

USING GENETIC DIVERSITY TO INFORM CONSERVATION EFFORTS FOR NATIVE CUTTHROAT TROUT OF THE SOUTHERN ROCKY MOUNTAINS

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Abstract—Recent research on native Cutthroat Trout *Oncorhynchus clarkii* of the southern Rocky Mountains suggests a convoluted taxonomy confused by stocking in the early 1900s that obscured the native distributions of these fish. DNA recovered from the few museum specimens collected 150 years ago shed light on the historical diversity and native ranges of lineages in Colorado. This study aims to characterize what remains of that diversity across the entire southern Rockies using a stratified random sampling design across the range of putative Colorado River Cutthroat Trout *O. c. pleuriticus*, Greenback Cutthroat Trout *O. c. stomias*, and Rio Grande Cutthroat Trout *O. c. virginialis*. Twenty-four biologists from four states collected 801 fish from 49 randomly selected conservation populations across Colorado, New Mexico, Utah, and Wyoming. Whole specimens were used to explore phenotypic differences in lineages suggested by molecular studies. Here, we used tissue samples collected prior to specimen preservation to describe mitochondrial haplotype diversity. These diversity patterns are critical to inform managers tasked with listing decisions for rare Cutthroat Trout lineages. Consistent with previous studies, four distinct lineages were recovered from sequence data on 648 base pairs of the ND2 mitochondrial gene. Substantial diversity was recovered in Rio Grande Cutthroat Trout (12 haplotypes), while only a single haplotype could be found in native Cutthroat Trout of the South Platte River basin. Within Colorado River Cutthroat Trout, nine haplotypes were recovered from 14 populations putatively native to the Upper Colorado, Gunnison, and Dolores basins (Green Lineage), but only six were found in 21 populations native to the Lower Colorado, Green, and Yampa basins (Blue Lineage). This was unexpected given the broad range of the Blue Lineage, and may suggest more recent ancestry of Green River basin fish. Rare haplotypes may indicate pockets of historical diversity. To avoid inadvertently “throwing away the pieces”, these conservation populations should be targeted for replication and protection to ensure their continued persistence.

INTRODUCTION

As the official state fish of seven western states and a prized game fish, Cutthroat Trout *Oncorhynchus clarkii* have long held the interest of anglers and managers alike. That interest in the taxonomy of native Cutthroat Trout was reignited several years ago by a study published in the journal *Molecular Ecology* suggesting there was a genetic basis for separating our native trout, and that earlier efforts to identify genetic markers for this purpose were hampered by a historical distribution patterns that were largely occluded by extensive stocking of native trout in the early part of the 20th century (Metcalf et al. 2007). That assertion was supported by more recent work that examined 150 year-old specimens housed in our nation’s most

prestigious museums (Metcalf et al. 2012). That study also suggested a richer diversity than is currently present on the landscape – at least than we are aware of, with six different lineages of Cutthroat Trout once calling Colorado home (Metcalf et al. 2012). The same colors used to describe the four extant lineages in that paper are also used here for consistency (Figure 1; see Bestgen et al. 2013 for color rendition), with blue representing Cutthroat Trout native to the Yampa, White, and Green River basins, green representing those native to the Colorado, Gunnison, and Dolores basins, orange for the Rio Grande Cutthroat Trout *O. c. virginialis*, and purple for the putative South Platte basin native.

Given that our native Cutthroat Trout occupy roughly just a tenth of their historic range (Alves et al. 2008, Hirsch et al. 2013), a loss of genetic diversity is not unexpected. It does however illustrate the importance of cataloging what remains, so that conservation efforts can target those populations that harbor remnant diversity, rather than ones that are already well replicated through historical stocking efforts.

New molecular methods have already been integrated in the routine management of Cutthroat Trout in the southern Rocky Mountains, with general tests of purity being used to evaluate which populations deserve “Conservation Population” status as outlined in conservation agreements and their associated strategies (UDWR 2000; CRCT Coordination Team 2006; Rogers 2008; Rogers 2012a; RGCT Conservation Team 2013). However, the United States Court of Appeals has affirmed that the U.S. Fish and Wildlife Service should continue to rely on morphology for identifying native trout in listing decisions (Campton and Kaeding 2005). While the primary focus of this large-scale cataloging effort was indeed to determine if differences implied by the DNA lineages described in Metcalf et al. (2012) are reflected in the physical characteristics of the populations they represent (Bestgen et al. 2013), it also provided an opportunity to characterize mitochondrial sequence diversity across the range of Cutthroat Trout in the southern Rocky Mountains at the same time. While the meristic work represents a critical step toward resolving the taxonomic uncertainty that will allow repatriation and restoration of these native trout to aboriginal habitats to resume, the molecular work described here can be used to inform conservation efforts that seek to determine which populations are most appropriate for those restoration activities.

