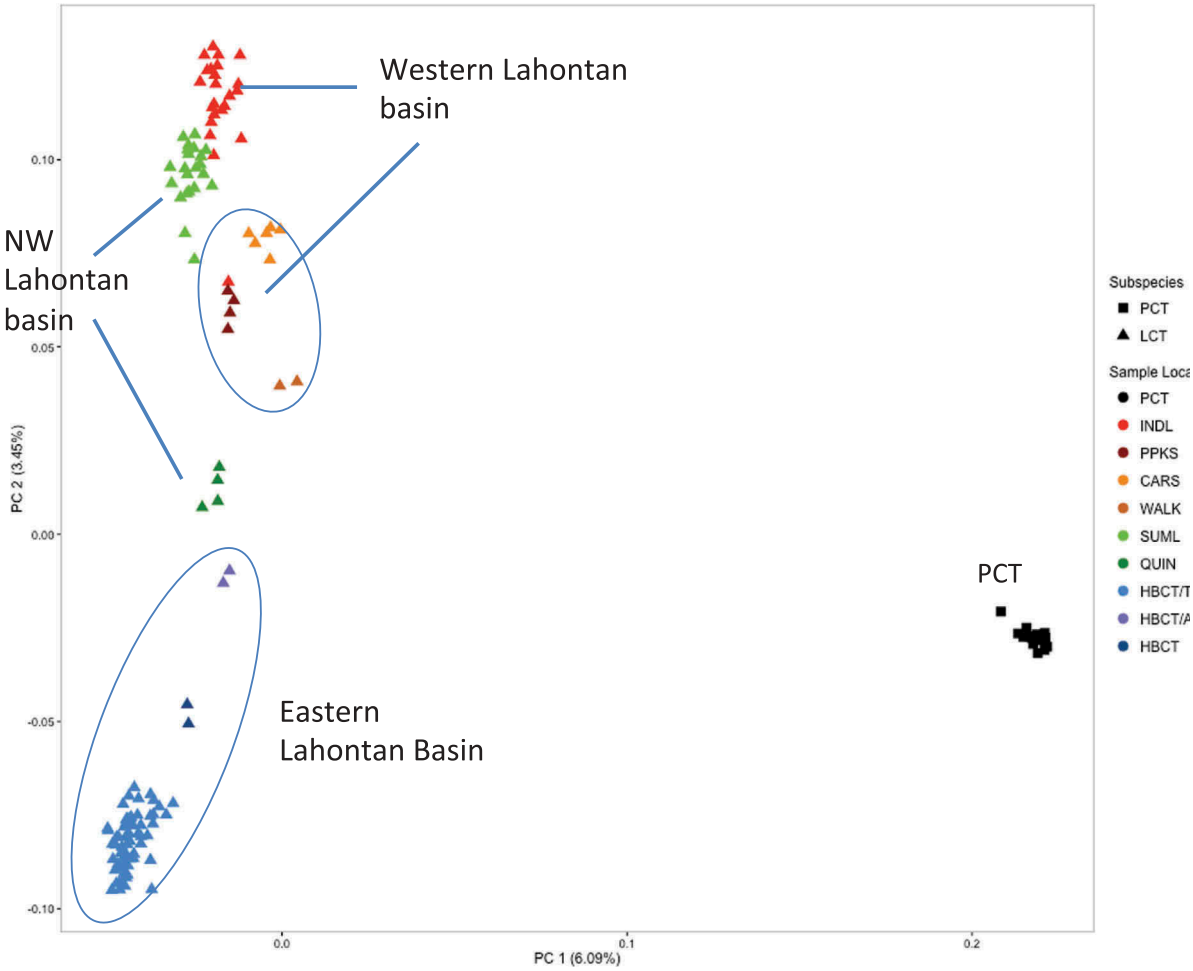

FIGURE 3. Principal components (PC) analysis plot created by using restriction-site-associated DNA sequencing data, depicting the relationships between Paiute Cutthroat Trout (PCT) and Lahontan Cutthroat Trout (LCT) populations in the three LCT management units (Western, Eastern, and Northwestern Lahontan Basin).

interrogated gene trees (i.e., trees estimated from the 100 independent scaffolds) converged on the same species/population tree. Discordance between gene tree and species/population tree topologies was observed only for lineages describing relationships among LCT populations. This was not surprising, as phylogenetic patterns of more recently diverged populations are likely to be heavily influenced by incomplete lineage sorting (Maddison and Knowles 2006). The absence of any evidence of incomplete lineage sorting between PCT and LCT indicates that the PCT can at least partially be treated as an independent evolutionary lineage.

