Phenotype predicts genotype for lineages of native cutthroat trout in the Southern Rocky Mountains

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Cover, top to bottom, depicts Bear Creek, Blue Lineage, Green Lineage, and Rio Grande cutthroat trout from the Southern Rocky Mountain region

IN MEMORIAM (1929-2013)

We recognize Dr. Robert J. Behnke as the driving force behind understanding the distribution, taxonomy, and conservation of salmonids in North America over the last half century. His work was particularly focused on description of taxa and conservation of the incredible diversity contained in the native trout of the Rocky Mountain west. We wonder what little might be left of that native diversity had "Doc" not dedicated his professional life to studying these fishes. His teachings and writings stimulated our interest in continuing to explore the taxonomy of cutthroat trout in the American West, and further his long-standing commitment to conserve these beautiful native fishes.

EXECUTIVE SUMMARY

Cutthroat trout *Oncorhynchus clarkii* is a widespread species with 14 recognized subspecies distributed across the western United States and Canada. Recent genetic investigations, using contemporary and historical museum specimens, have called into question the traditionally accepted taxonomic and systematic relationships of cutthroat trout in several of those subspecies found in the Southern Rocky Mountains. This was due, in part, to early and widespread distribution of hatchery-reared cutthroat trout across drainages, which obscured the true heritage and relationships of cutthroat trout across the region. Molecular studies on museum specimens suggested evidence of six historical lineages, but only the Blue Lineage (presumptive Colorado River cutthroat trout), Green Lineage, South Platte River basin native cutthroat trout (represented by Bear Creek fish, presumptive greenback cutthroat trout), and Rio Grande cutthroat trout are believed extant. Putatively pure specimens from populations that represent those extant lineages were collected, to determine if traditional morpho-merisitic approaches could better classify cutthroat trout distributions under the traditional Geographic Model (East Slope and West Slope of the Rockies, and Rio Grande basin) or if distributions followed the more recent genetics-based classifications under a newer Molecular Model.

The Molecular Model was more successful identifying groups (subspecies or lineages) of cutthroat trout based on within-lineage or taxa similarities in morphological traits than the traditional Geographic Model. This was true whether comparisons among groups were for individual meristic traits, groupings in the principal component analysis scatter plots using four or eight variables, or the discriminant function classification analysis. Further, individual traits and discriminant function analysis also showed substantial structuring within lineages, organized by major drainage (GMUs). As the cutthroat trout taxonomic literature suggests, the Geographic Model using a limited suite of morphological traits showed only moderate structuring of populations examined in this study and East and West Slope populations of cutthroat trout were similar in meristic traits (e.g., lateral series scale and gill rakers counts and spotting patterns). Bear Creek fish were distinct under each classification because of differences in several traits, as were Rio Grande cutthroat trout populations. Blue Lineage populations were distinct in the Molecular Model (100% classification success), unlike the same populations in the Geographic

Model, of which 44% were misclassified. Inconsistencies in classification of Green Lineage fish (individuals and populations) under the Molecular Model using discriminant analysis were due mostly to four Green Lineage populations found on the East Slope that showed distinct morphological and subtle genetic differences in traits relative to West Slope Green Lineage populations and Bear Creek fish.

How results presented here and recent molecular studies on cutthroat trout of the Southern Rocky Mountains will shape future management is not yet clear. A logical first step would be to determine if the four lineages studied here constitute recognizable and definable groups at a level of taxonomic organization such as subspecies. Previously published genetic studies suggest Bear Creek fish likely represent cutthroat trout native to the South Platte River basin (Metcalf et al. 2012). Those researchers also reasoned that Blue Lineage fish were likely best represented by fish recognized now as *O. c. pleuriticus*, but with a more restricted distribution than was historically recognized. Taxonomic status of Rio Grande cutthroat trout is largely unchanged by recent genetic and morphological studies, save for possible recognition of distinct population segments or evolutionary significant units. In the Southern Rocky Mountains, only Green Lineage fish seem to be largely unaccounted for in terms of assignment of an extant lineage to a recognized taxonomic entity. East and West Slope Green Lineage fish have distinct morphological and genetic differences that need additional investigation. Regardless of whether formal designation as a subspecies is warranted or if Green Lineage fish are simply recognized as an evolutionary significant unit or distinct population segment within Colorado River cutthroat trout, description of morphological variation of that and all other lineages is appropriate and needed. Minimally, this would assist managers with understanding historical and taxonomic origin of yet undiscovered or incompletely studied populations of cutthroat trout. This information would help focus conservation and recovery actions.

Combining traditional taxonomic metrics with molecular analyses of the same individuals and populations yielded robust study results upon which to weigh the merits of various taxonomic arrangements of cutthroat trout native to the Southern Rocky Mountains. Such an approach enabled censoring of individuals admixed with either rainbow trout or Yellowstone cutthroat trout that was not possible in previous morphometric studies, thus honing our classification ability. This advantage over traditional studies with limited taxonomic traits

and limited statistical treatment of data permitted substantively enhanced classification success of cutthroat trout to their appropriate lineages.

We conclude that historically-used meristic traits and spot counts, when combined with more sophisticated statistical techniques, were powerful tools for differentiating lineages and subspecies of cutthroat trout in the Southern Rocky Mountains. Recognition of those patterns was obscured from previous investigators by extensive historical stocking and unrecognized establishment of various lineages on the landscape outside of their historical ranges. Population structuring at the drainage basin level, as recognized with morphological techniques in this study, supports the long-held notion that population management and restoration activities should emphasize preservation of the unique genotypes that likely evolved in concert with the environment. Preservation of that genetic diversity, regardless of where it resides on the landscape, should be a guiding principle for future management.

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INTRODUCTION

Cutthroat trout *Oncorhynchus clarkii* is a widespread species with 14 recognized subspecies distributed across the western United States and Canada (Behnke 1992; Behnke 2002; Trotter 2008). Behnke (1992) suggested existence of four main cutthroat trout subspecies groups: coastal cutthroat *O. c.clarkii* that is native to coastal streams of the Pacific slope, Lahontan cutthroat trout *O. c. henshawi* and associated forms of the Great basin region, westslope cutthroat trout *O. c. lewisi*, and other forms from the upper Columbia and Missouri drainages including Yellowstone cutthroat trout *O. c. bouvieri*, and finally, subspecies such as Rio Grande cutthroat trout *O. c. virginalis*, from the Southern Rocky Mountains. Cutthroat trout in the Upper-Missouri and Southern Rocky Mountain areas, which are geographically the most removed from coastal ancestors, are thought to be closely related, based on geographic proximity, presumed recent isolation and evolution, and similarities of morphological and genetic traits (Leary 1987; Behnke 2002). The concept of four main subspecies groups was supported by Leary (1987), based on allozyme data, as well as more recent molecular studies (Utter and Allendorf 1994; Loxterman and Keeley 2012).

Cutthroat trout of the Southern Rocky Mountains in southwest Wyoming, eastern Utah, Colorado, northern New Mexico, and northeast Arizona were thought to be derived from southward expansion of Yellowstone cutthroat trout or eastward expansion of Bonneville cutthroat trout *O. c. utah* (Behnke 1992; 2002 in part) and historically were represented by four subspecies based on morphological differences, and geographic separation of major drainage basins. Colorado River cutthroat trout *O. c. pleuriticus* was thought native and restricted to the Colorado River basin in streams west of the Continental Divide. Colorado River cutthroat trout were assumed to be the likely ancestor to other forms to the south and east based on presumptive direction of invasions and similarities in morphology. Rio Grande cutthroat trout, the southernmost cutthroat trout subspecies, was thought to be native to the upper Rio Grande basin in southern Colorado and northern New Mexico, including Pecos and Canadian River drainages as well as streams in the Rio Grande proper. Greenback cutthroat trout *O. c. stomias* was thought to be native to streams on the East Slope of the Continental Divide in Colorado in the Arkansas and South Platte River basins. Greenback cutthroat trout were assumed to be sympatric with yellowfin cutthroat trout *O. c. macdonaldi* in the headwaters of the Arkansas River in Twin Lakes where the latter was considered endemic and very

restricted in distribution (Jordan 1891; Behnke 2002; but see Wiltzius 1985). Co-occurrence of yellowfin and greenback cutthroat trout represents the only known instance of sympatry in native cutthroat trout of North America (Behnke 1992; Behnke 2002; Trotter 2008).

Recent genetic investigations, using contemporary and historical museum specimens, have called into question the traditionally accepted taxonomic and systematic relationships of cutthroat trout in the Southern Rocky Mountains. Using a combination of mitochondrial and nuclear DNA analyses with contemporary cutthroat trout specimens from across the East and West slopes of Colorado, Metcalf et al. (2007) found Colorado River and greenback cutthroat trout subspecies not only in their presumed historical drainages, but also representatives of the former on the East Slope, and representatives of the latter on the West Slope. Metcalf et al. (2007) presumed presence of Colorado River cutthroats on the East Slope was due to widespread stocking, which was well underway by 1900, with fish from several sources in western Colorado (Metcalf et al. 2012). It was suggested that the putative "greenback" cutthroat trout in a Colorado River basin stream (West Antelope Creek in Gunnison River drainage) was also founded by stocking, though that assertion was problematic because few fish were stocked in West Slope streams from East Slope sources (Metcalf et al. 2012). Alleged presence of greenback cutthroat trout in a West Slope stream was a finding mostly overlooked because a larger issue emerged, that being the broodstock used for greenback cutthroat trout restoration on the East Slope appeared to be largely based on fish ultimately derived from the Trappers Lake region in the headwaters of the White River on the West Slope.

Because of early and widespread distribution of hatchery-reared cutthroat trout across drainages, and the potentially clouded nature of the heritage of cutthroat trout across Colorado, additional investigations were conducted using alcohol-fixed museum specimens collected prior to extensive stocking (Metcalf et al. 2012). That investigation was possible because old specimens (all > 100 years old) were not fixed first in formalin but instead were preserved directly in ethanol, which permitted use of "ancient" DNA techniques to resolve relationships (Metcalf et al. 2012). Despite low specimen numbers and degraded DNA, which limited the number of loci available for study, those investigations revealed even more complex relationships among cutthroat trout populations in Colorado than initially suspected. Historical specimens with genetic material consistent with Colorado River cutthroats were still found in their native West Slope range, but only in northwest Colorado in the Green, Yampa, and White River drainages. Those Colorado River

cutthroats were designated the “blue lineage” (here Blue Lineage), to reflect the uncertain taxonomic associations of cutthroats among various West Slope drainages even though they likely represent the archetypal *O. c. pleuriticus* (Metcalf et al. 2012). A second group, from the upper Colorado, Gunnison, and Dolores River drainages was designated the “green lineage” (here Green Lineage). This included the West Antelope Creek population from the Gunnison River drainage that Metcalf et al. (2007) had assigned to greenback cutthroat trout. Subsequent study suggested this population represented a lineage aboriginal to the West Slope (Rogers 2010), also referred to as Lineage GB. The Green Lineage also included fish from the Grand Mesa area of the Colorado-Gunnison River basin, where a major fish culture and distribution center was historically located, mostly to supply fish for other West Slope localities, as well as numerous locations east of the Continental Divide (Metcalf et al. 2012; Rogers 2012).

Alcohol-fixed museum specimens revealed more diversity. South of the range of Green Lineage fish a previously undetected genetic signature was found in the San Juan River drainage of southwestern Colorado, and indicated that area had a distinct lineage of cutthroat trout. That signature, however, was not detected in contemporary specimens, and the lineage is presumed extinct (Metcalf et al. 2012). Also, museum specimens from several South Platte River basin locations indicated a consistent genetic signature, but one that was not found in the Arkansas River basin, where greenback cutthroat trout was considered native. The archetypal taxon from the South Platte River drainage was traditionally considered greenback cutthroat trout (Behnke 1992) but contemporary specimens from sampled populations in the South Platte and Arkansas River drainages had genetic material consistent only with fish from either the Blue and Green lineages. The only extant population examined that had genetic material consistent with greenback cutthroat trout museum specimens from the South Platte River basin was found in Bear Creek, a small stream near Colorado Springs, Colorado, in the Arkansas River basin, outside of its native range. These Bear Creek fish were believed to have originated from a South Platte River source near the headwaters of the then fishless Bear Creek. Details of the Bear Creek cutthroat trout establishment were chronicled in Kennedy (2010), Metcalf et al. (2012), and Rogers (2012).

A final conclusion of the molecular analysis of museum specimens was the distinctness of one form of native Arkansas River cutthroat trout (Metcalf et al. 2012). As long postulated (Jordan 1891; Behnke 1992), specimens labeled as yellowfin cutthroat trout harbored a unique genetic signature which was also detected outside of Twin Lakes, suggesting a more widespread distribution

for yellowfin cutthroat trout, as espoused by the Colorado fish commissioner in the late 1800s, Gordon Land (Wiltzius 1985), rather than being endemic only to Twin Lakes (Jordan 1891; Behnke 2002). In addition, some fish from that same collection that were labeled greenback cutthroat trout also shared this unique yellowfin mitochondrial haplotype, suggesting that some of those specimens might have been misidentified. Metcalf (2012) found no evidence of South Platte River basin native cutthroat trout in the Arkansas River basin. The remaining "greenback cutthroats" in 1889 collections from Twin Lakes were genetically consistent with Green Lineage cutthroat trout to the west rather than the South Platte native form. Whether these Twin Lakes Green Lineage cutthroat trout were native or transplanted, even in 1889, remains unknown. It should be noted, however, that by that date, rainbow trout (*O. mykiss*), lake trout (*Salvelinus namaycush*), and Atlantic salmon (*Salmo salar*) had been introduced there. Regardless, yellowfin cutthroat trout of the Arkansas River basin has not been detected in contemporary samples and is therefore thought extinct. Thus, of six lineages detected by analysis of genetic material from ancient museum specimens, only Blue Lineage (presumptive Colorado River cutthroats), Green Lineage, South Platte River basin native cutthroat trout (presumptive greenback cutthroat trout, sensu Metcalf et al. 2012), and Rio Grande cutthroat trout are believed extant. Of surviving lineages, only the taxonomic status of the orange lineage (= Rio Grande cutthroat trout) of Metcalf (2012) remains unaffected by this recent work.

Assuming that the drainage definitions of lineages by Metcalf et al. (2012) are the best representation of what historically occurred, and given the early and large numbers (many millions) of fish that were distributed and established across the landscape from various hatcheries prior to detailed taxonomic investigations, it is difficult to imagine that ichthyologists working in Colorado with a few traditional meristic traits would or could emerge with a clear resolution of distribution of native trout taxa. Indeed, it has been repeatedly stated that Colorado River and greenback cutthroat trouts are impossible to differentiate morpho-meristically (Behnke 1992; Behnke 2002) or with allozyme analysis (Leary 1987). This is a logical conclusion given that the previously unknown lineages were already stocked widely and existed undiscovered across the landscape. Now that distributions of those lineages have been better defined and presumably pure specimens from populations that represent those lineages can be collected, this study was undertaken to determine if traditional morpho-merisitc approaches could confirm recent genetic-based classifications. Specifically, for the four lineages that survive, our task was to determine if traditional taxonomic techniques could be used to differentiate specimens of Blue Lineage, Green Lineage, Bear Creek

(presumptive South Platte native), and Rio Grande cutthroat trout. We then compared taxonomic traits of lineages defined by molecular studies (sensu Metcalf et al. 2012) to traits of traditionally recognized cutthroat trout subspecies (Behnke 1992) to see which taxonomic arrangement better explained cutthroat trout distributions in the Southern Rocky Mountains. We follow with discussion supporting the nuances uncovered in this analysis using traditional taxonomic traits, make suggestions for future studies, and clarify where data do and do not support the Molecular classification model and existence of the four discrete taxonomic groups of cutthroat trout across our study area.