METHODS

Tissue Collection

Both characterizing genetic diversity, and subsequent morpho-meristic treatments required an unbiased sampling of extant populations of Cutthroat Trout in the southern Rocky Mountains. This was achieved by randomly selecting Core Conservation Populations (sensu UDWR 2000) from Cutthroat Trout databases maintained by the Colorado River Cutthroat

Trout Conservation Team (Hirsch et al. 2013), Rio Grande Cutthroat Trout Conservation Team (Alves et al. 2008), and Greenback Cutthroat Trout Recovery Team (unpublished data). The sampling design was stratified across U. S. Geological Survey 4-digit Hydrologic Unit Code (HUCs) units that also serve as geographic Management Units (GMUs) by the conservation teams charged with securing the future of these three subspecies (Alves et al. 2008, Hirsch et al. 2013). Since genetic structuring if present, should contain a spatial element reflecting isolation by distance (Wright 1943, Whiteley et al. 2006, Pritchard et al. 2009), three candidate populations from each GMU were selected at random to ensure that both morphological and genetic diversity was well represented. Geographic bounds of trout lineages were based on the findings of Metcalf et al. (2007) with modifications from Metcalf et al. (2012) and supplementary information from unpublished data and Rogers (2010). Essentially, what was once termed the Colorado River Cutthroat Trout, *O. c. pleuriticus*, and formerly thought to occupy all Colorado drainages west of the Continental Divide, is now classified, in part, by Metcalf et al. (2012) as the Blue Lineage and is believed native only in the White, Yampa, Green and lower Colorado River drainages in northwestern Colorado, southwestern Wyoming, and eastern Utah. Remaining native trout in the Dolores, Gunnison, and upper Colorado basins are referred to as the Green Lineage. In HUCs where both blue and green lineages are present, up to three populations of each were selected. One exception to the protocol was in the upper Colorado River GMU where what was assumed to be a Blue Lineage population (Abrams Creek, Stream 25) was later determined to be a Green Lineage population. Thus, two Blue Lineage and four Green Lineage upper Colorado GMU populations were analyzed. In other drainages, limited numbers of populations of a certain lineage restricted the number of study streams (e.g., only one Blue Lineage population in each of the San Juan or Dolores River basins).

Inclusion of a stream in the study was also granted only for those meeting three additional criteria: (1) that a population from the same 8-digit HUC was not already selected, (2) molecular data was available to make a determination on the lineage present (Rogers 2008), and (3) estimated population size exceeded 150 adult Cutthroat Trout per mile to minimize

negative consequences of removing 12 or 24 fish from the population. Thus, the stream selection protocol generated a relatively unbiased sample of populations for inclusion in the study while minimally impacting relatively small populations of trout.

Twenty-four fish were collected from the first population selected for each GMU to characterize within population variability of morphometric and meristic characteristics (Bestgen et al. 2013). If that stream could not support removal of 24 fish because of small population size, only 12 fish were taken and another population was substituted for the larger sample. In several instances, sufficient numbers of fish could not be obtained from a stream and a substitute was identified, again based on a random draw from the remaining populations in that GMU. In one case, the only alternative was a lentic population, Henderson Horseshoe Pond, and was selected as an alternative to Steelman Creek. Only 12 fish were collected from subsequent populations within each GMU to characterize among-population variation. A small number of wild specimens and a larger number of hatchery fish were also available from Bear Creek in the Arkansas drainage, Colorado, which was noteworthy for its distinct genetic fingerprint (Proebstel et al. 1996; Evans and Shiozawa 2002; Metcalf et al. 2007; Metcalf et al. 2012).

Specimens were captured by electrofishing or hook and line. Tissue samples were obtained by clipping a 1-cm² piece of the right pelvic or upper caudal fin, which was then stored in 3.5-ml cryo-storage vials (Perfector Scientific, Atascadero, California) containing 80% ethanol. The blind nature of the study was maintained by labeling each vial with a unique code that was not shared with the molecular lab until after the study was complete.