The distinct phylogenetic signal of the PCT supports its evolutionary independence from LCT populations but does not shed light on historical events leading to the divergence of PCT in relation to the current distribution of LCT populations within the Lahontan basin. Genetic distances between PCT and all LCT populations were uniformly high and did not reflect current geography (i.e., PCT were not more closely related to Carson River LCT). Previous genetic analyses found a similar pattern; PCT shared mitochondrial DNA haplotypes with LCT from the Humboldt and Reese River watersheds (Eastern Lahontan Basin; Loxterman and Keeley 2012), and genetic differentiation index (F_{ST}) values between PCT and LCT from the Eastern Lahontan Basin were lower than values between other LCT populations (Nielsen and Sage 2002). These results led previous authors to propose that the PCT isolation event may have occurred sometime before the Eastern Lahontan Basin fish were isolated from the remainder of LCT populations in the Western Lahontan Basin due to the recession of Lake Lahontan (Benson and Thompson 1987; Behnke 1992). Higher genetic differentiation (i.e., F_{ST} values) between PCT and LCT populations in the geographically closer Western Lahontan Basin (Walker, Carson, and Truckee rivers) was attributed to rapid genetic drift caused by multiple population decline and expansion events (i.e., bottlenecks and founder effects), resulting in the numerous

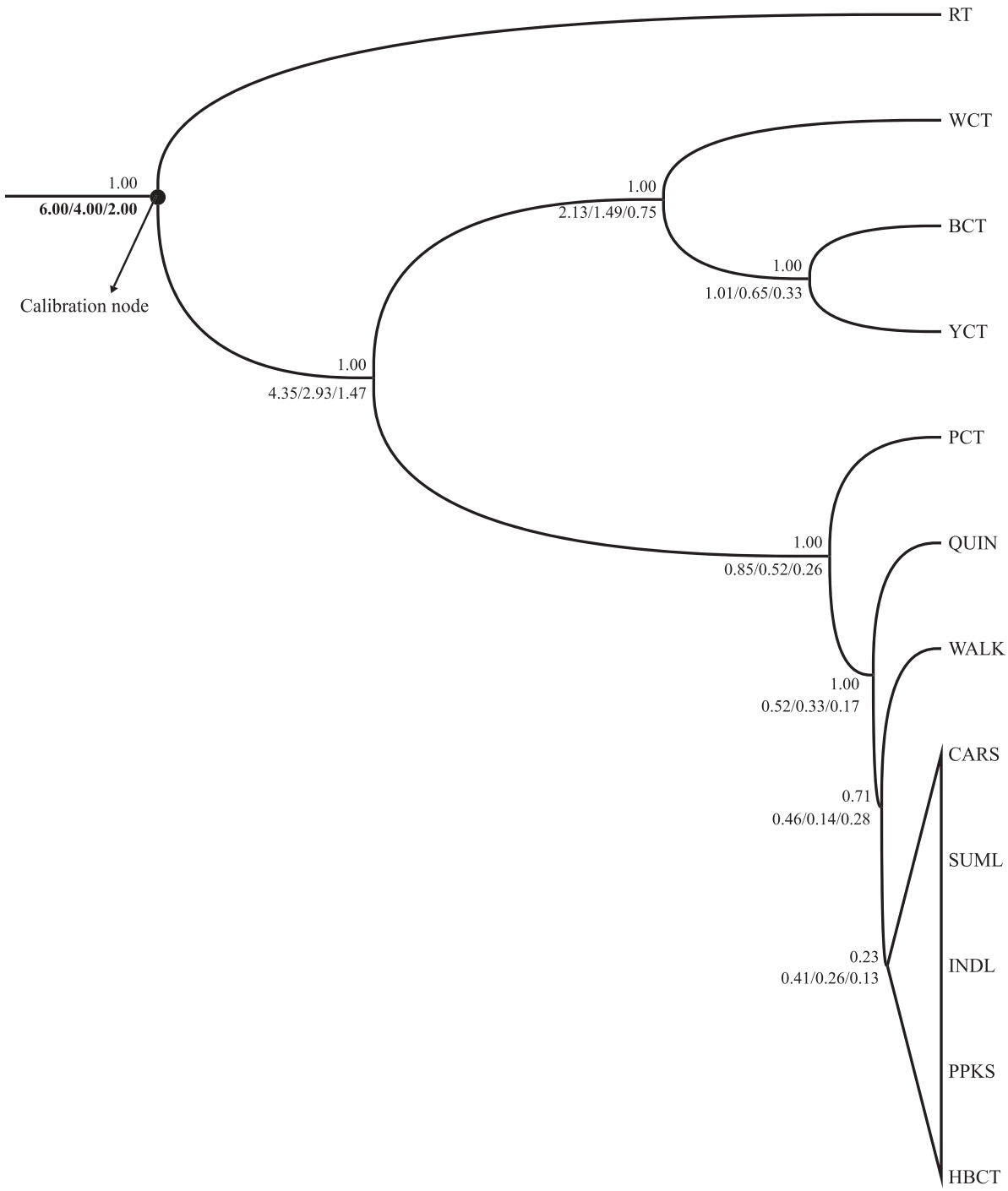

FIGURE 4. Phylogenetic relationship between Cutthroat Trout populations in and around the Lahontan basin as inferred from scaffold set 1 using *BEAST (population codes are defined in Table 1). Upper nodal values represent the posterior probability of each node, while lower nodal values represent divergence times depending on the assumed divergence between Cutthroat Trout and Rainbow Trout (2, 4, and 6 million years ago, respectively). For clarification, fixed divergence times are given in bold, whereas estimated divergence times are given in normal font.

small habitats observed in the Western Lahontan Basin today (Nielsen and Sage 2002; Peacock and Kirchoff 2007). Our trees show that PCT were least differentiated from Eastern Lahontan Basin (i.e., Humboldt River) LCT rather than the geographically closer Western Lahontan Basin LCT populations (Table 2). We suggest further work to formally test