Understanding the discriminating power of meristic traits to identify subspecies or lineages of cutthroat trout is critical as it drives conservation efforts for all forms listed or petitioned to be listed under the Endangered Species Act (ESA). While some have argued that molecular methods should drive that decision (Tautz et al. 2003, Allendorf et al. 2004, Allendorf et al. 2005), others have suggested that approach is too conservative because populations with any detectable (or suspected) levels of introgression would not be considered eligible for conservation and protection under ESA (O'Brien and Mayr 1991; Dowling and Childs 1992; Campton and Kaeding 2005). The U.S. Fish and Wildlife Service has concluded that introgressed populations warrant ESA protection if they "conform phenotypically" to the scientific description of the subspecies (USFWS 2003). This position was affirmed by the courts in its deliberation of the legal merits of listing westslope cutthroat trout (American Wildlands et al. vs. Kempthorne, U. S. Court of Appeals No. 07-5179). Because phenotypic traits are the standard upon which these taxonomic decisions are based, morphometric information will contribute to determining if the extant cutthroat trout lineages represent discrete subspecies or are simply genetic variants of a broadly distributed taxon.

METHODS

Population selection protocol. —A fundamental principle of this study was to ensure even and random representation of the range of variation of morpho-meristic characteristics present in cutthroat trout among the various lineages investigated. Geographic bounds of each lineage was 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 and Green and lower Colorado River drainages in northwestern Colorado, southwestern Wyoming, and eastern Utah. We presumed variation within the lineages would be spatially organized based on potential for isolation and differentiation in or across drainage basins. Thus, populations of each lineage were grouped within U. S. Geological Survey 4-digit Hydrologic Unit Code (HUCs) units. Those HUCs also served as geographic management units (GMUs) by the various conservation teams responsible for cutthroat trout management (Hirsch et al. 2006, Alves et al. 2008). Blue Lineage streams were in four GMUs; Yampa River drainage (including White River drainage), upper Green River drainage, lower Green River drainage, and lower Colorado River drainage.

The presumed native range of Green Lineage cutthroat trout was streams in the upper Colorado, Gunnison, and Dolores River drainages, located in southwestern Colorado and east-central Utah (Rogers 2010, Metcalf et al. 2012). Origin (native or introduced) of Green Lineage fish in the Arkansas River basin is uncertain. Metcalf et al. (2007) identified populations as West Slope lineage greenback cutthroat trout, given its similarity to what were believed to be the native cutthroat trout east of the Continental Divide in the South Platte and Arkansas River drainages. Subsequently, Rogers (2010) suggested these fish might be something other than greenback cutthroat trout and might be native to the West Slope, a finding that was confirmed by examining museum specimens collected prior to large-scale stocking activities (Metcalf et al. 2012). Those museum specimens also indicated a very restricted native range for greenback cutthroat trout that did not include West Slope streams. Thus, West Slope Green Lineage cutthroat trout populations were in three GMUs: one each for upper Colorado River, Dolores River, and Gunnison River drainages. South Platte River drainage native cutthroat trout were limited to the introduced populations in Bear Creek of the Arkansas River drainage (Metcalf et al. 2012), and Rio Grande cutthroat trout were only in the Rio Grande basin of Colorado and New Mexico and distributed among four GMUs, Canadian, Pecos, upper Rio Grande, and lower Rio Grande drainages.

Cutthroat trout databases maintained by the Colorado River Cutthroat Trout Conservation Team (Hirsch et al. 2006), Rio Grande Cutthroat Trout Conservation Team (Alves et al. 2008), and Greenback Cutthroat Trout Recovery Team (unpublished data) were used to identify

candidate populations for taxonomic investigation. Only core conservation stream populations (unaltered genetic status, but variously determined by recovery teams) were considered for inclusion in this study. Three candidate populations from each GMU were selected at random to ensure that morphological and genetic diversity was well represented and not influenced by personal knowledge of morphotypes or the perceived need to include a particular stream because it was unusual. If both Blue and Green lineages were present in a GMU, up to three of each was selected. One exception to the protocol was in the upper Colorado River GMU where one assumed Blue Lineage population selected (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 limited 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. 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).

Sample collection.—Because many streams that supported pure populations of native cutthroat are relatively cold and at high elevation, maximum fish size expected in those systems was relatively small. Thus, we intended to use only specimens in a comparatively narrow length range of 178 to 229 mm total length (TL) so that any variation in traits due to size differences was minimized; limited numbers of desired size fish in several instance necessitated using some smaller specimens. We assumed no temperature-induced differences of meristic or morphological traits, as most streams were at similar high elevations.

Specimens were captured by electrofishing or hook and line. Fin clips (upper caudal or right pelvic) were also collected for subsequent genetic analysis, and care was taken to ensure that tissue collection did not compromise specimens for morpho-meristic examination. After tissue collection, specimens were anaesthetized in MS-222, and placed in 10% formalin; a small incision in the right abdominal wall of the fish allowed formalin to preserve internal organs. Fish were fixed in formalin for at least 21 days after which they were rinsed and placed in successive washes of 25 and 50% ethanol for 4-5 days each, with a final preservative of 70% ethanol. Individual fish were tagged with a coded label and jars were similarly labeled, all by a third party, to ensure that collection locality of samples was unknown to investigators conducting morpho-meristic assessments. This strict blind protocol ensured that investigators collecting morpho-meristic data were not influenced by knowledge of the geographic locality of the stream or specimens. All specimens are housed at the Larval Fish Laboratory, Colorado State University.

Morpho-meristic data collection.—We selected traits to measure or count based in part on what was historically used in cutthroat trout taxonomic studies so comparisons could be made with information presented in the literature. Because few historical studies included strictly mensural traits (e.g., head length and body depth), presumably because of variation induced by environmental or other effects, we chose not to include those in this study. Meristic traits were made according to Hubbs and Lagler (1947) or Behnke (1992), with modifications as described below. Lateral series scales were counted 1-2 rows above the lateral line with the aid of a dissecting microscope (10X magnification), beginning with the scale posterior to the pectoral girdle and ending at the posterior end of the hypural plate. Scales were often embedded or covered in mucous and were difficult to count so the surface of the specimen was blotted and sometimes allowed to dry slightly. This caused the mucous directly above the scale to

differentially shrink and created an easier-to-count dimple in the scale surface, compared to the tissue surrounding the scale that did not dry as quickly. It was also sometimes helpful to identify the appropriate scales to count based on the presence and orientation of the scale row as it descended from the dorsal surface backwards toward the lateral line. Two investigators (RG and KRB) made replicate counts of lateral series scales on specimens in five samples of cutthroat trout (n = 37 total specimens) to determine if counts were consistent. The mean difference in scale counts for samples among investigators as a percentage of the mean number of scales counted by both investigators was small at 0.85% (0.15-1.88%); this equated to an average count difference of 1.6 scales (0.3 to 3.4) on specimens that had mean number of 182 scales. We used mean differences among samples (rather than specimens) because means of meristic characters among populations (streams) is the most often used method of comparison. The Pearson correlation coefficient of mean scale count among investigators for individual fish and the absolute value of the differences in scale counts among the investigators was low (r = 0.32). This suggested that number of scales was only weakly related to scale count differences; we examined this relationship because we thought differences among investigators might be affected by increased difficulty of obtaining accurate counts on specimens with more scales. Lateral series scale counts were sometimes difficult to obtain or became increasingly uncertain after repeated attempts because of damage to scales or irregular spacing. If a specimen in a sample had a missing lateral series scale count (n = 35 total), we substituted the mean number of lateral series scales for the remaining specimens in that sample. This allowed for use of data for other traits in statistical analyses that would otherwise have been excluded, without affecting the mean scale counts in the sample. However, mean lateral series scale counts for populations do not include substituted values.

We counted scales above the lateral line, a historically used trait (Behnke 1992), from the origin of the dorsal fin backwards to the lateral line. However, the dorsal surface and associated scales were typically covered with thick mucous or scales were especially deeply embedded which necessitated excavating them with a sharp probe, or estimating the count for a portion of the series that was difficult to detect. Because we were not confident of some of those counts, we excluded those data from further analysis. Pelvic fin rays (left fin) were counted, taking care not to miss the smallest rays nearer the body. Anterior gill rakers of the first arch (right side used to prevent disfiguring the opercle of the specimen on the left side) were counted on both the

upper and lower limbs; gill rakers on the posterior margin of the first arch (total) were also counted and included any obvious protuberances. Basibranchial tooth counts were obtained via inspection of the basibranchial area with a dissecting microscope (10X magnification) after staining the tissue overnight with a solution of Alizarin Red; we did not dissect or scrape soft tissue to reveal teeth. All pyloric caeca tips were counted by removing them from the main group after the stomach was removed from the fish and the intestine unwound. Inter-investigator differences in counts of gill rakers, basibranchial teeth, or pyloric caeca were not conducted because we believed those counts could be made without error.

Historically, spotting patterns of cutthroat trout, including the size, distribution, and number of spots, have been used to describe various taxa but only in a qualitative manner (Behnke 1992, but see Qadri 1959). We quantified numbers of spots on cutthroat specimens (using left side counts except as noted for top of head) by counting each spot (a pigment concentration at the surface of the skin, visible to the naked eye regardless of size, but not including deeper pigmentation concentrations such as parr marks) in seven areas; the lateral surface of the head and six regions of the trunk, excluding those on any fins (Figure 1). Head spots included those on the top of the head and opercle. The trunk of the fish in lateral view was divided into anterior, middle, and posterior thirds, with the anterior 1/3 consisting of the body (skin underlain with muscle tissue, and not the opercle) from just behind the opercle to origin of the dorsal fin, the middle 1/3 from origin of the dorsal fin to origin of the anal fin, the posterior 1/3 from origin of the anal fin to the end of the caudal peduncle, which was defined as the area just posterior to the hypural plate where caudal fin rays were clearly visible. No spots were counted on the caudal fin. The section boundaries were a vertical line at the respective origin of the dorsal and anal fin that was transverse to the long axis of the fish. Those thirds of body sections were each then divided into upper and lower halves by the lateral line. A spot was counted in a section if more than half of it was located inside the line of demarcation. The same majority rule was used for spots on the dorsal and ventral surfaces and spots on both the body and a fin; if more than half the spot was located in a particular section, it was counted. Section boundaries were demarcated with the edges of a paper towel during counting to aid in identifying the appropriate spots to be counted in each section. Presence (yes/no) of spots on the top of the head, a separate trait, was determined by examination of the top of the head on either side of the occipital division from a dorsal view.

Consistency of spots counts made by a single investigator was compared among three separate counts taken at different times for total counts per fish (n = 137 individuals; all trunk sections were summed, but not including the head). Variation in replicated spots counts was low. The difference in maximum and minimum counts for a single fish, as a percentage of the mean total number of spot counts per fish, was 2.0 % (0-6.1%); the average spot count per fish was 173 (28 - 498). The Pearson correlation coefficient between the mean spot count per fish and the difference between the maximum and minimum number of spots per fish was low (r = 0.04), which suggested that larger differences in spot counts were not induced by the potential increased difficulty of counting more dense and smaller spots.

Quantification of spot numbers by section allowed not only for total spot counts on the trunk, but also quantification of spot distribution patterns (relative coverage) over the body. This was done by summing the number of spots in all upper and all lower sections and dividing the former by the latter. Because cutthroat trout spots are typically concentrated dorsally the resulting ratio was nearly always > 1; a ratio close to 1 suggested a relatively even spot distribution from the dorsal to the ventral direction and a number less than 1 suggested concentration of spots ventrally. Other ratios to describe spot distribution were calculated by dividing total number of spots in the anterior-most two sections (one above and one below the lateral line) and the two middle-body sections by total spot counts in the upper and lower posterior-most sections that included the caudal peduncle area (fore-spot and mid-spot ratios, respectively). Each of those ratios was typically < 1 because spots on cutthroat trout are usually concentrated posteriorly; a ratio near 1 suggested a more even spot distribution from anterior to posterior while a number greater than 1 suggested spots relatively concentrated anteriorly.

The final spot characteristic quantified was largest mean spot size. This was estimated by measuring, with a dial caliper (nearest 0.1 mm), the greatest diameter of the three largest spots on the lateral surface, spots that typically occurred on or near the caudal peduncle. Sometimes spots were large as a result of two or more partially merged spots (e.g., a snowman shape) that were treated as a single spot; the mean size of the three largest spots was used as an analysis trait. Replicate spot size measurements were made on individual fish in three samples (n = 36 individuals) with relatively different sizes of spots among them, by two separate investigators, to determine accuracy and precision of spot size measurements. This necessarily included potential bias induced not just by measuring the spots correctly, but also by choosing which spots were the

largest on the fish. Absolute differences in mean spot size between investigators among the three samples compared were relatively small and averaged 4.8% of the mean of spot diameters among investigators (0.6 to 32.4%); two large outliers were the result of different interpretations of whether a pigment mass was one spot or two separate spots; other variation was due to measuring a relatively small structure (e.g., usually < 4 mm diameter) and the potential for small differences to be a large percentage of the total measurement. For example, a small 0.2 mm variation in spot size measurements among investigators for a 4 mm diameter spot results in a 5% difference in spot size. The Pearson correlation coefficient between the absolute difference of the mean spot size per fish among investigators and the mean spot size among investigators was low (r = 0.11), which suggested that larger differences in spot size measurements were not biased by mean spot size. The mean spot size measured among investigators was 4.24 mm (2.0-8.1 mm).

Molecular data collection.—The DNA from fin clips of each specimen were isolated in a blind fashion using a proteinase K tissue lysis and spin-column DNA purification protocol per manufacturer specifications (Qiagen DNeasy Kit). 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 assignment. 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 (ME) 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 ME tree was searched using the Close-Neighbor-Interchange (CNI) 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. Though all conservation populations were assumed to be pure, admixture with potential hybridizing taxa (rainbow trout and

Yellowstone cutthroat trout) in the nuclear genome was explored with amplified fragment length polymorphisms (AFLP). Fragment size was evaluated on an ABI 3130 sequencer (Applied Biosystems, Foster City, California). A genetic fingerprint was produced for each individual sample using the program Genemapper 4.0 (Applied Biosystems), by scoring for the presence or absence of a standardized set of 119 markers between 50 and 450 base pairs in size generated from reference cutthroat trout populations. The genetic fingerprints of individuals in the test population were compared to those found in the reference populations (Rogers 2008; Rogers and Heydinger in prep) using a Bayesian approach for identifying population clusters (Pritchard et al. 2000). The program STRUCTURE 2.2 (Falush et al. 2007; Pritchard et al. 2007) was used to determine similarity between the test individuals and reference populations. Reference populations were selected and grouped by their mtDNA lineage (Metcalf et al. 2007), and not by geographic or historic subspecies classifications. The similarity or dissimilarity was scored as the admixture proportion, or the probability that each test individual shared a genetic background with each of the cutthroat subspecies reference population groups. These proportions are expressed as q values for each subspecies. These q values were obtained by running STRUCTURE ten times for each population of interest using a burn-in of 50,000 steps followed by 50,000 Monte Carlo Markov Chain replicates. Average q values from the run with the highest log likelihood (Pritchard et al. 2007) were used to generate the admixture proportions for the unknown population. Confidence intervals around admixture were generated with the software application QSTRAP Version 3.1 (Rogers 2008b).