DNA Isolation and Evaluation

Tissue samples were delivered to Pisces Molecular (Boulder, Colorado) for DNA isolation and sequencing. A proteinase K tissue lysis and spin-column purification protocol following manufacturer specifications (Qiagen DNeasy Kit) was used to isolate DNA from the fin clip samples. Sample DNA was amplified using primers specific to a region of the NADH dehydrogenase subunit 2 (ND2) mitochondrial gene, generating a 648 bp fragment that falls within the fragment cited in previous studies (Metcalf et al. 2007, Loxterman and Keeley 2012), which allowed

us to confirm lineage assignments as well as identify unique haplotypes. Samples were run on a capillary sequencer (Applied Biosystems 3130 Genetic Analyzer, Foster City, California). Sequence reads were assembled using the Contig Express program (Vector NTI 11, Invitrogen, Carlsbad, California). The assembled contiguous sequence chromatograms were examined for sequence quality and accuracy, and the primer sequences removed from the ends of the fragments. Sequences were aligned in ClustalW (Thompson et al. 1994) and the evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei 1992) in MEGA4 (Tamura et al. 2007). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was calculated (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). The tree was searched using the Close-Neighbor-Interchange algorithm (Nei and Kumar 2000) at a search level of one. The Neighbor-joining algorithm (Saitou and Nei 1987) was used to generate the initial tree. Aligned sequence data was exported from MEGA to Arlequin using PGDSpider (Lischer and Excoffier 2012) where pairwise distances between haplotypes were calculated (Excoffier 2005). That table was then imported into HapStar (Teacher and Griffiths 2011) to generate a minimum spanning network.

RESULTS

The stream selection protocol resulted in a relatively even representation of populations from throughout the ranges and GMUs of the respective lineages and recognized subspecies (Table 1, Figure 1). In some instances, we could not select three populations of each lineage for a given GMU, usually because insufficient numbers of available Core Conservation Populations existed from which to draw from. This was true for Blue Lineage Cutthroat Trout in the San Juan and Dolores GMU's where only one population was drawn from each, and Green Lineage populations in the South Platte and Arkansas River GMU's, where only two populations were drawn from each. One of the sobering results from this effort was just how few Core Conservation Populations of Cutthroat Trout are present east of the Continental Divide in the South Platte and Arkansas River basins (Figure 1), when compared to basins west of the Continental Divide or in the Rio Grande drainage.

Table 1: Sample location information for the 49 populations of Cutthroat Trout used in this study. Geographic Management Units (GMU) reflect 4-digit USGS Hydrologic Unit Codes as portrayed in the study area map (Figure 1.)