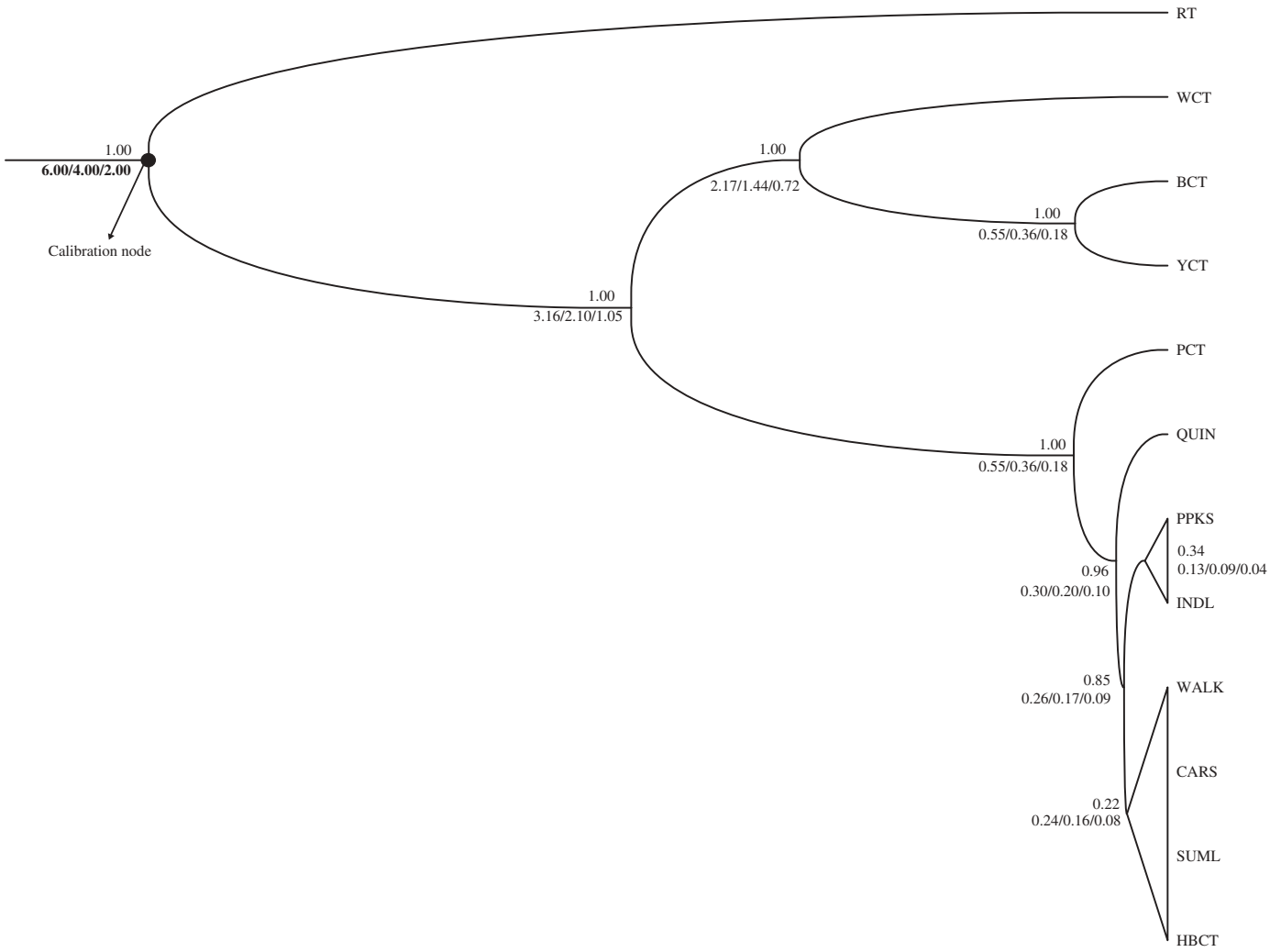

FIGURE 5. Phylogenetic relationship between Cutthroat Trout populations in and around the Lahontan basin as inferred from scaffold set 2 using *BEAST (population codes are defined in Table 1). Upper nodal values represent the posterior probability of each node, while lower nodal values represent divergence times depending on the assumed divergence between Cutthroat Trout and Rainbow Trout (2, 4, and 6 million years ago, respectively). For clarification, fixed divergence times are given in bold, whereas estimated divergence times are given in normal font.

TABLE 2. Pairwise genetic differentiation index (F_{ST}) values between geographic groups of Lahontan Cutthroat Trout (LCT) and Paiute Cutthroat Trout (PCT), calculated using ANGSD software. Groups are based on geographic and genetic similarity. The following LCT groups are included: Independence Lake (INDL), Pilot Peak Hatchery strain (PPKS), Carson River drainage (CARS), Walker River drainage (WALK), Summit Lake (SUML), Quinn River drainage (QUIN), and Humboldt River Cutthroat Trout (HBCT).

Group	PCT	INDL	PPKS	CARS	WALK	SUML	QUIN
INDL	0.323	–					
PPKS	0.499	0.124	–				
CARS	0.391	0.146	0.266	–			
WALK	0.483	0.139	0.321	0.110	–		
SUML	0.315	0.131	0.141	0.094	0.112	–	
QUIN	0.473	0.137	0.290	0.237	0.348	0.128	–
HBCT	0.308	0.142	0.153	0.135	0.133	0.150	0.119

different demographic models and their influence on patterns of genetic variation to gain more insight into the evolutionary history of LCT and PCT.

Conclusions

Molecular studies using genomic data are steadily elucidating hitherto unresolved relationships between closely related taxa with complicated phylogenetic histories. Cutthroat Trout in particular present an evolutionary puzzle in which phylogenetic relationships have real management implications. We used genomic data to examine the genetic relationships between the PCT and its closest relative, the LCT. Overall, we found high genetic differentiation between PCT and all LCT populations and a clear phylogenetic signal showing the PCT to be a separate evolutionary lineage. Indeed, the

phylogenetic placement of PCT was as robust as that of any other Cutthroat Trout subspecies in and around the Lahontan basin (each had a probability of 1). Furthermore, PCT were more differentiated from LCT than any groups of LCT were from each other, and PCT are phenotypically distinct (Trotter and Behnke 2008). Our data strongly support the continued recognition of the PCT as a distinct Cutthroat Trout group that is worthy of significant conservation efforts.