Data analysis.—We asked how similar the morphotypes of the various cutthroat populations were, assuming the traditional geographic distribution model (Geographic classification model, hereafter Geographic Model) alignment of subspecies was correct (e.g., Behnke 1992) and compared that to lineage distributions described by genetic data (Molecular classification model, hereafter Molecular Model). The Geographic Model had groups consistent with recognized subspecies from the East Slope of the Continental Divide, Colorado (*O. c. stomias*), the West Slope of the Continental Divide (*O. c. pleuriticus*) in Wyoming, Utah, and Colorado, and the Rio Grande basin (*O. c. virginalis*) in Colorado and New Mexico; we also included in this classification a fourth group from Bear Creek, to have consistency with the alignment suggested by Molecular Model (Metcalf et al. 2007; Metcalf et al. 2012) as that classification also recognized the Bear Creek and Rio Grande basin groups, in addition to the

Blue and Green lineages previously described. This analysis provided a multi-trait view of historical cutthroat taxonomy, which has been conducted only infrequently for those groups, and provided a comparison to other analyses that assumed the lineages outlined by Metcalf et al. (2007; 2012) represent the historical diversity of cutthroat trout. Cutthroat trout morpho-meristic characteristics are known to possess considerable variation, so classification used both traits for individual specimens as well as population metrics based on mean values of traits for populations from individual streams. Use of means for traits by stream is consistent with historical analyses (e.g., Wernsman 1973; Behnke 1992) and also allowed for comparisons to information presented in the literature.

We first compared basic summary data for the main morphological traits (e.g., mean, range, and frequency distributions) for groups organized by the Geographic Model as well as for groups consistent with the Molecular Model (Metcalf et al. 2007; Metcalf et al. 2012). We focused on eight character traits for most analyses. Four were traditional measures; lateral series scale counts, total number of anterior gill rakers, number of basibranchial teeth, and number of pyloric caeca, which allowed comparisons with assessments in the literature. We also chose four non-traditional metrics; total trunk spots, fore-spot ratio, mid-spot ratio, and mean largest spot size. Trait selection was based, in part, on the largest *F*-values for traits obtained from linear discriminant function analyses (SAS PROC DISCRIM) using all specimen data. Other characters not used in multivariate analyses were of interest and will also be discussed.

We examined how well 12 (small) and 24 (large) fish samples represented the morphological variation within and among cutthroat trout populations in each of two ways. First, we compared coefficients of variation (CVs) for various traits from samples in a GMU where a large sample was collected (23-26 specimens). We then calculated CVs of morphological traits for the large sample as well as for two small samples (9-13 specimens) from the same GMU. We then compared means of CVs from large and small samples to determine if variation was similar.

Second, we determined if 12 specimens from a 24 fish sample adequately represented variation of three meristic traits. Lateral series scale counts and basibranchial tooth counts had the highest and lowest variation (respectively) among lineages, so were selected for analysis. We chose total number of trunk spots to complement the two traditional characters. From each of the large samples we drew 1,000 small samples (without replacement) and calculated means

and 95% confidence limits for each sample. We then determined how many of the confidence intervals included the trait mean from the 24-fish sample and calculated confidence interval coverage as the total number of samples that included the large sample trait mean. That number was divided by 1,000, and multiplied by 100 to yield a percent. Based on $\alpha = 0.05$, nominal confidence interval coverage should be 95%, which suggests that the true population mean is captured by the confidence limit for 12 fish samples 95% of the time, consistent with statistical expectations. This analysis was useful for determining if small samples adequately captured variation in meristic traits for populations compared to large samples and may also be useful to guide future sampling efforts where meristic traits are evaluated on specimens from additional populations.

Although we constrained the size range of specimens used in this study to reduce the chance of size-dependency in spot attributes (e.g., larger fish have more or larger spots), we used correlation or regression analysis to determine if the restricted standard length (SL) size range of fish used in analyses affected number of trunk spots and mean spot size, and if mean spot size and spot number were correlated. This allowed us to test if fish size differences were responsible for differences in spot measurement among classification groups. Because selection of specimens retained for study was based on field determined total length, we used SL = -3.755 + 0.861*TL (derived from all study specimens, n = 744) to obtain SL, which allowed us to use the more reliable and traditional SL (Hubbs and Lagler 1947) in subsequent analyses.

We conducted a principal components analysis (PCA, PROC PRINCOMP, with correlation matrix) of trait data, to assign populations to Geographic or Molecular models. This allowed groups to cluster in the principal component space without *a priori* assignment of streams to specific taxa or lineages. This also allowed further clarification of the overlap of morphotypes previously reported and more importantly, permitted us to assess which, if any, groups of populations were relatively distinct from each other in the PCA for both Geographic and Molecular models. The exterior-most dots in PCA space, each of which represented mean component scores by stream population (not individuals), were enclosed in ellipses; we allowed for up to one outlier in each to be excluded (except for Bear Creek), which seemed reasonable given the wide variation in populations. Thus, an hypothesis under the Geographic Model would be that East and West Slope populations would broadly overlap based on presumed similarities and Rio Grande and Bear Creek populations would fall out somewhat separate based on reported

differences in lateral series scale, basibranchial teeth, and pyloric caeca counts. Similarly, a hypothesis for the Molecular Model would be more separation among groups within the Blue and Green lineages as well as preservation of distinction of Rio Grande and Bear Creek cutthroat trout. Examination of overlap among groups is clearly a subjective technique, but importantly does not make assumptions regarding where populations should be assigned *a priori*. We conducted the PCA analyses using only traditional meristic data (gill raker, lateral series scale, pyloric caeca, and basibranchial teeth counts) to test whether using traditional traits yielded informative patterns. We then included the more detailed spot count, spot pattern, and spot size information to determine if that approach improved distinctness of groups under each of the two classification models. This was done to illustrate the similarities and differences that would have been evident if using only traditional traits.

We then used discriminant function analysis to assess classification rates for individual fish as well as populations, again using either the Geographic Model or the Molecular Model. A discriminant classification function (SAS PROC DISCRIM) based on the eight morphological traits was used to determine classification rates for specimens from different groups. Covariance matrices were not equal among groups, so within-group covariance matrices and quadratic functions were used for classification (SAS Institute 1988). Constructing discriminant functions from a data set and then obtaining classification rates using the same data can lead to inflated classification rates. Therefore, the CROSSVALIDATE option (a jacknife resubstitution procedure) was used to test the discriminatory ability of the functions. This procedure is nearly unbiased (SAS Institute 1988) because observations are individually removed and the discriminant function is then rerun to reassess classification rates. Additionally, it is more robust and conservative than the Resubstitution procedure in PROC DISCRIM for classification, which yields overly optimistic classification rates (Lance et al. 2000). We used the same procedure to determine classification rates of populations under the assumptions of the Geographic and Molecular models. This was accomplished by using the mean trait values for each population as the cross-validation data to determine successful classification rates when the data from individual fish provided the training dataset. In other words, the entire sample of individual fish within a lineage or taxa was the basis for variation, and means of individual populations were then tested to determine if they classified within the populations they came from.

We conducted an additional discriminant classification analysis to characterize similarity and variation among populations within lineages. All individuals within a lineage were used to establish the training dataset and then cross-validation was used with individuals to determine classification rates of specimens to GMUs within the lineage area. Population means were also used to determine classification rates of streams to the correct GMU within the particular lineage.

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 2). Three populations of each lineage were not always chosen from each GMU, especially for transplanted populations, mainly because of insufficient numbers of available core conservation populations. 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. The relative paucity of conservation populations present east of the Continental Divide in the South Platte and Arkansas River basins was evident, when compared to basins west of the Continental Divide or in the Rio Grande drainage. Absence of 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. Density of coldwater streams is higher in West Slope locations and upper Rio Grande, Colorado, compared to other locations.

Specimen and stream censoring—A total of 837 specimens was available for study, including 72 from Bear Creek. Only 36 Bear Creek fish were selected leaving 801 total specimens. One Bear Creek sample (n = 24) was composed of 12 wild fish and 12 hatchery-reared fish (1st generation). An additional 12 hatchery mortalities, which were in good condition and in the desired size category, were selected for a second "population" to enable discriminant classification analyses that requires two or more samples of each lineage, and to increase sample size to assess morphological variation in this population. Additional Bear Creek individuals or "populations" were not used so that the presumed unique lineage was not overrepresented.

All specimens were screened with molecular methods to confirm that they fit within their anticipated clades using mitochondrial sequence data, then with amplified fragment length polymorphisms (AFLP) markers to discern evidence of introgressive hybridization in the nuclear genome (Table 2). We recovered 32 unique ND2 mitochondrial haplotypes in the 801 fish sampled from 49 populations that were distributed among five distinct clades 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, we recovered 12 Rio Grande haplotypes, nine Green Lineage haplotypes and six Blue Lineage haplotypes (Figure 3). The ND2 sequence data suggested that 47 of 49 populations were assigned to their anticipated lineages (Table 2, Figure 2). One of the two exceptions was Abrams Creek, where ND2 sequence data were more similar to a Green Lineage population rather than a Blue Lineage. 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 (n = 25). This population was removed from subsequent analysis. Genetic information for Irish Canyon specimens was supported by meristic data as mean number of lateral series scales (186; range = 174-194), pyloric caeca (n = 40; 28-49), and gill raker (19; 17-21) counts conformed well with Yellowstone cutthroat trout (Behnke 1992) rather than Blue Lineage/Colorado River cutthroat trout analyzed in this study.

Although only core conservation populations identified in the Cutthroat Databases were considered during the selection process, we detected several possible instances of introgressive hybridization of native cutthroat trout specimens with rainbow trout or Yellowstone cutthroat trout with the AFLP screening. If AFLP data suggested that native cutthroat trout individuals displayed > 0.5% admixture with either alien taxon, those individuals were eliminated from further analysis. Interestingly, 4 of 5 fish eliminated from Little Taylor Creek, a West Slope, Colorado, population from the Dolores River drainage (a Green Lineage population based on molecular data) were identified *a priori* as having rainbow trout influence based on relatively low lateral series scale counts (mean = 181, 174-191) in a population that otherwise had a mean scale count of 202 (178-219). Remaining putative pure specimens from Blue Lineage streams (n = 349 individuals, 21 streams) that were initially chosen for study were reduced by 15

individuals from Steel Creek (Stream 7, n = 2 specimens), Tabegauche Creek (Stream 12, n = 6), South Fork Cache la Poudre (Stream 13, n = 4), and Johnson Fork (Stream 45, n = 3). Thus, a total of 334 Blue Lineage specimens from 21 populations were available for further study (Table 2, Figure 2). We did not eliminate any specimens that had evidence of admixture among the native cutthroat lineages, which allowed for the potential that the slight admixture might represent rare alleles in a pure population.

A total of 229 Green Lineage specimens from 14 populations were available for initial analysis. Specimens from Nate Creek (Stream 8, n = 1), Little Taylor Creek (Stream 18, n = 5, mentioned above), Big Red Canyon Creek (Stream 21, n = 4), and Henderson Horseshoe Pond (Stream 34, n = 1), were eliminated because each possessed 0.5% or greater admixture of either rainbow trout or Yellowstone cutthroat trout alleles as measured by AFLPs. Thus, a total of 218 Green Lineage specimens from 14 populations were available for analysis. Those Blue and Green Lineage specimens and streams (n = 552 specimens, 35 streams) were also the same fish represented in the Geographic Model as follows; 25 streams and 386 specimens were allocated to West Slope populations and 10 streams and 166 specimens were allocated to East Slope streams. All 156 specimens of Rio Grande cutthroat trout from 12 populations were retained for analysis. Thus, a total of 744 specimens from 49 populations (including two groups of fish from stream 49, Bear Creek) were used in this analysis. Bear Creek was retained as distinct from other East Slope populations in the Geographic Model because it likely would not have been identified as a presumably pure population (e.g., rated as Grade B, indicative of some mixing with rainbow trout or other taxon, instead of a Grade A, "pure" population; Proebstel et al. 1996), and so it is consistently and similarly represented in both the Geographic and Molecular models.

Variation among small and large samples and fish length effects—We compared coefficients of variation (CVs) for morphological traits from 10 GMU's, each with one larger sample of 24 cutthroat trout and two smaller samples of 12 trout (30 total samples). For the eight main traits examined in this study, we found that variation in small samples for basibranchial tooth number exceeded that for large samples mainly because a few individuals in small samples had disparately high tooth counts. Of the seven remaining traits where mean CVs for the small samples were less than those for the large samples, small sample CVs averaged only 12% smaller (3-18%) compared to large sample CVs, despite having only about half the specimens of

the larger samples. Thus, comparisons of CVs indicated that small samples represented nearly 90% of character state variation present in larger samples.

Correlation of SL and spot number was weak and positive, but generally consistent among various Molecular classification groups (mean for classification units: $r = 0.30$; range, $r = 0.11$-0.43, Table 3). Only Green Lineage specimens were an outlier ($r = 0.11$), indicating that number of spots increased less as a function of specimen length than for other classification groups. For example, the regression equation of spot number as a function of SL for the representative Blue Lineage fish ($r = 0.35$) showed that spot numbers for specimens in specimen length range of 149 to 193 mm SL increased from 176 to 227 (difference of 51 spots; Spot number = 4.73 + 1.15*SL [mm]). Green Lineage fish showed a lower overall spot count and a smaller increase from 108 to 127 spots over the same specimen size range (difference of 19 spots; Spot number = 41.89 + 0.44*SL [mm]).

Correlation of SL and spot size was positive, and consistently correlated among classification units (mean among units; $r = 0.49$, range, $r = 0.35$-0.60). The relatively large-spotted specimens from Green Lineage and Rio Grande classification groups showed the lowest rate of increase of spot size with fish length, suggesting more stable spot size. This also suggested that as fish size increases, differences in spot size among lineages with large or small spots would continue to increase.

There was nearly no correlation of spot size with spot number in Bear Creek, East Slope, West Slope, and Blue Lineage fishes (mean over classification units; $r = 0.0$, range, $r = -0.13$-0.09). Spot size and number were negatively and weakly correlated for Green Lineage and Rio Grande specimens, indicating that as spot size increased spot number declined slightly.

Individual character comparisons—Mean specimen size, as SL, was similar across samples and presumptive taxa (Table 4). Minimal variation in mean specimen size across samples from various lineages and taxa reduced the chance of any size-dependent effects on morpho-meristic traits.

Lateral series scale counts were lowest for Bear Creek (mean 180.8) and Rio Grande (182.7) classification groups (Table 4; Figure 4). The similarity of mean scale counts for East and West Slope groups (198.4 and 201.5, respectively) was supported by overlapping 95% confidence limits. Mean scale counts were different for Blue and Green Lineage groups (198.4

and 203.4, respectively) and differences were supported by non-overlapping 95% confidence limits.

Anterior gill raker counts were lowest for Bear Creek specimens (mean = 16.4; Figure 5). The remaining classification groups had mean anterior gill raker counts of about 19, but were slightly lower for Green Lineage and Rio Grande specimens.

Basibranchial tooth counts varied substantially across groups (Figure 6). Bear Creek had the lowest mean count (mean = 1.5) and the highest incidence of specimens with no basibranchial teeth (61%). Rio Grande specimens had the next lowest mean basibranchial tooth counts at 5.9. That mean, however, represented populations that had individuals with relatively high (mean = 9.1, 6 populations) and low tooth counts (mean = 2.1, 6 populations). Geographic structuring for that character was evident as 5 of 6 populations with high counts were from the Rio Grande drainage proper (upper and lower Rio Grande GMUs) and one was from the Pecos River (Rio Valdez). Mean counts for Blue and Green Lineage fish were 8.3 and 8.5, respectively. The East Slope group had a lower mean tooth count than West Slope (6.2 vs. 9.3).