| Drainage | GMU | Stream | Number | Coordinates | |
|----------------|------------------|------------------------|--------|-------------|-----------|
| | | | | Latitude | Longitude |
| Arkansas | Arkansas | South Apache Creek | 6 | 37.85 | -104.94 |
| Arkansas | Arkansas | North Taylor Creek | 26 | 38.11 | -105.62 |
| Arkansas | Arkansas | Graneros Creek | 43 | 37.89 | -104.95 |
| Arkansas | Arkansas | Hayden Creek, S. Prong | 3 | 38.30 | -105.81 |
| Arkansas | Arkansas | Severy Creek | 19 | 38.89 | -104.99 |
| Arkansas | Arkansas | Bear Creek | 49 | 38.80 | -104.90 |
| Colorado River | Upper Colorado | Little Green Creek | 29 | 40.30 | -106.63 |
| Colorado River | Upper Colorado | Mitchell Creek | 42 | 39.57 | -107.37 |
| Colorado River | Upper Colorado | Abrams Creek | 25 | 39.59 | -106.85 |
| Colorado River | Upper Colorado | Cunningham Creek | 31 | 39.33 | -106.55 |
| Colorado River | Upper Colorado | Horseshoe Pond | 34 | 39.83 | -106.08 |
| Colorado River | Upper Colorado | Brush Creek, W. Fk | 46 | 39.34 | -107.84 |
| Colorado River | Dolores | Tabeguache Creek | 12 | 38.45 | -108.47 |
| Colorado River | Dolores | Little Taylor Creek | 18 | 37.58 | -108.20 |
| Colorado River | Dolores | Big Red Canyon Creek | 21 | 38.26 | -108.20 |
| Colorado River | Dolores | Deep Creek, E. Fk | 24 | 37.97 | -107.90 |
| Colorado River | Gunnison | Nate Creek | 8 | 38.18 | -107.60 |
| Colorado River | Gunnison | Deep Creek | 11 | 38.97 | -107.30 |
| Colorado River | Gunnison | Doug Creek | 47 | 38.65 | -107.53 |
| Colorado River | Upper Green | Steel Creek | 7 | 40.95 | -110.48 |
| Colorado River | Upper Green | South Beaver Creek | 41 | 42.44 | -110.38 |
| Colorado River | Upper Green | Irish Canyon Creek | 2 | 42.66 | -109.36 |
| Colorado River | Lower Green | Little West Fk | 16 | 40.44 | -111.09 |
| Colorado River | Lower Green | South Brownie Creek | 38 | 40.69 | -109.77 |
| Colorado River | Lower Green | Johnson Fk | 44 | 39.93 | -111.01 |
| Colorado River | Yampa | Milk Creek | 23 | 40.15 | -107.62 |
| Colorado River | Yampa | Snell Creek | 30 | 40.07 | -107.34 |
| Colorado River | Yampa | Deep Creek | 35 | 41.21 | -107.17 |
| Colorado River | Lower Colorado | Pine Creek | 5 | 37.97 | -111.65 |
| Colorado River | Lower Colorado | Right Fk U M Creek | 40 | 38.68 | -111.59 |
| Colorado River | Lower Colorado | West Fk Boulder Creek | 45 | 38.04 | -111.49 |
| Colorado River | San Juan | East Fk Piedra River | 28 | 37.49 | -107.08 |
| Rio Grande | Canadian | West Fk Luna Creek | 22 | 36.21 | -105.36 |
| Rio Grande | Canadian | McCrystal Creek | 36 | 36.78 | -105.13 |
| Rio Grande | Canadian | Leandro Creek | 39 | 36.88 | -105.19 |
| Rio Grande | Pecos | Rio Valdez | 9 | 35.93 | -105.53 |
| Rio Grande | Pecos | Dalton Creek | 10 | 35.68 | -105.76 |
| Rio Grande | Pecos | Macho Creek | 14 | 35.69 | -105.72 |
| Rio Grande | Upper Rio Grande | West Indian Creek | 1 | 37.43 | -105.21 |
| Rio Grande | Upper Rio Grande | Osier Creek | 15 | 37.02 | -106.33 |