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REFERENCES

Behnke, R. 1992. Native trout of western North America. American Fisheries Society, Monograph 6, Bethesda, Maryland.

Behnke, R. 2002. Trout and salmon of North America. Free Press, New York.

Behnke, R. J., and M. Zarn. 1976. Biology and management of threatened and endangered western trouts. U.S. Forest Service General Technical Report RM-28.

Benson, L. V., and R. S. Thompson. 1987. Lake-level variation in the Lahontan Basin for the Past 50000 years. Quaternary Research 28:69–85.

Berthelot, C., F. Brunet, D. Chalopin, A. Juanchich, A. Bernard, N. Benjamin, P. Bento, C. da Silva, K. Labadie, A. Alberti, J.-M. Aury, A. Louis, P. Dehais, P. Bardou, J. Montfort, C. Klopp, C. Cabau, C. Gaspin, G. H. Thorgaard, M. Boussaha, E. Quillet, R. Guyomard, D. Galian, J. Bobe, J.-N. Volff, C. Genêt, P. Wincker, O. Jaillon, H. R. Crollius, and Y. Guiguen. 2014. The Rainbow Trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nature Communications 5:3657.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLOS (Public Library of Science) Computational Biology [online serial] 10(4):e1003537.

Britton, T., C. L. Anderson, D. Jacquet, S. Lundqvist, and K. Bremer. 2007. Estimating divergence times in large phylogenetic trees. Systematic Biology 56:741–752.

Busack, C. A., and G. A. E. Gall. 1981. Introgressive hybridization in populations of Paiute Cutthroat Trout (*Salmo clarki seleniris*). Canadian Journal of Fisheries and Aquatic Science 38:939–951.

Carstens, B. C., and L. L. Knowles. 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* grasshoppers. Systematic Biology 56:400–411.

Cordes, J. F., J. A. Israel, and B. May. 2004. Conservation of Paiute Cutthroat Trout: the genetic legacy of population transplants in an endemic California salmonid. California Fish and Game 90:101–118.

Edwards, S. V., and P. Beerli. 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution 54:1839–1854.

Finger, A. J., M. R. Stephens, N. W. Clipperton, and B. May. 2009. Six diagnostic single nucleotide polymorphism markers for detecting introgression between Cutthroat and Rainbow trouts. Molecular Ecology Resources 9:759–763.

Fumagalli, M., F. G Vieira, T. Linderoth, and R. Nielson. 2014. ngsTools: methods for population genetics analyses from next-generation sequencing data. Bioinformatics 30:1486–1487.

Gaggiotti, O. E., and L. Excoffier. 2000. A simple method of removing the effect of a bottleneck and unequal population sizes on pairwise genetic distances. Proceedings of the Royal Society B 267:81–87.

Heled, J., and A. J. Drummond. 2010. Bayesian inference of species trees from multilocus data. Molecular Biology and Evolution 27:570–580.

Huang, H., and L. L. Knowles. 2016. Unforeseen consequences of excluding missing data from next-generation sequences: simulation study of RAD sequences. Systematic Biology 65:357–365.

Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. Mammalian Protein Metabolism 3:21–132.

Knowles, L. L., and B. C. Carstens. 2007. Delimiting species without monophyletic gene trees. Systematic Biology 56:887–895.

Korneliussen, T. S., A. Albrechtsen, and R. Nielsen. 2014. ANGSD: analysis of next generation sequencing data. BMC Bioinformatics [online serial] 15:356.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948.

Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27:2987–2993.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754–1760.

Loxterman, J. L., and E. R. Keeley. 2012. Watershed boundaries and geographic isolation: patterns of diversification in Cutthroat Trout from western North America. BMC Evolutionary Biology [online serial] 12:38.

Maddison, W., and L. Knowles. 2006. Inferring phylogeny despite incomplete lineage sorting. Systematic Biology 55:21–30.

McCormack, J. E., J. Heled, K. S. Delaney, A. T. Peterson, and L. L. Knowles. 2011. Calibrating divergence times on species trees versus gene trees: implications for speciation history of *Aphelocoma* jays. Evolution 65:184–202.

McKay, S. J., M. J. Smith, and R. H. Devlin. 1996. Phylogeny of Pacific salmon and trout based on growth hormone type-2 and mitochondrial NADH dehydrogenase subunit 3 DNA sequences. Canadian Journal of Fisheries and Aquatic Sciences 53:1165–1176.