Mean number of pyloric caeca was similar (36 to 38) for most groups (Table 4; Figure 6), but Rio Grande specimens had a comparatively high mean number of pyloric caeca (41), and the 95% confidence limits for this trait did not overlap those for any other group. Mean pyloric caeca counts differed by about 2 for East and West Slope, but were practically identical for Blue and Green Lineages.

Trunk spot counts varied substantially among most classification groups (Figure 8); only Green Lineage and Rio Grande groups were similar (mean = 114 each). Bear Creek specimens had the highest mean trunk spot counts (218), followed by Blue Lineage (187), West Slope (163), and East Slope (147). Confidence intervals for East Slope-West Slope and Bear-Blue overlapped slightly, that for Green-Rio Grande was almost complete, but other pairings did not overlap.

The fore-trunk spot ratio also showed considerable differences among the Molecular classification groups, with Bear Creek the highest (mean = 0.71), followed by Blue Lineage at 0.58, Green Lineage at 0.42, and Rio Grande the lowest at 0.33 (Figure 9). The 95% CI's for the fore-trunk spot ratio overlapped for Bear Creek and Blue Lineage fish but not for other group combinations. East and West Slope group fore-trunk ratios were similar (0.55 and 0.51, respectively) and confidence limits overlapped.

The mid-trunk spot ratios were typically higher than the fore-trunk spot ratios and showed essentially the same pattern for Molecular classification groups except the Blue Lineage was highest (mean = 0.81), followed by Bear Creek (0.70), Green Lineage (0.57), and Rio Grande (0.43; Figure 10). The 95% CIs for those four groups did not overlap. The ratios for East and West Slope specimens revealed similar pattern of greater spot concentration posteriorly; although ratios were similar (mean = 0.76 and 0.69, respectively), their 95% CIs did not overlap.

Finally, mean largest spot size varied little among any of the groups, except that of Bear Creek was considerably less (Figure 11). Rio Grande specimens had the largest mean spot size, but were only slightly larger than those for Blue or Green Lineage fishes. Mean largest spot size for East and West Slope groups were also similar (3.90 and 3.76, respectively) and confidence limits overlapped.

Additional traits not included in multi-variate analyses also showed variation. For example, posterior gill raker counts were low for Bear Creek specimens (mean = 4.5, Figure 12) and raker count confidence limits did not overlap those for Rio Grande fish. The mean posterior gill raker counts for other Molecular groups were higher, ranging from 6.0 to 8.8. The Geographic classification groups for East and West Slope had similar mean posterior gill raker counts at 8.8 and 7.3, respectively, but confidence limits did not overlap.

The frequency of specimens with spots on top of the head in the classification groups essentially followed the pattern for trunk spot counts. Bear Creek (0.47) and Blue Lineage (0.56) groups had the highest proportion of specimens with spots on top of the head and Green Lineage (0.39) and Rio Grande (0.22) groups had fewer specimens with spots on top of the head (Figure 13); the 95% confidence limits did not overlap among those groups except for Bear Creek and Blue Lineage fish. East Slope and West Slope groups had similar percentages of specimens with spots on top of the head at 42% and 52%, respectively; the 95% confidence limits for those groups overlapped.

Intra-lineage variation-comparison of GMUs—Variation among the two populations of Bear Creek fish, one with wild and hatchery fish, and the other wholly hatchery fish, showed minor differences in lateral series scale (181 and 175, respectively) and mean basibranchial tooth (1.2 and 2.2, respectively) counts, but otherwise few differences were evident. Trunk spot count differences (218 and 227, respectively) might be due to the slightly larger size of fish from the latter population (140.9 versus 175.2 mm SL).

Blue Lineage fish were found in every GMU where fish were collected in this study except the Gunnison River drainage and the Rio Grande, and showed only modest variation in meristic traits examined (Table 5). For example, mean lateral series scales for populations from its presumed native range in the Green, Yampa, and lower Colorado River was slightly lower than for transplanted populations (mean of GMU population means was 196 and 200, respectively). Similarly, posterior gill raker counts were substantially lower in the native range of Blue Lineage fish compared to elsewhere (6.5 and 10.6, respectively). Finally, mean trunk spot counts were higher for presumed native Blue Lineage fish than transplanted populations (208 and 171, respectively), and similar to Bear Creek fish (221). Mean trunk spots were lowest in Blue Lineage fish from the Arkansas and South Platte River GMUs (144 and 165, respectively). Other traits varied (e.g., basibranchial tooth counts), but no geographic pattern was evident. The substantially higher basibranchial tooth count of cutthroat trout from the lower Colorado River GMU was notable.

Green Lineage fish were available only from their putative native range in the Colorado (upper Colorado River only), Dolores, and Gunnison River GMUs; no populations of Green Lineage cutthroat trout were available from streams in the Green, Yampa, lower Colorado, or San Juan River drainages. Populations were available from the Arkansas and South Platte River GMUs east of the Continental Divide. Several traits showed geographic structuring such that presumed native West Slope Green Lineage fish differed from East Slope Green Lineage fish (Table 6). For example, lateral series scale counts (Figure 14) were higher in their presumed native range (means for GMU means = 208) than in East Slope populations (mean = 194). Lateral series scale counts of East Slope populations were between that for West Slope Green Lineage fish and Bear Creek fish (mean = 179). A similar pattern was evident for basibranchial tooth counts as native Green Lineage populations had highest mean counts (11.0), presumably transplanted East Slope Green Lineage populations were lower (3.9), but higher than Bear Creek fish (1.7). Mean trunk spot counts were lowest for presumed native Green Lineage fish (mean = 96, although Upper Colorado River GMU fish were high with a mean of 156), higher in East Slope populations (mean = 136; Figure 14), and highest in Bear Creek fish (221). Similarly, fore-spot and mid-spot ratios were lowest for native Green Lineage fish (0.35 and 0.50, respectively), intermediate for East Slope Green Lineage fish (0.54 and 0.68 respectively), and

highest for Bear Creek fish (0.71 and 0.69, respectively). Other traits showed mixed patterns or little variation.

Rio Grande cutthroat trout also showed geographic structuring by GMUs, with lower and upper Rio Grande GMUs grouping together and having comparatively high counts and ratios for several traits compared Pecos and Canadian River GMUs (Table 7). For example, Rio Grande GMU fish had mean lateral line scale counts of 186 (mean of GMU means) compared to 180 for the Pecos and Canadian River GMUs (Pecos GMU was especially low). Comparisons among mean Rio Grande GMUs and the Pecos and Canadian River drainage GMUs showed substantial differences for basibranchial tooth counts (7.7 and 3.5, respectively), pyloric caeca (45 and 37, respectively), and total trunk spots (142 and 80, respectively), as well as for fore-spot (0.38 and 0.27, respectively) and mid-spot (0.45 and 0.39, respectively) ratios.

Side-by-side comparisons of East Slope Green and Blue Lineage GMUs (same data as in tables 5 and 6, but portrayed here for ease of comparisons in Table 8) showed lateral series scale counts of Blue Lineage fish were slightly higher than Green Lineage (203 versus 194) as was anterior gill raker count. Additionally, posterior gill raker, basibranchial tooth, and total trunk spot counts were modestly lower for Green than Blue Lineage fish, and each was substantially different than Bear Creek fish. Other traits for East Slope Green and Blue Lineages were similar (e.g., mean spot size differences), but were substantially different than Bear Creek. Blue and Green Lineage populations from the East Slope also had several traits that were different from populations of the same lineage on the West Slope. For example, East Slope Blue Lineage populations had higher lateral series scale counts and fewer spots than their West slope counterparts. Conversely, East Slope Green Lineage populations had lower lateral series scale counts and higher spot numbers than their West Slope counterparts. Those patterns made Blue and Green Lineage East Slope populations relatively similar to each other.

Multivariate analyses-PCA—Principal components (PC) 1 and 2 for stream population means that included the four historically used morphological traits and the Geographic Model accounted for 70% of total variation in the data (Table 9). The Bear Creek populations were widely separated from all other populations along PC 1, except Rio Grande, and the two samples from Bear Creek grouped closely together (upper left panel, Figure 15). Separation of Bear Creek populations from others was mainly due to its relatively low lateral line scale counts, low anterior gill raker counts, and low number of basibranchial teeth; slight separation of Bear Creek

and Rio Grande populations along PC 2 was due to relatively low numbers of pyloric caeca. Rio Grande populations were moderately distinct from the East and West Slope groups and Bear Creek in principal component space. Rio Grande populations separated along axis PC 1 based on relatively low lateral line scale counts and moderate basibranchial tooth counts, and along PC 2 based on relatively high pyloric caeca counts. Two Rio Grande cutthroat trout population outliers (Macho Creek, Pecos River drainage, above and left of Bear Creek and not in ellipse, and McCrystal Creek, Canadian River drainage, below and right of Bear Creek centroid, and in ellipse) forced the ellipse and the centroid for that taxon towards the negative region of both PC 1 and 2. Macho and McCrystal creeks aligned relatively closely with Bear Creek based on lower than average mean counts for: lateral series scales (170 and 181, respectively), anterior gill rakers (17.7 and 17.4 respectively), basibranchial teeth (0 and 1.3, respectively), and pyloric caeca (37.9 and 31.4).

East Slope and West Slope populations were relatively distinct from Bear Creek and most Rio Grande populations but showed much overlap among themselves in principal component space, as population centroids were close together and population ellipses overlapped broadly. East and West slope streams grouped together because of similar and relatively high lateral series scale, gill raker, and basibranchial tooth counts along PC 1; the centroid and a few populations were slightly separated along PC 2 because East Slope streams had slightly lower basibranchial tooth counts.

A similar PCA using streams organized by the Molecular classification technique showed relationships for Bear Creek and Rio Grande populations (upper right panel, Figure 15) similar to those portrayed by geographic classification. Centroids and population ellipses for these populations were nearly identical because none of those streams changed classification groups. A moderate difference noted for Blue and Green Lineage streams in the Molecular classification scheme was a tighter cluster of Blue Lineage streams (all Blue streams included in its elliptical space) that overlapped less with Green Lineage ellipse than under the Geographic Model. Nonetheless, group centroids remained similarly located in PC space. This was caused mainly by the negative eigenvector for lateral series scale counts on PC2, which provided modest separation of some low scale count Green Lineage populations from higher scale count Blue Lineage populations.

The PCA for stream population means with four historically used morphological traits plus four spot traits using PC 1 and PC 2 accounted for 53% of total variation in the data (Table 9, lower panels of Figure 15). In the Geographic Model, Bear Creek populations were again well-separated from all other streams and groups mainly along PC 2, due to relatively low lateral series scale, basibranchial tooth, gill raker counts, as well as relatively small mean spot size. Most Rio Grande populations overlapped broadly with East and West Slope populations in the Geographic classification, even though its population centroid was well separated from East and West Slope populations. That separation was mainly along PC1 and due mostly to lower lateral series scale counts, low number of trunk spots, and low fore-spot and mid-spot ratios. Distinctiveness of Rio Grande populations was enhanced by outliers, Macho and McCrystal creeks, whose placement in the extreme negative area of PC 1was due to very low mean trunk spot counts (69 in both populations).

Higher scale and trunk spot counts and higher fore-spot and mid-spot ratios separated East and West Slope centroids and populations from Rio Grande centroid and populations on PC1 in the Geographic Model. Near complete overlap of East Slope and West Slope, both for centroids and population ellipses, was based mainly on similarity of spot counts and similar fore-spot and mid-spot ratios.

A PCA using all eight traits with streams organized by the Molecular Model showed wide separation along PC2 of Bear Creek populations from all others. Unlike the Geographic Model, the Rio Grande centroid and streams were well separated from Blue Lineage populations on PC 1, based on lower lateral line scale counts but particularly lower trunk spot counts and smaller fore-spot and mid-spot ratios. Green Lineage populations were more closely aligned with the Rio Grande centroid and streams along PC 1 in the Molecular than Geographic Model, mainly a consequence of mean trunk spot count similarities, as well as Green Lineage fore-spot and mid-spot ratios that were between those of Rio Grande and Blue Lineages. Exclusion of the four East Slope Green Lineage populations with comparatively low lateral series scale counts and high trunk spot counts (Figure 14) from West Slope native Green Lineage populations (the dashed ellipse in the lower right panel, Figure 15) resulted in their complete separation from Blue Lineage populations. The Blue Lineage was separated in space along PC 1 by relatively high lateral series scales counts, and more importantly, high trunk spot counts and high fore-spot and mid-spot ratios.

Multivariate analyses-DFA for Geographic and Molecular models—The *F*-statistics and *p*-values produced in the discriminant function analysis (DFA) supported the overall use of the eight trait variables used to describe and classify populations of cutthroat trout in the Southern Rocky Mountains (Table 10). Among traditional variables, lateral series scales had the highest *F*-values, followed by anterior gill raker, basibranchial tooth, and pyloric caeca counts. Spot characters had relatively high *F*-values, which supported preliminary assessments of the higher discriminating power of spot traits relative to traditional taxonomic traits (analysis not presented). Among spot traits, mid-spot ratio and trunk spot counts had highest discriminating value, followed by spot size and fore-spot ratio.

The DFA supported the findings of individual trait analysis and PCAs that various cutthroat trout taxa were less structured for the Geographic Model than for lineages in the Molecular Model. Classification success of individual fish under the Geographic Model, using the cross-validation procedure when the training dataset is all other individual fish in the lineage, was comparatively high for Bear Creek and Rio Grande specimens (86 and 89%, respectively, Table 11); misclassified fish for each taxon were assigned to all other groups. Classification success of East Slope and West Slope individuals to their correct group was lower at 68 and 64%, respectively. Most misclassified East Slope fish were classified as West Slope fish, although 10% of East Slope fish were classified as Rio Grande cutthroat trout. Similarly, West Slope fish were most often misclassified as East Slope fish (28%), with a smaller proportion misclassified as Rio Grande (7%) or Bear Creek (1%) fish. Overall classification success for individual fish was 71%; collectively, 65% of East and West Slope fish were correctly classified.

Classification success of populations, based on mean trait values (i.e., one value per population) to their correct lineage under the Geographic Model was slightly higher than for individuals. Both Bear Creek populations and all Rio Grande and East Slope populations were correctly classified. Successful classification of the 10 East Slope streams was unexpected, but likely due to the comparatively low basibranchial tooth counts and similar lateral series scale and spot counts for those streams (e.g., Table 8). Only 56% of West Slope populations were correctly classified and 44% were classified as East Slope populations. Overall classification success for populations in the Geographic Model was 78%; 69% of East and West Slope populations were correctly classified.

Classification success of individual fish under the Molecular Model, using the cross-validation procedure, was comparatively high for Bear Creek and Rio Grande specimens (89% each, Table 11). Misclassified Bear Creek fish were classified mostly as Rio Grande cutthroat trout (8%) and misclassified Rio Grande cutthroat trout were mostly classified as Green Lineage fish (9%). Classification success of individual Blue and Green Lineage fish to their correct group was 86% and 68%, respectively. Most misclassified Blue Lineage fish were classified as Green Lineage fish (10%). Misclassified Green Lineage fish were most often misclassified as Blue Lineage fish (17%) or Rio Grande cutthroat trout (13%). Overall classification success for individual fish was 81%; 79% of Blue and Green Lineage fish were correctly classified.