Table 1: Continued

| Drainage | GMU | Stream | Number | Coordinates | |
|--------------|------------------|----------------------|--------|-------------|-----------|
| | | | | Latitude | Longitude |
| Rio Grande | Upper Rio Grande | Carnero Creek, M | 27 | 37.98 | -106.42 |
| Rio Grande | Lower Rio Grande | El Rito | 4 | 36.53 | -106.27 |
| Rio Grande | Lower Rio Grande | Columbine Creek | 20 | 36.65 | -105.51 |
| Rio Grande | Lower Rio Grande | Policarpio Creek | 33 | 36.14 | -105.45 |
| South Platte | South Platte | S Fk Cache la Poudre | 13 | 40.54 | -105.60 |
| South Platte | South Platte | Roaring Creek | 32 | 40.75 | -105.76 |
| South Platte | South Platte | Hunters Creek | 48 | 40.21 | -105.58 |
| South Platte | South Platte | Como Creek | 17 | 40.02 | -105.51 |
| South Platte | South Platte | Fern Creek | 37 | 40.34 | -105.67 |

Absence of Core Conservation Populations in the southern portion of the South Platte River basin is notable and few exist in the western portion of the Arkansas River basin. Anthropogenic influences are not entirely responsible for the paucity of conservation populations east of the Continental Divide. The density of coldwater streams is simply higher on the West Slope and upper Rio Grande basin.

All specimens were screened with molecular methods to confirm that they fit within their anticipated clades using mitochondrial sequence data (Figure 2). We recovered 32 unique ND2 mitochondrial haplotypes in the 801 fish sampled from 49 populations that were distributed among five distinct clades consistent with those identified in earlier studies (Loxterman and Keeley 2012; Metcalf et al. 2012). Twenty-six haplotypes occurred in more than one individual, and 15 were shared among two or more populations. In addition to four haplotypes commonly found in Yellowstone Cutthroat Trout *O. c. bouvieri* that represent instances of admixture, we recovered 12 Rio Grande haplotypes, nine Green Lineage haplotypes and six Blue Lineage haplotypes (Figure 2). The ND2 sequence data suggested that 47 of 49 populations were assigned to their anticipated lineages (Figure 2). One of the two exceptions was Abrams Creek, where ND2 sequence data suggested it was a Green rather than Blue Lineage population. The other was Irish Canyon (Stream 2, SW Wyoming, Upper Green River GMU) where all fish had a pair of common Yellowstone Cutthroat Trout haplotypes, a finding corroborated by AFLP data which also indicated this population was Yellowstone Cutthroat Trout (Bestgen et al. 2013).

DISCUSSION

Critical to the integrity of this study was to adequately represent the genetic diversity of the various taxonomic entities of Cutthroat Trout across the southern Rocky Mountains. We were largely successful to that end, as representatives from each of the groups were selected at random from each of the 14 GMUs that collectively encompass the range of these Cutthroat Trout. This coordinated sampling effort across a four-state area ensured that basic spatial sampling design considerations were fulfilled, which was different from historical efforts that used opportunistically obtained samples, and also ensured that bias associated with over or under representation of one or more groups was minimized. The blind data acquisition protocol ensured that investigators were not influenced by knowing location or heritage of samples or specimens. This was guaranteed by a coding system for streams and specimens that was not revealed until after data collection was complete.