Metcalf, J. L., V. L. Pritchard, S. M. Silvestri, J. B. Benkins, J. S. Wood, D. E. Cowley, P. R. Evans, D. K. Shiozawa, and A. M. Martin. 2007. Across the great divide: genetic forensics reveals misidentification of endangered Cutthroat Trout populations. Molecular Ecology 16:4445–4454.

Miller, M. R., J. P. Dunham, A. Amores, W. A. Cresko, and E. A. Johnson. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. Genome Research 17:240–248.

Neville, H. M., J. B. Dunham, and M. M. Peacock. 2006. Landscape attributes and life history variability shape genetic structure of trout populations in a stream network. Landscape Ecology 21:901–916.

Nielsen, J. L., and G. K. Sage. 2002. Population genetic structure in Lahontan Cutthroat Trout. Transactions of the American Fisheries Society 131:376–388.

Peacock, M. M., and N. Dochtermann. 2012. Evolutionary potential but not extinction risk of Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*) is associated with stream characteristics. Canadian Journal of Fisheries and Aquatic Science 69:615–626.

Peacock, M. M., and V. Kirchoff. 2007. Analysis of genetic variation and population genetic structure in Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*) extant populations. Final Report submitted to the U.S. Fish and Wildlife Service, Reno, Nevada.

Pritchard, V. L., N. R. Campbell, S. R. Narum, M. R. Peacock, and J. C. Garza. 2012. Discovery and characterization of novel genetic markers for use in the management of Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*). Molecular Ecology Resources 13:276–288.

R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Ryan, J. H., and S. J. Nicola. 1976. Status of the Paiute Cutthroat Trout, *Salmo clarki seleniris* Snyder, in California. California Department of Fish and Game, Inland Fisheries Branch, Administrative Report 76-3, Sacramento.

Saglam, I. K., J. Baumsteiger, M. J. Smith, J. Linares-Casenave, A. L. Nichols, S. M. O'Rourke, and M. R. Miller. 2016. Phylogenetics support an ancient common origin of two scientific icons: Devils Hole and Devils Hole Pupfish. Molecular Ecology 25:3962–3973.

Smith, G. R., T. W. Dowling, K. W. Gobalet, T. Lugaski, D. Shiazawa, and R. P. Evans. 2002. Biogeography and timing of evolutionary events among Great Basin fishes. Smithsonian Contributions to the Earth Sciences 33:75–234.

Snyder, J. O. 1933. Description of *Salmo seleniris*, a new California trout. Proceedings of the California Academy of Sciences 20:471–472.

Snyder, J. O. 1934. A new California trout. California Department of Fish and Game 20:105–112.

Song, S., L. Liu, S. V. Edwards, and S. Wu. 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proceedings of the National Academy of Sciences of the USA 109:14942–14947.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30:2725–2729.

Trotter, P. C. 2008. Cutthroat Trout: native trout of the west, 2nd edition. University of California Press, Berkeley.

Trotter, P. C., and R. J. Behnke. 2008. The case for *humboldtensis*: a subspecies name for the indigenous Cutthroat Trout (*Oncorhynchus clarkii*) of the Humboldt River, Upper Quinn River, and Coyote basin drainages, Nevada and Oregon. Western North American Naturalist 68:58–65.

USFWS (U.S. Fish and Wildlife Service). 1995. Lahontan Cutthroat Trout (*Oncorhynchus clarki henshawi*) recovery plan. USFWS, Portland, Oregon.

USFWS (U.S. Fish and Wildlife Service). 2009. Five-year review: summary and evaluation, Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*). USFWS, Region 8, Sacramento, California.

USFWS (U.S. Fish and Wildlife Service). 2013. Five-year review: summary and evaluation, Paiute Cutthroat Trout (*Oncorhynchus clarkii seleniris*). USFWS, Region 8, Sacramento, California.

Wilson, W. D., and T. F. Turner. 2009. Phylogenetic analysis of the Pacific Cutthroat Trout (*Oncorhynchus clarki* ssp.: Salmonidae) based on partial mtDNA ND4 sequences: a closer look at the highly fragmented inland species. Molecular Phylogenetics and Evolution 52:406–415.