Classification success of populations (based on mean trait values) to their correct lineage under the Molecular Model was 100% for Bear Creek, Blue Lineage, and Rio Grande populations. Only 64% (9 of 14 populations) of Green Lineage populations were correctly classified, with 29% (4 populations) misclassified as Blue Lineage populations and one (7%) misclassified as Rio Grande cutthroat trout. Overall classification success for populations under the Molecular Model was 90%; 86% of Blue and Green Lineage populations were correctly classified. One Green Lineage population misclassified as a Blue Lineage population was from Nate Creek in Gunnison River drainage and the remaining four were all found in East Slope streams (Como Creek and Fern Creek [founded from Como Creek fish] in South Platte River drainage and Severy Creek in Arkansas River drainage) and one (South Prong Hayden Creek, Arkansas River basin) was misclassified as Rio Grande cutthroat trout. Each of the four had relatively similar traits, including low lateral series scale counts and high spot counts (Table 8).

Multivariate analyses-DFA within lineages by GMU—Mean classification rates of individual Blue Lineage fish to their respective GMUs was 46% (range 25-58%). Misclassified individuals were distributed broadly, with no apparent pattern, across other GMUs. Mean classification of Blue Lineage populations to GMUs was higher at 86% (18 of 21 correct). Arkansas River (n =3), Dolores River (n =1), lower Colorado River (n = 3), San Juan River (n = 1), upper Colorado River (n = 2), and upper Green River (n = 2) populations were correctly classified to their respective GMUs. One population in each of the remaining GMUs was misclassified: lower Green River GMU (n = 3, 1 to upper Colorado River GMU), South Platte River GMU (n = 3, 1 to San Juan River GMU), and Yampa River GMU (n = 3, 1 to Dolores

River GMU). Thus, two of the three misclassified Blue Lineage populations were from within their presumptive native range and one was not.

Some structuring was evident even among specimens from variable GMUs. For example, East Slope Blue Lineage fish from the Arkansas River and South Platte River basins had the lower mean trunk spot counts than other GMUs (mean =144 and 165, respectively); average of mean trunk spot counts from other GMUs was 197. Among the West Slope Blue Lineage GMUs, native populations averaged 210 trunk spots, whereas introduced populations had an average of 188. Trunk spot counts of Lake Nanita cutthroat trout, a population founded from Trappers Lake stock in the early 1900s (as were most Blue Lineage introductions, Metcalf et al. 2012), averaged a low 174 spots (unpublished data) and was more similar to some introduced East Slope Blue Lineage populations than native West Slope populations.

Mean classification rates of individual Green Lineage fish to their respective GMUs was 68% (range 50-83%). Misclassified individuals were distributed broadly across other GMUs with no apparent pattern. Classification rate of the 14 populations to their five GMUs, South Platte, Arkansas, Dolores, Gunnison, and upper Colorado River drainages, was 100%.

Structuring was evident for populations of Green Lineage cutthroat trout within GMUs, even for populations outside of their putative natural range. For example, East Slope Green Lineage populations in the Arkansas River basin had a relatively high mean trunk spot count of 126 and few basibranchial teeth (mean = 4), whereas West Slope Green Lineage fish collectively possessed comparatively few spots. Similarly, South Platte Green Lineage fish averaged 146 trunk spots and 4 basibranchial teeth. The high classification rates of West Slope Green Lineage populations to their respective GMUs were also due to relatively distinctive within-GMU traits. For example, most Dolores River basin fish consistently possessed few and relatively large spots (mean trunk spots = 68, mean spot size = 4.5 mm) but had many basibranchial teeth (mean = 16). Gunnison River fish also had few spots but were more variable in number and smaller (mean = 63, 12-253, mean spot size = 3.6 mm, 2.8-4.5), and those fish averaged only 7 basibranchial teeth (0-24). The other GMU where Green Lineage fish were sampled was the Upper Colorado River; those fish had a higher number of relatively small trunk spots (mean trunk spots = 156, 15-314; mean spot size = 3.1 mm, 2.1-4.7) and moderate numbers of basibranchial teeth (mean = 10, 0-21).

Mean classification rate of individual Rio Grande cutthroat trout to their respective GMUs was 64% (range 36-81%). Misclassified individuals from the Canadian River GMU (58% correct) were most often placed with lower Rio Grande individuals (36%), misclassified individuals from Pecos River GMU (81% correct) were most often grouped with Canadian River fishes (17%), misclassified individuals from lower Rio Grande GMU (36% correct) were most often classified as upper Rio Grande individuals (33%), and misclassified individuals from the upper Rio Grande GMU (79% correct) were most often identified as lower Rio Grande individuals (17%). Eleven of 12 populations (92%) were correctly classified to their respective GMUs; one Canadian River population (Leandro Creek) was misclassified to the lower Rio Grande GMU.

Similar to Green Lineage fish, structuring was evident for Rio Grande cutthroat within GMUs and river basins. This was especially evident for fish within the Rio Grande proper, as 69% and 96 % of fish from the lower and upper Rio Grande GMUs, respectively, were classified as Rio Grande origin fish rather than from Pecos or Canadian rivers. Lower and upper Rio Grande basin fish had relatively high spot counts (means = 130 and 154, respectively) and high numbers of pyloric caeca (means = 43 and 47, respectively). This is in contrast to Pecos River fish that had fewer trunk spots (mean = 66) and fewer pyloric caeca (mean = 38), or Canadian River fish that had moderate number of trunk spots (mean = 94) and few pyloric caeca (mean = 36).

DISCUSSION

Recent genetic investigations, using contemporary and historical museum specimens, have called into question the traditionally accepted taxonomic and systematic relationships of cutthroat trout in the Southern Rocky Mountains (Metcalf et al. 2007; 2012). Those studies suggested evidence of six lineages, but only the Blue Lineage (presumptive Colorado River cutthroats), Green Lineage, South Platte River basin native cutthroat trout (represented by Bear Creek fish, presumptive greenback cutthroat trout, sensu Metcalf et al. 2012), and Rio Grande cutthroat trout were believed extant. Presumably pure specimens from populations that represent those better-defined lineages were collected, to determine if traditional morpho-merisitic approaches could better classify

cutthroat trout distributions under the traditional Geographic Model or if distributions followed the more recent, genetics-based, classifications under the Molecular Model. Because such phenotypic traits are typically the standard upon which taxonomic decisions are based, morphometric information will contribute to determining if the extant cutthroat trout lineages represent discrete subspecies or are simply genetic variants of a broadly distributed taxon.

The Molecular Model was more successful identifying groups (subspecies or lineages) of cutthroat trout based on within-lineage or taxa similarities in morphological traits than the traditional Geographic Model. This was true whether comparisons among groups were for individual meristic traits, groupings in the principal component analysis scatter plots using four or eight variables, or the discriminant function classification analysis. Further, individual traits and discriminant function analysis also showed substantial structuring within lineages, organized by drainage (GMUs). As the cutthroat trout taxonomic literature suggests (Behnke 1992; Behnke 2002; Trotter 2008), the Geographic Model using a limited suite of morphological traits showed only moderate structuring of populations examined in this study and East and West Slope populations of cutthroat trout were similar in meristic traits (e.g., lateral series scale and gill rakers counts and spotting patterns). Bear Creek fish were distinct under each classification because of differences in several traits, as were Rio Grande cutthroat trout populations. Blue Lineage populations were distinct in the Molecular Model (100% classification success), unlike the same populations in the Geographic Model, of which 44% were misclassified. Inconsistencies in classification of Green Lineage fish (individuals and populations) under the Molecular Model using discriminant analysis were due mostly to four Green Lineage populations found on the East Slope that showed distinct morphological and subtle genetic differences traits relative to West Slope Green Lineage populations and Bear Creek fish. The following discussion supports nuances uncovered in this analysis using traditional taxonomic traits, clarifies where data do and do not support the Molecular Model and existence of four discrete taxonomic groups of cutthroat trout, including Bear Creek, Green and Blue Lineages, and Rio Grande cutthroat trout, across our study area, and makes suggestions for future studies.

Distinctiveness of lineages and comparisons—Individual morphologic traits, PCA, DFA, and mitochondrial ND2 data all suggested that Bear Creek specimens were substantially different from any other lineage or subspecies of cutthroat trout examined in this study. The low basibranchial tooth counts as well as many individuals with no basibranchial teeth, low gill raker

and lateral series scale counts, and numerous small spots that were evenly distributed over the trunk were noted by others (Proebstel et al. 1996, Behnke 2002) and prompted them to suspect introgressive hybridization with rainbow trout. Regardless, distinctive traits and absence of rainbow or Yellowstone cutthroat trout alleles in specimens (Evans and Shiozawa 2002, Metcalf et al. 2007) ensured protection of this population before contemporary genetic analyses of museum specimens linked this population to the native cutthroat trout of the South Platte basin (Metcalf et al. 2012).

It is difficult to compare traits of contemporary Bear Creek fish to historical samples for Front Range cutthroat trout. First, as has been recently discovered, the original description for *O. c. stomias* greenback cutthroat trout, the archetypal subspecies from the South Platte River basin (Jordan 1891), appears to have been based on Rio Grande cutthroat trout (Metcalf et al. 2012; Rogers 2012). Further, morphological analysis of museum specimens, whose DNA was used by Metcalf et al. (2012), was not possible in this study because of the need to travel to museums and examine specimens. Inspection of those specimens would be beneficial for determining what the ranges of morphological traits might have been for greenback cutthroat trout and whether the Bear Creek population, which may have been genetically bottle-necked over time, represents that variation. The few museum specimens available for any putative taxa would also limit inferences about variation and character states among basins for archetypal cutthroat trout. Based on genetic and morphological evidence, however, there is no doubt that Bear Creek fish are distinct and deserving of taxonomic recognition at some level.

Morphological analyses confirmed the wide overlap of traits for East and West Slope cutthroat trout noted by earlier investigators under the Geographic Model (Behnke 1992; Behnke 2002). Among the historically used traits we examined, only basibranchial tooth numbers were slightly different between East and West Slope cutthroat trout populations. Similarities among East and West Slope fish based on lateral series scale counts and pigmentation patterns with an overall theme of extremely wide variation in patterns, led Behnke (2002) to reasonably suggest that such groups could in fact be considered the same subspecies. He retained the taxa under the Geographic Model classification system because it was “convenient for management” and facilitated conservation actions where the prevailing and correct paradigm was (and is) to preserve the genetic diversity where it existed on the landscape (Behnke 1972; Behnke 1992).

The widespread stocking of two potentially separate taxonomic entities on both sides of the Continental Divide (Blue and Green lineages of Metcalf et al. 2012) in the late 1800s and early 1900s, would have made separation of each lineage by early taxonomists difficult. Their distinguishing repertoire was limited to several meristic characters and qualitative spot traits. This challenge was illustrated by our comparison of individual traits, PCA, and discriminant function analyses. The minimum information required by early scientists to define existing variation was to understand the native occurrence of Green and Blue lineages prior to their stocking across the landscape and detailed descriptions of trait variation within each lineage prior to dispersal by humans. Neither was available. Finding various lineages scattered about the landscape was possible only with the aid of reasonably fine-scale genetic analyses. Nor is this the first discovery of cryptic taxa by molecular techniques (Shaffer et al. 2004; Egge and Simons 2006), but morphological studies to verify genetic diversity is uncommon (Vredenburg et al. 2006; Berendzen et al. 2009).

Under the Molecular classification system, all 21 populations of Blue Lineage fish grouped together in the discriminant function analysis using phenotypic characteristics. Given the broad distribution of these populations and potential for considerable morphological variation, this high classification rate was not anticipated. Limited variation of Blue Lineage fish appears to be supported by the mitochondrial genome as well, with recovery of only six Blue Lineage haplotypes in 340 samples across those 21 streams, compared to nine Green Lineage haplotypes recovered in only 14 populations and eight Rio Grande cutthroat trout haplotypes recovered from 12 streams. Slightly lower lateral series scale counts and especially the relatively high number of spots distributed evenly over the body (high fore-spot and mid-spot ratios) discriminated Blue Lineage populations from Green Lineage populations. High classification success of Blue Lineage fish was partially a consequence of widespread stocking of Trappers-Lake-derived fish. The perception of low morphological variation among Blue Lineage fish was also supported by comparing CVs for morphological traits among all lineages/taxa examined (Table 12). Blue Lineage fish had the lowest CVs for 8 of 10 traits (except lateral series scales and pyloric caeca). Low trait variation likely enhanced classification success of populations. The notion of genetic similarity of Blue Lineage populations outside their native range was also supported with all populations examined having a single dominant Trappers Lake mitochondrial haplotype.

Our analysis of West Slope cutthroat trout populations, using DFA under the Geographic classification system, supported the notion of two extant lineages in that region. Correct classification of these populations was low; nearly half of the 25 West Slope origin populations (the 26 dots on Figure 2 include Irish Canyon, a Yellowstone cutthroat population excluded from analyses), examined were classified as East Slope fish, which would perhaps be expected if two or more lineages were scattered across the landscape. The correct classification of all 10 East Slope populations is likely because East Slope Blue populations (n = 6) had spotting characteristics and lateral series scale counts (both powerful classification traits) that were intermediate between West Slope Green and Blue Lineage populations. East Slope Green Lineage populations (n = 4) were distinct, having higher spots counts and higher fore-spot and mid-spot ratios (Tables 3 and 8).

Fewer populations and individuals of Green Lineage fish were assigned to their correct lineage. This may have been because native Green Lineage populations were morphologically intermediate between Blue Lineage populations, and Rio Grande cutthroat trout. For example, Green Lineage fish were similar to Blue Lineage fish in having comparable number of lateral series scales, basibranchial teeth, anterior gill rakers, and pyloric caeca, but differed in having fewer spots that were more concentrated posteriorly. Alternatively, based on similarities in spotting characteristics, Green Lineage populations were more similar to Rio Grande cutthroat trout than Blue Lineage populations, as was suggested by mitochondrial DNA (e.g. Figure 3). However, Green Lineage fish were different from Rio Grande fish in having fewer pyloric caeca, and more lateral series scales and basibranchial teeth. Intermediacy was demonstrated also by PCA results (Figure 15, lower right panel), which showed the Green Lineage centroid between Blue Lineage and Rio Grande cutthroat trout populations. Lower classification rates were also likely a function of the relatively high trait variation of Green Lineage fish, especially for anterior gill raker and trunk spot counts and fore-spot and mid-spot ratios.

A main reason for lower classification rates of Green Lineage populations and individuals was also revealed when the geographic location of misclassified populations was considered. All but one of the misclassified Green Lineage populations was found on the East Slope; Nate Creek in Gunnison River drainage was the exception and it was classified as a Blue Lineage population. Each of the other four East Slope Green Lineage misclassifications was a result of atypical Green Lineage traits, including higher spots counts and higher fore-spot and mid-spot ratios. There are

two possible explanations. Green Lineage fish produced at Grand Mesa hatchery may have been morphologically uniform (e.g., similar to the Trappers Lake situation), but different from extant native Green Lineage populations. Grand Mesa derived fish might have been replaced with other strains in their native range, but persisted in East Slope streams in what were likely historically fishless waters. Or, translocated populations initiated with a small number of individuals from a single source might have given rise to distinct phenotypes and mitochondrial haplotypes through founder effects.

Alternatively, distinctness of East Slope Green Lineage fish might be the result of those populations representing archetypal native cutthroat trout diversity, invading during the late Pleistocene from West Slope sources and persisting despite extensive stocking of Green Lineage fish from Grand Mesa stock (Metcalf et al. 2012). This explanation is supported, in part, by presence of a relatively rare but dominant haplotype found in Como (Stream #17) and Fern creeks in (Stream #37, founded with Como Creek fish) South Platte River drainage and Severy Creek (Stream #19) in Arkansas River drainage (Figure 3). This haplotype has also been found in other East Slope populations not included in this study (Rogers, unpublished data). The Severy Creek haplotype has also been reported in one location in the western portion of Rocky Mountain National Park (A. Martin, Univ. of Colorado, Boulder), in the Colorado River drainage, and represents the only known West Slope localities for the haplotype. That location is near the Continental Divide and East Slope Green Lineage populations, and it unclear if that West Slope population is native or stocked; apparently few or no Park cutthroat trout populations are thought to be unaffected by stocking (pers. comm.., C. Kennedy, U. S. Fish and Wildlife Service). Even more compelling is the presence of a unique haplotype recovered in South Prong Hayden Creek (Stream 3) in the Arkansas River drainage found nowhere else other than from a pair of cutthroat trout collected in 1889 from Twin Lakes which lie in the headwaters of the Arkansas River (Metcalf et al. 2012). Presence of rare haplotypes and morphological consistencies of East Slope Green Lineage fish provides justification for managing these populations as discrete entities pending more detailed examination of their respective genomes.