Four putatively native distinct lineages were recovered from ND2 sequence data after those that fell into the Yellowstone Cutthroat Trout clade were discounted. These lineages are consistent with those described in earlier studies (Metcalf et al. 2007, Loxterman and Keeley 2012, Metcalf et al. 2012). Substantial diversity was recovered in Rio Grande Cutthroat Trout (12 haplotypes), while only a single haplotype could be found in native Cutthroat Trout of the South Platte River basin. Within Colorado River Cutthroat Trout, nine haplotypes were recovered from 14 populations presumed to be native to the Upper Colorado, Gunnison, and Dolores basins

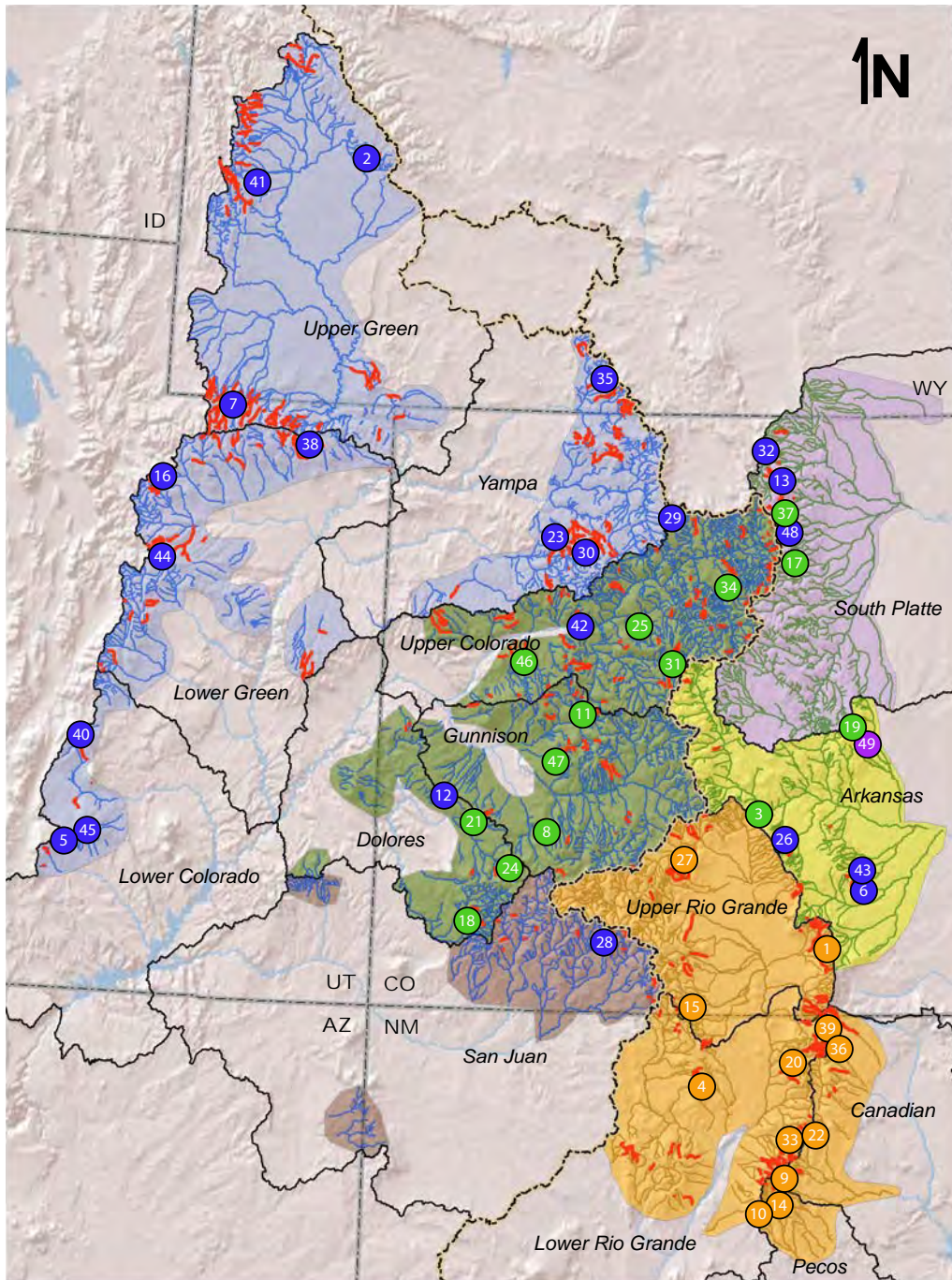


Figure 1: Fourteen hydrologic units from five western states that comprise the accepted historical range of Colorado River Cutthroat Trout (blue labeled streams), Greenback Cutthroat Trout (green streams), and Rio Grande Cutthroat Trout (orange streams) are named in italics. Core Conservation Populations from which our study populations were randomly drawn are highlighted in red. The presumed historical ranges of lineages described in Metcalf et al. (2012) are represented by shading: the Blue Lineage (Yampa River, upper and lower Green River, and lower Colorado River GMU's) is shaded blue, the Green Lineage (upper Colorado River, Gunnison River, and Dolores River drainage GMU's) is shaded green, San Juan River drainage (and GMU) is shaded brown, Rio Grande Cutthroat Trout (upper and lower Rio Grande, Pecos River and Canadian River GMU's) are shaded orange, yellowfin Cutthroat Trout (Arkansas River GMU) is shaded yellow, and South Platte native cutthroat lineage (South Platte River GMU) lineage is shaded purple. Dots represent the populations sampled in this study and are colored as per the lineage defined by the ND2 clade using the same color scheme above. The numbers within each dot indicate the stream sampled.

(Green Lineage), but only six were found in 21 populations native to the Lower Colorado, Green, and Yampa basins (Blue Lineage) despite covering a much broader geographical area. Perhaps this is a reflection of a more recent evolutionary past, or greater connectedness in the stream systems they inhabit. Presence of Blue Lineage Cutthroat Trout in the lower Colorado River basin GMU was unexpected, given presence of presumably native Green Lineage fish in Dolores and upper Colorado River basin GMUs

upstream. Headwater dispersal from proximate lower Green River basin GMU Blue Lineage stocks may explain presence of Blue Lineage fish in the lower Colorado River GMU. The unique haplotypes found in these populations (Streams 5, 40, and 45) and nowhere else (Figure 2) might suggest that these populations were not founded by stocking, but rather represent aboriginal genetic diversity.

Invasion of West Slope Colorado River basin streams south of the presumed native distribution