The genetic structure and morphological patterns of yellowfin cutthroat trout, and their distribution and interaction with other potentially native cutthroat trout taxa in the Arkansas River drainage is not well understood. Yellowfin cutthroat trout, long believed extinct (Juday 1906; Behnke 1992; Behnke 2002), had many fine spots covering the body in a relatively

uniform manner, and represented the other extreme of the spotting pattern spectrum from the relatively sparse pattern typical of Green Lineage fish (Jordan 1891). Further, scale counts for Severy Creek fish were also intermediate between typical Green Lineage populations and those for yellowfin cutthroat trout (Jordan 1891; Behnke 2002). Unfortunately, comparison of other traits is not possible because we were not able to examine the few yellowfin cutthroat trout museum specimens available. Destructive sampling (pyloric caeca counts) or even minimal disfiguring (gill rakers counts, basibranchial tooth staining) has been discouraged on the rare and valuable historical specimens.

Individual traits, PCA, and DFA techniques supported distinction of Rio Grande cutthroat trout. Low basibranchial tooth counts (lower only for Bear Creek specimens), many individuals with no basibranchial teeth, low lateral series scale counts, high pyloric caeca counts, and low overall spot counts and spot ratios separate them from other Southern Rocky Mountain cutthroat trout. Its distinction was also supported by the literature (Jordan 1891; Behnke 1992; Behnke 2002; Pritchard et al. 2008) and confirmed that Rio Grande cutthroat trout were relatively stable in terms of classification. This clarity was doubtless facilitated by fewer hatchery-produced fish being stocked in the native range of Rio Grande cutthroat trout compared to that for Green and Blue Lineage fish. Pritchard et al. (2008) also discussed the apparent relative resiliency of the genetic signature of those populations, whereby native forms may have survived better or failed to intermix genetically with stocked fish (Pritchard et al. 2008).

Multivariate analyses-DFA within lineages by GMU—Classification rates for individual fish to their respective GMU was relatively low for all taxa, which was not unexpected given the wide range of variation among specimens from each population. Classification rates for individual Blue Lineage fish were the lowest, which may reflect the many populations included in this study and inclusion of several from outside their native range. Mixing of native and transplanted fish resulting in mixing of genetic heritages might also reduce classification success. Nonetheless, classification rates of populations to GMUs were high (86% or 18 of 21), which suggested that largely, if not entire, native signatures were retained. High classification rates of East Slope Blue Lineage populations and other populations established outside their native ranges to their respective GMUs may also indicate trait consistency among them. This might be a function of stocking of morphologically consistent Trappers Lake-derived fish. This

consistency was supported by presence of the single dominant ND2 haplotype in every Blue Lineage population established outside of its presumptive native range.

Unlike Blue Lineage fish, high classification rates were observed for individual Green Lineage fish to their respective GMU, which contributed to correct classification of all 14 populations to their correct GMU. This was not expected because several East Slope Green Lineage populations were misclassified as Blue Lineage fish under the Molecular Model. Correct classification of East Slope Green Lineage populations to their respective GMUs indicates consistent traits among them. This might be a function of stocking primarily, or only, Grand Mesa-derived hatchery fish. With only 14 populations upon which to interpret intra-lineage variation, conclusions are limited, especially because only 10 streams were from the presumed native range of the Green Lineage.

Regardless, the structuring of morpho-meristic traits at the level of the GMU for both Blue and Green Lineage fish deserves additional investigation with larger numbers of samples from throughout the range of the lineage. The apparent morphological structuring of those populations also suggests that management of cutthroat trout should proceed at the level of the GMU until additional information suggests otherwise.

High classification rates of individual Rio Grande cutthroat trout was consistent with correct classification of nearly all (11 of 12) populations to their correct GMU. The pattern of structuring, based on morpho-meristic data, by Rio Grande cutthroat trout within its GMUs was supported by microsatellite data (Pritchard et al. 2008) and our AFLP analysis. Most upper and lower Rio Grande populations grouped together (northern and central New Mexico groups), as did most Pecos and Canadian River populations. Populations from the latter two drainages shared more traits than they did with Rio Grande populations, but were also distinct unto themselves. Pritchard et al. (2008) also found similarity of upper Canadian River fish (e.g., Ricardo Creek) with lower Rio Grande populations, which also was evident in our data as expressed by the high number of individual Canadian River fish misclassified as lower Rio Grande fish. Pritchard et al. (2008) suggested Rio Grande-Canadian linkages may be a function of stocking “New Mexico cutthroat”, an apparently Rio Grande-derived population from Costilla Creek, across the range of cutthroat trout in New Mexico, including streams in the Canadian River drainage. Although the number of populations and specimens imposes some limitations on

interpretations of our results, structuring by GMU and major river basin is a feature of Rio Grande cutthroat trout.

Study design considerations—An important underpinning of this study was to adequately represent the genetic diversity of the various taxonomic entities of cutthroat trout across the Southern Rocky Mountains, under both the Geographic and Molecular models. 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 cutthroat trout in the Southern Rocky Mountains. This coordinated sampling effort across a four-state area ensured that basic spatial sampling design considerations were fulfilled, which was perhaps different from historical efforts that used opportunistically obtained samples, and also ensured that bias associated with over or underrepresentation of one or more groups was minimized. Strong inference from a study, such as this, is possible only with a sound sampling design (Manly 1992; Burnham and Anderson 2002).

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 both morphological and molecular data collection was completed. To further reduce potential for investigator bias, traditional meristic counts were done by one person unfamiliar with issues surrounding cutthroat trout taxonomy and systematic relationships.

We chose a single investigator to gather all traditional meristic data, across the 20-month data-gathering portion of the study, to ensure consistency. Other approaches, whereby multiple investigators gather data on the same specimens, to assess inter-investigator variability in trait counts and measurements and incorporate that into analyses have been used (e.g., Conner and Shenk 2003), but the large number of samples in this study and the nature of some traits (e.g., raised scales) precluded that approach in this instance. For example, lateral series scale counts were difficult due to small scale size and their embedded nature, and sometimes required lifting (not removing) individual scales with a needle to keep an accurate count and correct path along the length of specimen. That approach left evidence of what was counted and where, which may have biased a second investigator. Our replicated scale counts for consistency checks were somewhat biased in that regard, although we deliberately chose some specimens that did not require scale lifting to ensure that those multiple counts were least biased. Also, pyloric caeca

counts required removing individual cecum tips from the intestine wall (these were cataloged with specimens), which would obviously influence counts by a second investigator. Spot count data were gathered by a second trained investigator. Finally, an investigator unfamiliar with the purpose of the study gathered spot size data after traditional meristic and spot counts were completed. All investigators were trained by one individual (KRB) and multiple assessments were made to ensure accurate and unbiased data collection.

We conducted inter-investigator and intra-investigator consistency checks for traits we thought might be prone to systematic miscounts, measurement error, or have high variability. Counts for lateral series scales and spots and spot measurements were consistent among replicated counts. Another design feature that reduced potential for inaccurate trait count and measurement was using a narrow size range of specimens. This also helped ensure that if there were any biases they would be uniform.

Comparison of trait variation, using CVs of 24 and 12 fish samples within GMUs, showed that specimens from smaller samples contained about 90% of the variation present in specimens from larger samples. Preliminary analyses using random draws of 12 fish from larger 24-fish samples showed that confidence interval coverage of small samples was about 90%, when an expectation for random draws of 24 fish samples would be 95%. These analyses gave us confidence that small samples adequately portrayed variation in morphology of cutthroat trout from our samples.

Length-dependent variation of traits has rarely been assessed for trout in taxonomic studies. Those dependencies could be examined either by assessing trait differences over a length range of specimens or by repeated measures of traits collected on individual fish through ontogeny (e.g., spot counts). Each approach has difficulties in terms of ease of data collection and reliability, and repeated assessment of traits would almost certainly involve captive-reared specimens, introducing yet another source of variation that might be inconsistent with the natural environment. Spot counts and size are traits that certainly change over ontogeny, especially among cutthroat trout, and continued use of those traits in future studies would need to account for size-dependencies or control for specimen size. Investigation of size (or age) dependent traits in spotting patterns would be useful to understand such variation.

Finally, screening populations and individuals with ND2 and AFLP molecular analyses ensured that populations and individuals represented the native cutthroat trout present at each

sampling location. To assess if individuals screened by AFLP tests would affect classification rates, we added back fish originally excluded by the AFLP analysis (15 Blue Lineage fish from four streams, 11 Green Lineage fish from four streams) and reran the discriminant function analysis using eight variables under the Molecular Model. Population assignments and classification rates were identical with and without the AFLP screened individuals, demonstrating that the classification was robust to the presence of a few mildly admixed individuals in those populations.

Management—How results presented herein and recent molecular studies on cutthroat trout of the Southern Rocky Mountains (Metcalf et al. 2007; Rogers 2010; Loxterman and Keeley 2012; Metcalf et al. 2012) will shape future management is not evident. A logical first step would be to determine if the four lineages studied here constitute recognizable and definable groups at a level of taxonomic organization such as subspecies. Metcalf et al. (2012) suggested that Bear Creek fish likely represent cutthroat trout native to the South Platte River basin, which seems reasonable. They also reasoned that Blue Lineage fish were likely best represented by fish recognized now as *O. c. pleuriticus*, but with a more restricted distribution than was historically accepted. Certainly, a redescription of those lineages, based on larger contemporary samples and a more correct view of their distribution patterns, is warranted.

Taxonomic status of Rio Grande cutthroat trout is largely unchanged by recent genetic and morphological studies, save for possible recognition of distinct population segments or evolutionary significant units (sensu Moritz 1994), as proposed by Pritchard et al. (2008). A more complete description based on better-understood geographical variation of traits may assist managers in the future.

Yellowfin cutthroat trout have long been recognized as a subspecies, but are thought extinct. Similarly, an apparently unique genetic lineage of cutthroat trout from the San Juan River drainage of southwest Colorado, northwestern New Mexico, and possibly regions of abutting Utah and Arizona (Metcalf et al. 2012) may also be extinct. Given that no extant populations of those taxa exist, understanding morphological variation in terms of subspecies designation (for the San Juan group) seems a lower priority, at least for management purposes. Emergence of more museum specimens or discovery of remnant populations would necessitate additional investigations.

In the Southern Rocky Mountains, only Green Lineage fish seem to be largely unaccounted for in terms of assignment to a recognized taxonomic entity. Regardless of whether formal designation as a subspecies is warranted or if Green Lineage fish are simply recognized as an evolutionary significant unit or distinct population segment, description of morphological variation is appropriate and needed. Minimally, this would assist managers with understanding historical and taxonomic origin of yet undiscovered or incompletely studied populations of cutthroat trout. This information would help focus conservation and recovery actions.

Phylogeny—Invasions of cutthroat trout to the Southern Rocky Mountain region likely emanated from the Yellowstone Lake area. Given geographic proximity, and drainage patterns, the likely invasion route was down the Green River drainage, including Yampa and White rivers, which is believed the native distribution of Blue Lineage fish. Some similarities exist among Yellowstone cutthroats and Blue Lineage fish, particularly presence of relatively large numbers of smaller spots on the trunk and a relatively even spot coverage. Yellowstone cutthroats have fewer lateral series scales and at least among Yellowstone Lake fish, more anterior gill rakers (20-22) and basibranchial teeth (average 20 or more) than typical Blue Lineage fish. Presence of Blue Lineage cutthroat 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. 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. Distinctiveness of lower Colorado River Blue lineage fish was supported by their grouping together the phylogenetic tree.

Invasion of West Slope Colorado River basin streams south of the presumed native distribution of Blue Lineage fish likely occurred along several fronts and resulted in a cutthroat trout with relatively fewer and larger spots, and slightly more scales. The subsequent invasion of Green Lineage fish into the Rio Grande in Colorado is supported by similarities among populations of the two groups for several morphological traits, particularly presence of relatively few and larger spots and lower numbers of basibranchial teeth. Rio Grande populations reflect additional divergence in having fewer lateral series scales and more pyloric caeca, at least for some populations. Behnke (1992; 2002) and Pritchard et al. (2008) discussed the distribution of Rio Grande cutthroat trout among various drainages and as our data support, suggested wide

differentiation among Rio Grande populations, and those residing in the Pecos and Canadian River drainages.

Origin of East Slope populations of cutthroat trout in South Platte River and Arkansas River drainages is uncertain. If native South Platte drainage cutthroat trout are represented well by Bear Creek fish, they have strongest affinities with Blue Lineage fish in terms of spotting patterns. However, mtDNA analyses (Figure 3) do not support such a relationship and instead suggest clustering of Bear Creek and Green Lineage populations with Rio Grande cutthroats as ancestral to those. Yellowfin cutthroat trout of the Arkansas River drainage might have derived from any of three sources based on abutments to other drainages and include Green Lineage fish, Rio Grande cutthroat trout, or South Platte River drainage native cutthroat trout.

Morphologically, yellowfin cutthroat trout are most similar to Bear Creek cutthroat trout (native South Platte basin cutthroat trout) and are similar in having a large number of small spots and comparatively few lateral series scales (Wernsman 1973; Behnke 1992). Yellowfin cutthroat trout are similar also with Rio Grande cutthroat trout, based on lateral series scale counts, but not for coloration or spotting patterns. Yellowfin cutthroat trout are dissimilar to Green Lineage fish by nearly all measures.

The historical presence of large-spotted trout in Twin Lakes (Jordan 1891; Behnke 2002) presented additional complications for our analyses. Their provenance as either a native or transplanted population is uncertain, but Metcalf et al. (2012) established that some of those large-spotted specimens were Green Lineage fish, but others were not. Given the highly managed nature of the Twin Lakes fishery, where by 1889 brook trout, Atlantic salmon, lake trout, and rainbow trout had been introduced (Jordan 1891; Juday 1906) and the absence of sympatric cutthroat trout subspecies in other drainages in western North America, it is doubtful these large-spotted trout were native. The presence on the East Slope, however, of a morphologically unusual Green Lineage fish and it possessing a rare mitochondrial haplotype speaks to the need to fully explore the nuclear genome of extant populations. In addition, morphological traits of the old museum fish should be reexamined to determine if differences among the yellowfin cutthroat trout specimens are evident and consistent with differing mitochondrial genomes. Further exploration of the nuclear genome in these museum specimens would be beneficial as well.

Conclusions—Combining traditional taxonomic metrics with molecular analyses of the same individuals and populations yielded a robust study upon which to weigh the merits of various taxonomic arrangements of cutthroat trout native to the Southern Rocky Mountains. Such an approach enabled censoring of individuals admixed with either rainbow trout or Yellowstone cutthroat trout that was not possible in previous morphometric studies, thus honing our classification ability. This advantage over traditional studies with limited taxonomic traits and limited statistical treatment of data permitted substantively enhanced classification success of cutthroat trout to their appropriate lineages.

The hypothesis of four extant lineages represented by Bear Creek, Blue Lineage, Green Lineage, and Rio Grande cutthroat trout, as proposed by Metcalf et al. (2012), was supported in this study, compared to more traditional alignments of subspecies (e.g., Behnke 2002). Under the Molecular Model, much more structure was evident and populations grouped more consistently not only when individual traits were compared, but also for PCA and DFA procedures. This is especially true when potential reasons for misclassification of Green Lineage fish were better understood. Those misclassified and relatively distinctive populations, and perhaps other populations that represent historical or present-day genetic diversity on the East Slope, suggest the need for additional study. These assertions are based on the assumption that the genetic relationships and lineages proposed by Metcalf et al. (2012) are correct. We recognize the limited number of specimens and small DNA fragment sizes used in Metcalf et al. (2012) and suggest that additional specimens be used, perhaps with other molecular techniques, to further clarify relationships among these lineages.

We conclude that historically-used meristic traits, as well as spot counts, when combined with appropriate statistical techniques, were powerful tools for differentiating lineages and subspecies of cutthroat trout in the Southern Rocky Mountains. Recognition of those patterns was obscured from previous investigators by extensive historical stocking and unrecognized establishment of various lineages on the landscape outside of their historical ranges. Population structuring at the drainage basin level, as recognized with morphological techniques in this study, supports the long-held notion that population management and restoration activities should emphasize preservation of the unique genotypes that likely evolved in concert with the environment (Behnke 1972; 2002). Preservation of that genetic diversity, regardless of where it resides on the landscape, should be a guiding principle for future management.

Recommendations—A number of recommendations emerged from this study. They are paraphrased below; see Discussion above for fuller explanation and justification.

- Conduct additional stream surveys to better understand distribution of various lineages of cutthroat trout on the landscape.
- Resolve taxonomic status of all lineages, particularly, East Slope Green Lineage populations.
- Until more complete information is available to describe their affinities, populations of East Slope Green Lineage cutthroat trout, as well as other rare morphological and genetic types in all lineages, should be replicated and protected.
- Nuclear genome characterization may assist with understanding lineage relationships.
- Redescription of lineages, based on larger contemporary samples and a more correct view of their distribution patterns, is needed.
- Structuring of morpho-meristic traits at the level of the GMU for both Blue and Green Lineage fish deserves additional investigation with larger numbers of samples from throughout the range of the lineage. The apparent morphological structuring of those populations also suggests that management of cutthroat trout should proceed at the level of the GMU until additional information suggests otherwise.
- Examine morpho-meristic traits of old museum specimens to obtain a better view of the taxonomy and variation of lineages as they are presently defined which will assist with better understanding relationships within and among lineages. Examination of additional South Platte River basin specimens is especially needed.
- Investigate size (or age) dependent traits in spotting patterns and other traits to evaluate their utility.

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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 2).

Drainage	GMU	Stream	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	Henderson 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 Fork	16	40.44	-111.09
Colorado River	Lower Green	South Brownie Creek	38	40.69	-109.77
Colorado River	Lower Green	Johnson Fork	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 Fork U M Creek	40	38.68	-111.59
Colorado River	Lower Colorado	West Fork Boulder Creek	45	38.04	-111.49
Colorado River	San Juan	East Fork Piedra River	28	37.49	-107.08
Rio Grande	Canadian	West Fork 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
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. Fork Cache la Poudre River	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

Table 2. Sample location information and associated lineage designations based on mitochondrial haplotypes and amplified fragment length polymorphisms for the 49 populations of cutthroat trout used in this study. Geographic Management Units (GMU) are portrayed in the study area map (Figure 2). Lineage designations are per Metcalf et al. (2012): Blue is the lineage thought native to the Yampa, Green and lower Colorado River GMU's, Green is the lineage thought native to the Upper Colorado, Gunnison, and Dolores River GMUs, S. Platte is thought native to the South Platte River basin GMU, followed by Yellowstone cutthroat trout, Rio Grande cutthroat trout, and rainbow trout.

			Stream		AFLP (% purity)				
Drainage	GMU	Stream	number	Lineage	Blue	Green	Rio Grande	Yellowstone	Rainbow
Arkansas	Arkansas	South Apache Creek	6	Blue	100				
Arkansas	Arkansas	North Taylor Creek	26	Blue	100				
Arkansas	Arkansas	Graneros Creek	43	Blue	100				
Arkansas	Arkansas	Hayden Creek, S. Prong	3	Green	96	4			
Arkansas	Arkansas	Severy Creek	19	Green		100			
Arkansas	Arkansas	Bear Creek	49	S. Platte					
Colorado River	Upper Colorado	Little Green Creek	29	Blue	100				
Colorado River	Upper Colorado	Mitchell Creek	42	Blue	100				
Colorado River	Upper Colorado	Abrams Creek	25	Green		100			
Colorado River	Upper Colorado	Cunningham Creek	31	Green		100			
Colorado River	Upper Colorado	Henderson Horseshoe Pond	34	Green		100			
Colorado River	Upper Colorado	Brush Creek, W. Fk	46	Green	7	93			
Colorado River	Dolores	Tabeguache Creek	12	Blue	99			1	
Colorado River	Dolores	Little Taylor Creek	18	Green	1	95			3
Colorado River	Dolores	Big Red Canyon Creek	21	Green	7	88		3	3
Colorado River	Dolores	Deep Creek, E. Fk	24	Green	11	89			
Colorado River	Gunnison	Nate Creek	8	Green	1	98		1	
Colorado River	Gunnison	Deep Creek	11	Green		100			
Colorado River	Gunnison	Doug Creek	47	Green		100			

Colorado River	Upper Green	Steel Creek	7	Blue	98			2
Colorado River	Upper Green	South Beaver Creek	41	Blue	100			
Colorado River	Upper Green	Irish Canyon Creek	2	Yellowstone	2			98
Colorado River	Lower Green	Little West Fork	16	Blue	100			
Colorado River	Lower Green	South Brownie Creek	38	Blue	96	2	2	
Colorado River	Lower Green	Johnson Fork	44	Blue	99	1		
Colorado River	Yampa	Milk Creek	23	Blue	100			
Colorado River	Yampa	Snell Creek	30	Blue	100			
Colorado River	Yampa	Deep Creek	35	Blue	100			
Colorado River	Lower Colorado	Pine Creek	5	Blue	100			
Colorado River	Lower Colorado	Right Fork U M Creek	40	Blue	100			
Colorado River	Lower Colorado	West Fork Boulder Creek	45	Blue	100			
Colorado River	San Juan	East Fork Piedra River	28	Blue	100			
Rio Grande	Canadian	West Fork Luna Creek	22	Rio Grande	1	2	97	
Rio Grande	Canadian	McCrystal Creek	36	Rio Grande			100	
Rio Grande	Canadian	Leandro Creek	39	Rio Grande	1		99	
Rio Grande	Pecos	Rio Valdez	9	Rio Grande			100	
Rio Grande	Pecos	Dalton Creek	10	Rio Grande			100	
Rio Grande	Pecos	Macho Creek	14	Rio Grande			100	
Rio Grande	Upper Rio Grande	West Indian Creek	1	Rio Grande			99	
Rio Grande	Upper Rio Grande	Osier Creek	15	Rio Grande			100	
Rio Grande	Upper Rio Grande	Carnero Creek, M	27	Rio Grande			100	
Rio Grande	Lower Rio Grande	El Rito	4	Rio Grande			100	
Rio Grande	Lower Rio Grande	Columbine Creek	20	Rio Grande	2		98	
Rio Grande	Lower Rio Grande	Policarpio Creek	33	Rio Grande	1		99	
South Platte	South Platte	S. Fk. Cache la Poudre River	13	Blue	99			1
South Platte	South Platte	Roaring Creek	32	Blue	100			
South Platte	South Platte	Hunters Creek	48	Blue	100			

South Platte	South Platte	Como Creek	17	Green	99	1
South Platte	South Platte	Fern Creek	37	Green	98	2

Table 3. Pearson correlation coefficients of spot number and spot size with SL, and spot size and spot number for cutthroat trout lineages/taxa from the Southern Rocky Mountains. East and West Slope data are for cutthroat trout under the Geographic classification (G) and Blue and Green Lineage cutthroat trout conform to those groups as defined by Metcalf et al. (2012) for the Molecular classification (M); Bear Creek and Rio Grande groups are the same under each classification.

Classification	n	SL and spot number	SL and spot size	Spot size and spot number
Bear Creek	36	0.24	0.60	-0.13
East Slope	164	0.43	0.51	0.09
West Slope	388	0.25	0.55	-0.03
Blue Lineage	334	0.35	0.51	0.05
Green Lineage	218	0.11	0.39	-0.30
Rio Grande	156	0.38	0.35	-0.28
mean		0.29	0.49	-0.10

Table 4. Mean, range, standard deviation, and 95% confidence limits (up to down) for length and 10 traits for lineages/taxa of cutthroat trout from the Southern Rocky Mountains. Numbers of streams and total specimens are in parentheses below lineage designations. East and West Slope data are for cutthroat trout under the Geographic classification (G) and Blue and Green Lineage cutthroat trout conform to those groups as defined by Metcalf et al. (2012) for the Molecular classification (M); Bear Creek and Rio Grande groups are the same under each classification.

Trait	Bear Creek (1, 24)	East Slope (G) (10, 164)	West Slope (G) (25, 388)	Blue Lineage (M) (21, 334)	Green Lineage (M) (14, 218)	Rio Grande (12, 156)
Standard length	140.9	147.2	160.8	159.2	152.9	162.1
	105-187	96-212	121-213	113-213	96-207	95-192
	21.4	19.8	18.4	19.7	19.4	16.8
	131.9-149.9	144.1-150.2	158.9-162.6	157.1-161.3	150.3-155.5	159.5-164.8
Scales in lateral series	180.8	198.4	201.5	198.4	203.8	182.7
	159-196	167-234	170-240	170-234	167-240	159-220
	10.9	12.6	13.4	12.4	13.9	12.9
	176-185.6	196.3-200.4	200.2-202.9	197.1-199.8	202.0-205.7	180.6-184.7
Anterior gill rakers, total	16.4	19.0	19.1	19.3	18.7	18.7
	15-19	15-22	15-23	16-23	15-23	15-22
	0.97	1.38	1.32	1.19	1.45	1.21
	16.0-16.8	18.8-19.2	18.9-19.2	19.2-19.4	18.5-18.9	18.5-18.9
Posterior gill rakers, total	4.5	8.8	7.3	8.7	6.3	6.0
	1-8	2-15	1-16	1-16	1-15	0-12
	1.93	2.99	3.26	3.23	2.69	2.79
	3.7-5.4	8.3-9.2	7.0-7.6	8.2-9.0	5.9-6.6	5.6-6.4
Basibranchial teeth	1.5	6.2	9.3	8.3	8.5	5.9
	0-9	0-21	0-33	0-33	0-33	0-23
	0.38	4.79	7.18	6.36	7.23	5.21
	0.5-2.5	5.4-6.9	8.6-10.0	7.6-9.0	7.5-9.4	5-6.7
Pyloric cecae	37.0	35.6	37.8	37.2	37.4	41.2
	27-48	22-52	23-55	22-55	23-53	20-58
	5.1	4.9	5.5	5.1	6.0	6.8
	34.8-39.1	34.8-36.3	37.3-38.4	36.4-37.5	36.6-38.2	40.1-42.2
Trunk spots	217.7	147.4	163.0	187.5	113.7	114.1
	108-496	32-300	12-462	55-462	12-314	25-293

	99.2	52.8	83.8	64.7	70.6	49.4
	175.8-259.6	139.3-155.6	154.6-171.4	180.6-194.5	104.3-123.1	106.3-121.9
Fore-trunk	0.71	0.55	0.51	0.59	0.42	0.33
spot ratio	0.20-1.90	0.09-1.68	0.00-1.27	0.08-1.27	0.00-1.68	0.00-0.95
	0.37	0.25	0.28	0.23	0.28	0.19
	0.56-0.87	0.52-0.59	0.48-0.54	0.57-0.62	0.38-0.45	0.30-0.36
Mid-trunk	0.70	0.76	0.69	0.81	0.57	0.43
spot ratio	0.43-1.05	0.21-1.32	0.00-1.30	0.34-1.30	0.00-1.32	0.07-0.97
	0.18	0.21	0.24	0.19	0.22	0.16
	0.62-0.77	0.73-0.80	0.67-0.72	0.79-0.83	0.54-0.60	0.40-0.45
Mean largest	2.27	3.90	3.76	3.90	3.66	4.09
spot size (mm)	1.5-4.57	2.23-5.91	2.06-6.94	2.37-6.94	2.06-6.82	2.41-8.21
	0.63	0.66	0.80	0.75	0.76	0.92
	2.00-2.53	3.80-4.00	3.68-3.84	3.82-3.98	3.56-3.76	3.94-4.23
Spot presence,	0.54	0.42	0.52	0.56	0.38	0.22
top of head	0-1	0-1	0-1	0-1	0-1	0-1
	0.51	0.50	0.50	0.50	0.49	0.41
	0.33-0.76	0.34-0.50	0.47-0.57	0.51-0.62	0.32-0.45	0.15-0.28

Table 5. Mean, range, standard deviation, and 95% confidence limits (up to down) for 10 traits for Blue Lineage cutthroat trout from various Geographic Management Units (GMUs) from the Southern Rocky Mountains. The GMUs are portrayed in the study area map (Figure 2). Blue Lineage fish are thought native to the Upper and Lower Green River, Yampa River, and lower Colorado River GMUs and introduced elsewhere; comparative data are shown for Bear Creek fish. Numbers of streams and total specimens are in parentheses below GMU names.