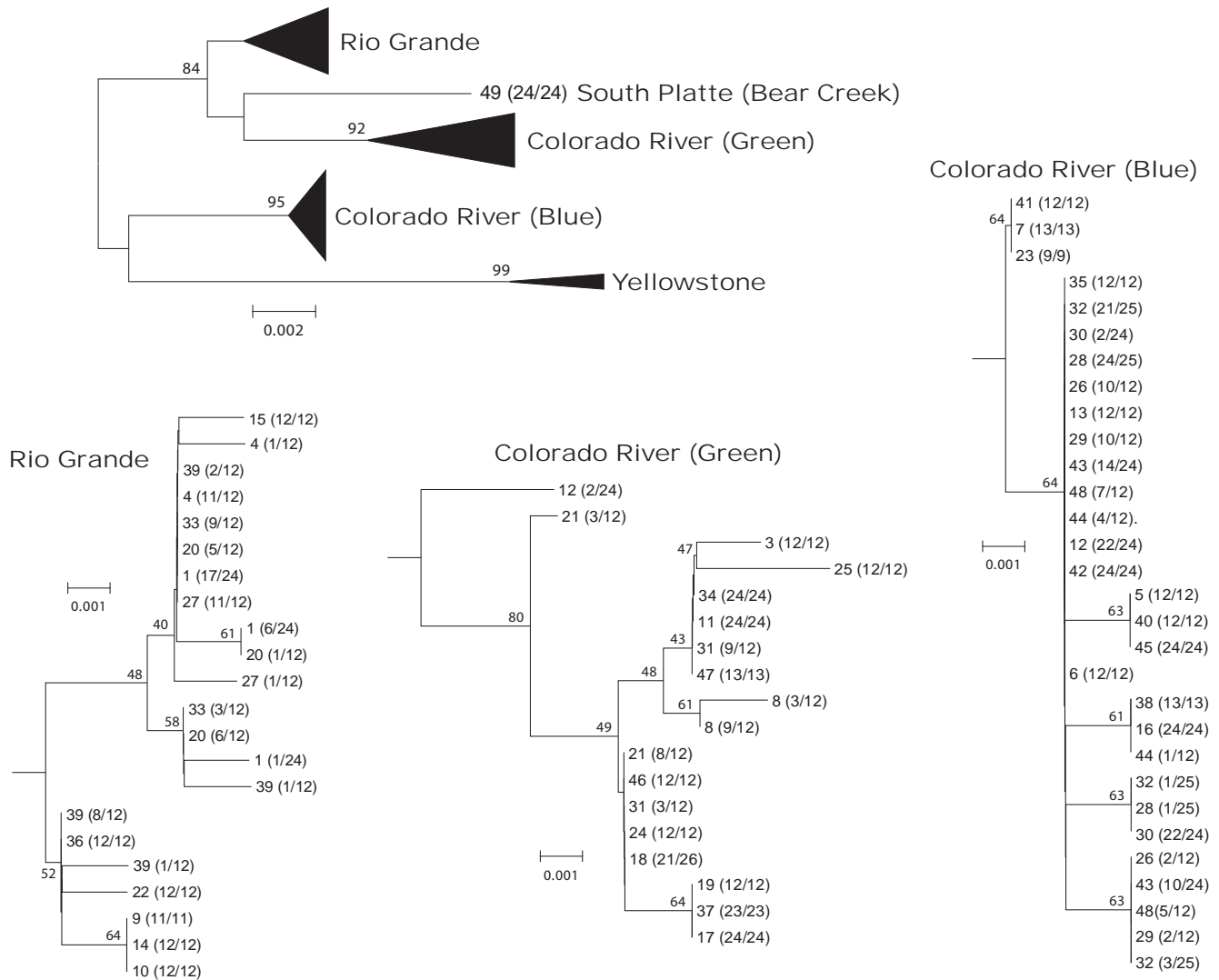


Figure 2: Phylogenetic relationships inferred from 648 base pairs of the mitochondrial ND2 gene for Cutthroat Trout from the Southern Rocky Mountains. The evolutionary history was developed with the Minimum Evolution method. Percent branching support was evaluated with 500 bootstrap replicates with values exceeding 40% indicated above the tree branches. Major clades relevant for this study are broken into separate sub-trees. Stream numbers are listed first, followed by (in parentheses) the number of fish with a given haplotype out of the total number sampled in each population. A Rainbow Trout haplotype was detected in a single fish in Stream 21, and from five fish in Stream 18 – these were not included in the tree. Four Yellowstone Cutthroat Trout haplotypes were also detected in two populations (Stream 2 and 44). Phylogenetic analyses were conducted in MEGA4 with evolutionary distance units representing the number of base substitutions per site.

of Blue Lineage fish may have occurred at multiple times during their evolutionary history, resulting in apparently closely related Cutthroat Trout on both sides of the Continental Divide, with perhaps Green Lineage trout radiating into the Rio Grande basin to give rise to the Rio Grande Cutthroat Trout before invading streams to the north in the Arkansas and South Platte basins. The haplotype tree (Figure 2) does hint at a common ancestor for Rio Grande, Green Lineage, and South Platte native, which is supported by a minimum spanning haplotype network (Figure 3). This implies that these fish made it across the Divide at some point in their evolutionary history. There is no compelling reason to believe that would have been an isolated incident.

Haplotypes representing Rio Grande Cutthroat Trout were recovered from all of the putative Rio Grande Core Conservation Populations sampled but not anywhere else outside of their native range. Substantial structure was indicated among these populations (Figure 2) consistent with earlier work on the subspecies (Behnke 1992; Behnke 2002; Pritchard and Cowley 2006; Pritchard et al. 2009) that showed significant differentiation between fish in the Pecos and Canadian drainages compared to those from the upper and lower Rio Grande basins. Our data are consistent with those findings, with a unique endemic haplotype found in the Pecos drainage (Streams 9, 10, and 14). Populations from the Canadian River drainage (Streams 22 and 36) also harbor some unique

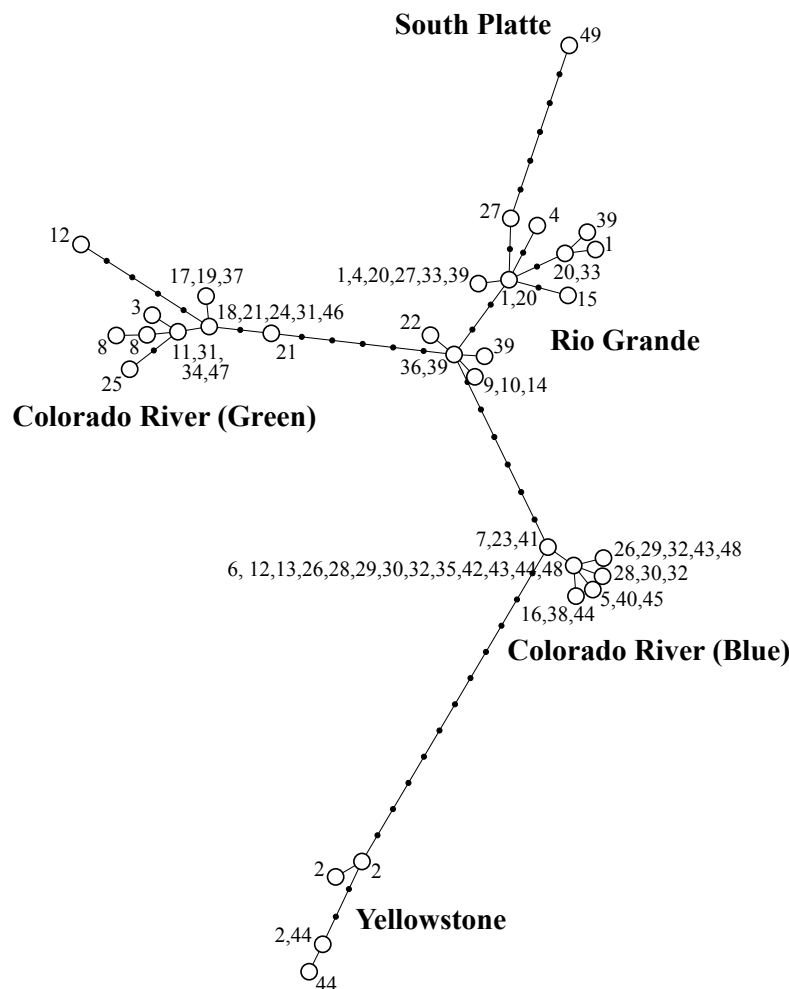


Figure 3: A minimum spanning network generated for haplotypes recovered (open circles) from a 648 bp variable region of the ND2 mitochondrial gene. Line segments represent a single mutation and black dots represent unsampled haplotypes. Numbers identify the population from which a given haplotype was detected.

haplotypes that appear to align with the Pecos drainage clade. Two of twelve trout collected from Leandro Creek (Stream 39, NE New Mexico, Canadian River GMU) displayed a more common “main basin” Rio Grande haplotype. This population was founded from Ricardo Creek stock (not part of this study), that also showed similar haplotypic diversity that Pritchard et al. (2009) suggested might reflect past anthropogenic transplants.

Of particular interest were the haplotypes recovered among Green Lineage fish. While clearly members of the same clade, it is interesting to note that those recovered East of the Divide were not the same as those recovered on the West Slope. This was unexpected since the current paradigm suggests that those Green Lineage fish found east of the Divide were founded from early stocking efforts in the very early 1900s that derived their fish from West Slope sources (Metcalf et al. 2012). As such, we should expect them to share haplotypes with other West Slope populations, which they do not. In fact, the only trout that share the haplotype found in the South Prong of Hayden Creek (Stream 3, SE Colorado, Arkansas River GMU) are a pair of specimens collected by David Starr Jordan in 1889 from Twin Lakes in the headwaters of the Arkansas basin, now housed at the Smithsonian (Metcalf et al. 2012). Although numerous species of nonnative salmonids had already been stocked into Twin Lakes by 1889, it does suggest the possibility at least, that Green Lineage fish may have again found their way across the Divide, into the Arkansas basin during the recent Pleistocene and begun to differentiate.

While sequence divergence among the different lineages of Cutthroat Trout is perhaps more subtle compared to other recognized coldwater fish species of the Rocky Mountains (Whiteley et al. 2006, Young et al. 2013), it is clear that enough structure exists to begin to suggest phylogenetic relationships in addition to identifying where remnants of past diversity might remain. Rare haplotypes (those found in only a single population) were recovered from three of the four lineages. Like earlier studies (Metcalf et al. 2012), the haplotype that matched those historically found in the South Platte basin were only recovered from Bear Creek (Stream 49, SE Colorado, Arkansas River GMU) on the eastern flanks of Pikes Peak, reconfirming the value of this population for

conservation efforts. Rare haplotypes recovered in both Rio Grande Cutthroat Trout and Green Lineage populations tended to occur around the periphery of their respective ranges or in marginal habitats lacking headwater lakes that may have attracted the attention of early fish culturists.

Only Green Lineage fish seem to be largely unaccounted for in terms of assignment to a recognized taxonomic group in the southern Rocky Mountains. Regardless of whether formal designation as a subspecies is warranted or if Green Lineage fish simply come to be known as an evolutionary significant unit or distinct population segment within Colorado River Cutthroat Trout, it is critical that we seek to preserve the substantial diversity contained in this lineage. With only 60 conservation populations identified to date (Rogers 2012b), these fish clearly deserve our attention if we are to preserve that diversity for future generations. Our hope is that management efforts will focus less on what trout from a given location are called, and more on preserving the native genetic diversity contained in them, regardless of where it is found on the landscape.

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