		Geographic Management Unit								
		Introduced blue lineage populations					Native blue lineage populations			
	Bear Creek	Arkansas	S.Platte	Dolores	U. Colo.	San Juan	L. Green	U. Green	Yampa	L. Colo.
Trait	(2, 36)	(3, 38)	(3, 45)	(1, 18)	(2, 36)	(1, 25)	(3, 46)	(2, 23)	(3, 45)	(3, 48)
Scales, lateral series	178.7	197.8	208.2	188.0	197.2	206.3	198.0	191.4	193.6	200.7
	158-196	181-216	181-234	177-200	171-216	184-225	170-228	171-212	175-226	182-227
	10.5	9.6	14.4	5.9	9.7	11.4	12.3	11.7	11.4	11.1
	175.0-182.4	194.8-200.7	203.4-213.1	185.0-190.9	193.9-200.5	201.5-211.2	194.3-201.6	186.1-196.8	190.2-197.1	197.4-204.0
Anterior gill rakers, total	16.2	19.4	19.4	18.8	19	19.7	19.7	19.7	18.9	19.1
	14-19	18-22	16-22	16-21	17-21	19-21	17-21	17-22	16-22	16-23
	1.04	1.01	1.30	1.17	0.93	0.74	0.97	1.18	1.47	1.35
	15.8-16.5	19.1-19.7	19.0-19.7	18.2-19.4	18.7-19.3	19.4-20.0	19.4-19.9	19.2-20.2	18.4-19.3	18.7-19.5
Posterior gill rakers, total	4.4	9.7	10.4	8.9	11.9	11.9	6.0	6.0	8.0	6.1
	0-8	4-14	5-15	4-13	8-14	6-16	1-11	3-11	3-13	2-12
	2.01	2.41	2.68	2.52	1.70	2.74	2.28	2.14	2.17	2.64
	3.7-5.1	9.0-10.4	9.6-11.3	7.6-10.1	11.3-12.5	10.8-13.0	5.4-6.7	5.1-7.0	7.4-8.7	5.3-6.9
Basibranchial teeth	1.7	8.3	7.5	2.4	8.3	8.4	9.1	4.2	5.0	15.5
	0-11	3-20	0-21	0-7	0-20	1-15	0-25	0-12	0-14	1-33
	2.85	3.55	5.58	2.01	6.25	3.67	6.44	3.28	3.63	8.10
	0.8-2.7	7.3-9.3	5.9-9.2	1.5-3.4	6.2-10.4	6.9-9.9	7.2-11.0	2.8-5.6	4.0-6.2	13.1-17.8

Pyloric cecae	36.7	34.4	36.9	37.9	36.75	36.5	35.5	40.7	39.8	36.8
	27-48	22-47	30-44	32-48	29-45	27-46	24-47	32-55	32-54	24-49
	5.1	4.5	3.8	4.3	3.82	4.4	5.8	6.0	4.7	5.1
	34.9-38.4	33.1-35.7	35.7-38.0	35.8-40.1	35.5-38.0	34.7-38.3	33.8-37.2	38.1-43.3	38.3-41.3	35.3-38.3
Trunk spots	220.9	143.7	165.1	168.2	174.6	201.2	209.6	198.0	222.2	203.6
	99-496	64-237	55-281	102-327	96-351	113-287	123-462	99-276	89-441	104-388
	98.8	36.7	53.7	53.6	45.2	37.7	72.9	47.1	89.7	62.7
	187.4-254.3	133.0-154.3	148.9-181.2	141.5-194.8	159.3-189.9	185.7-216.20	187.9-231.2	177.7-218.4	195.2-249.1	185.4-221.8
Fore-trunk spot ratio	0.71	0.45	0.61	0.80	0.52	0.60	0.49	0.69	0.77	0.59
	0.22-1.90	0.17-0.96	0.17-1.04	0.24-1.23	0.13-0.91	0.32-0.91	0.08-1.13	0.28-1.06	0.38-1.20	0.29-1.27
	0.32	0.20	0.20	0.30	0.20	0.15	0.25	0.20	0.20	0.19
	0.60-0.81	0.39-0.51	0.55-0.67	0.65-0.95	0.46-0.59	0.54-0.66	0.41-0.56	0.60-.077	0.71-0.83	0.54-0.65
Mid-trunk spot ratio	0.69	0.78	0.82	0.97	0.76	0.68	0.70	0.89	0.92	0.81
	0.34-1.05	0.40-1.11	0.41-1.21	0.65-1.3	0.38-1.23	0.44-0.94	0.34-1.22	0.57-1.09	0.69-1.28	0.55-1.16
	0.18	0.18	0.19	0.21	0.21	0.14	0.18	0.12	0.13	0.15
	0.63-0.75	0.73-0.83	0.77-0.88	0.86-1.07	0.69-0.84	0.62-0.74	0.65-0.75	0.84-0.94	0.88-0.96	0.76-0.85
Mean largest spot size (ln mm)	0.87	1.3	1.46	1.26	1.28	1.5	1.18	1.21	1.31	1.46
	0.41-1.52	1.09-1.69	1.14-1.78	1.09-1.50	0.98-1.59	1.35-1.75	0.86-1.53	0.88-1.59	1.04-1.67	1.19-1.94
	0.24	0.16	0.15	0.12	0.17	0.11	0.19	0.20	0.14	0.16
	0.79-0.95	1.29-1.39	1.42-1.51	1.20-1.32	1.23-1.34	1.48-1.57	1.13-1.24	1.12-1.30	1.27-1.35	1.41-1.51
Spot presence, top of head	0.47	0.27	0.44	0.94	0.81	0.84	0.28	0.52	0.58	0.77
	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
	0.51	0.45	0.50	0.24	0.40	0.37	0.46	0.51	0.50	0.43
	0.30-0.64	0.14-0.40	0.29-0.60	0.83-1.06	0.67-0.94	0.69-0.99	0.15-0.42	0.30-0.74	0.43-0.73	0.65-0.90

Table 6. Mean, range, standard deviation, and 95% confidence limits (up to down) for 10 traits of Green Lineage cutthroat trout from GMUs from the Southern Rocky Mountains. Geographic Management Units (GMU) are portrayed in the study area map (Figure 2). Green Lineage cutthroat trout are thought native to the Upper Colorado River, Gunnison River, and Dolores River GMUs and introduced elsewhere (Metcalf et al. (2012). Comparative data are shown for Bear Creek fish. Numbers of streams and total specimens are in parentheses below GMU names.

		Geographic Management Unit				
Trait	Bear Creek (2, 36)	Arkansas (2, 24)	S. Platte (2, 47)	Dolores (3, 41)	Gunnison (3, 47)	U. Colo. (4, 59)
Scales, lateral series	178.7	194.8	192.9	203.6	205.3	214.9
	158-196	177-209	167-217	178-228	187-235	187-240
	10.5	7.8	11.2	12.7	10.4	12.0
	175.0-182.4	191.4-198.1	189.6-196.3	199.5-207.6	202.2-208.5	211.8-218.0
Anterior gill rakers, total	16.2	18.6	18.3	19.1	19.6	17.9
	14-19	15-21	15-21	17-22	17-23	15-20
	1.04	1.53	1.45	1.30	1.17	1.21
	15.8-16.5	18.0-19.3	17.9-18.7	18.7-19.5	19.3-20.0	17.6-18.2
Posterior gill rakers, total	4.4	8.0	6.5	6.0	5.9	5.9
	0-8	4-12	2-14	1-12	1-15	1-12
	2.01	2.21	2.67	2.54	2.90	2.59
	3.7-5.1	7.1-8.9	5.7-7.3	5.2-6.8	5.0-6.7	5.3-6.6
Basibranchial teeth	1.7	4.0	3.8	15.7	7.4	9.8
	0-11	0-14	0-19	3-33	0-24	0-21
	2.85	4.23	3.9	7.57	7.27	5.46
	0.8-2.7	2.2-5.8	2.7-5.0	13.3-18.1	5.3-9.6	8.4-11.2
Pyloric cecae	36.7	34.0	36.3	36.8	37.7	40.0
	27-48	26-42	24-52	24-49	30-49	23-53
	5.1	4.3	6.1	5.1	4.8	6.9
	34.9-38.4	32.2-35.8	34.4-38.7	35.2-38.4	36.2-39.1	38.2-41.8
Trunk spots	220.9	125.5	145.5	68.4	62.5	155.7
	99-496	58-243	32-300	22-121	12-253	15-314
	98.8	48.4	63.1	22.7	45.1	79.6
	187.4-254.3	105.0-145.9	127.0-164.0	61.3-75.6	49.2-75.7	135.0-176.5

Fore-trunk	0.71	0.41	0.68	0.53	0.26	0.26
spot ratio	0.22-1.90	0.09-0.75	0.17-1.68	0.00-1.27	0.00-0.78	0.00-0.64
	0.32	0.19	0.28	0.30	0.17	0.14
	0.60-0.81	0.33-0.49	0.60-0.76	0.43-0.63	0.21-0.30	0.22-0.30
Mid-trunk	0.69	0.57	0.78	0.57	0.50	0.44
spot ratio	0.34-1.05	0.22-0.91	0.21-1.32	0.10-1.00	0.00-0.88	0.15-0.86
	0.18	0.21	0.23	0.20	0.154	0.17
	0.63-0.75	0.48-0.66	0.72-0.85	0.51-0.64	0.45-0.54	0.43-0.52
Mean largest	0.87	1.29	1.28	1.50	1.27	1.12
spot size	0.41-1.52	1.12-1.48	0.80-1.52	1.18-1.92	1.03-1.51	0.72-1.54
(ln mm)	0.24	0.11	0.15	0.19	0.13	0.17
	0.79-0.95	1.24-1.33	1.23-1.32	1.44-1.56	1.23-1.31	1.08-1.17
Spot presence,	0.47	0.13	0.70	0.68	0.06	0.27
top of head	0-1	0-1	0-1	0-1	0-1	0-1
	0.51	0.34	0.46	0.47	0.247	0.45
	0.30-0.64	-0.02-0.27	0.57-0.84	0.53-0.83	-0.01-0.14	0.15-0.39

Table 7. Mean, range, standard deviation, and 95% confidence limits (up to down) for 10 traits for Rio Grande cutthroat trout from various GMUs from the Southern Rocky Mountains. Geographic Management Units (GMU) are portrayed in the study area map (Figure 2). Rio Grande cutthroat trout is native to four GMUs of the Rio Grande basin. Comparative data are shown for Bear Creek fish. Numbers of streams and total specimens are in parentheses below GMU names.

		Geographic Management Unit			
	Bear Creek	Canadian	Pecos	U. Rio Gr.	L. Rio Gr.
Trait	(2, 36)	(3, 36)	(3, 36)	(3, 48)	(3, 36)
Scales,	178.7	183.7	175.5	182.3	189.1
lateral series	158-196	171-206	159-196	160-200	159-220
	10.5	8.7	9.1	10.5	18.1
	175.0-182.4	180.8-186.6	172.4-178.6	179.1-185.4	182.9-195.2
Anterior gill	16.2	18.4	18.5	19.3	18.5
rakers, total	14-19	15-21	16-20	17-22	16-20
	1.04	1.36	0.97	1.20	1.08
	15.8-16.5	17.9-18.8	18.1-18.8	18.9-19.6	18.1-18.9
Posterior gill	4.4	5.9	6.8	6.1	5.2
rakers, total	0-8	1-11	0-11	1-12	0-11
	2.01	2.56	2.90	2.49	3.20
	3.74-5.10	5.02-6.76	5.8-7.7	5.34-6.79	4.14-6.31
Basibranchial	1.7	2.70	4.30	8.90	6.60
Teeth	0-11	0-12	0-22	1-23	0-18
	2.85	3.26	5.90	4.40	4.84
	0.8-2.7	1.6-3.8	2.3-6.3	7.6-10.1	5.0-8.2
Pyloric cecae	36.7	35.6	37.8	46.6	42.9
	27-48	20-46	30-49	36-56	33-58
	5.1	6.0	4.5	4.4	6.0
	34.9-38.4	33.6-37.7	36.2-39.3	45.3-47.9	40.8-44.9

Trunk spots	220.9	93.5	66.1	153.8	129.8
	99-496	40-163	25-114	75-293	45-256
	98.8	30.0	19.3	44.3	41.1
	187.4-254.3	83.3-103.7	59.5-72.6	140.9-166.7	115.8-143.7
Fore-trunk	0.71	0.26	0.28	0.38	0.38
spot ratio	0.22-1.90	0.04-0.60	0.06-0.57	0.01-0.95	0.00-0.95
	0.32	0.14	0.13	0.25	0.19
	0.60-0.81	0.22-0.31	0.23-0.32	0.31-0.45	0.31-0.44
Mid-trunk	0.69	0.39	0.40	0.47	0.43
spot ratio	0.34-1.05	0.08-0.89	0.07-0.71	0.19-0.97	0.15-.68
	0.18	0.15	0.13	0.18	0.13
	0.63-0.75	0.34-0.44	0.35-0.44	0.42-0.53	0.39-0.48
Mean largest	0.87	1.31	1.59	1.37	1.28
spot size	0.41-1.52	0.95-1.61	1.33-2.11	1.00-1.80	0.88-1.71
(ln mm)	0.24	0.16	0.21	0.15	0.16
	0.79-0.95	1.25-1.36	1.52-1.66	1.32-1.41	1.23-1.34
Spot presence,	0.47	0.22	0.14	0.33	0.14
top of head	0-1	0-1	0-1	0-1	0-1
	0.51	0.42	0.35	0.48	0.35
	0.30-0.64	0.08-0.37	0.02-0.26	0.20-0.47	0.02-0.26

Table 8. Mean, range, standard deviation, and 95% confidence limits (up to down) for 10 traits for Blue and Green Lineage cutthroat trout from South Platte and Arkansas River GMUs from the Southern Rocky Mountains. Geographic Management Units (GMU) are portrayed in the study area map (Figure 2). Blue and Green Lineage cutthroat trout are thought introduced to the South Platte and Arkansas River (Metcalf et al. 2012). Comparative data are shown for Bear Creek fish. Numbers of streams and total specimens are in parentheses below GMU names.

		Blue lineage		Green lineage	
		Geographic Management Unit			
Trait	Bear Creek (2, 36)	Arkansas (3, 38)	S. Platte (3, 45)	Arkansas (2, 24)	S. Platte (2, 47)
Scales, lateral series	178.7 158-196 10.5 175.0-182.4	197.8 181-216 9.6 194.8-200.7	208.2 181-234 14.4 203.4-213.1	194.8 177-209 7.8 191.4-198.1	192.9 167-217 11.2 189.6-196.3
Anterior gill rakers, total	16.2 14-19 1.04 15.8-16.5	19.4 18-22 1.01 19.1-19.7	19.4 16-22 1.30 19.0-19.7	18.6 15-21 1.53 18.0-19.3	18.3 15-21 1.45 17.9-18.7
Posterior gill rakers, total	4.4 0-8 2.01 3.7-5.1	9.7 4-14 2.41 9.0-10.4	10.4 5-15 2.68 9.6-11.3	8.0 41376 2.21 7.1-8.9	6.5 2-14 2.67 5.7-7.3
Basibranchial teeth	1.7 0-11 2.85 0.8-2.7	8.3 3-20 3.55 7.3-9.3	7.5 0-21 5.58 5.9-9.2	4.0 0-14 4.23 2.2-5.8	3.8 0-19 3.9 2.7-5.0
Pyloric cecae	36.7 27-48 5.1 34.9-38.4	34.4 22-47 4.5 33.1-35.7	36.9 30-44 3.8 35.7-38.0	34.0 26-42 4.3 32.2-35.8	36.3 24-52 6.1 34.4-38.7
Trunk spots	220.9 99-496 98.8 187.4-254.3	143.7 64-237 36.7 133.0-154.3	165.1 55-281 53.7 148.9-181.2	125.5 58-243 48.4 105.0-145.9	145.5 32-300 63.1 127.0-164.0

Fore-trunk spot ratio	0.71 0.22-1.90 0.32 0.60-0.81	0.45 0.17-0.96 0.20 0.39-0.51	0.61 0.17-1.04 0.20 0.55-0.67	0.41 0.09-0.75 0.19 0.33-0.49	0.68 0.17-1.68 0.28 0.60-0.76
Mid-trunk spot ratio	0.69 0.34-1.05 0.18 0.63-0.75	0.78 0.40-1.11 0.18 0.73-0.83	0.82 0.41-1.21 0.19 0.77-0.88	0.57 0.22-0.91 0.21 0.48-0.66	0.78 0.21-1.32 0.23 0.72-0.85
Mean largest spot size (ln mm)	0.87 0.41-1.52 0.24 0.79-0.95	1.3 1.09-1.69 0.16 1.29-1.39	1.46 1.14-1.78 0.15 1.42-1.51	1.29 1.12-1.48 0.11 1.24-1.33	1.28 0.80-1.52 0.15 1.23-1.32
Spot presence, top of head	0.47 0-1 0.51 0.30-0.64	0.27 0-1 0.45 0.14-0.40	0.44 0-1 0.50 0.29-0.60	0.13 0-1 0.34 -0.02-0.27	0.70 0-1 0.46 0.57-0.84

Table 9. Eigenvectors for principal components analysis for four traditional meristic traits used to describe cutthroat trout from the Southern Rocky Mountains and for those traits plus four more spot traits (8 total) for the Geographic and Molecular models (scores are same for each, the only difference is to which classification model populations are assigned). All non-ratio trait data were transformed (l_n) to normalize distributions. The % variation explained is the cumulative total for principal component (PC) axes 1 and 2.

Traits	PC 1	PC 2
4 trait models		
Lateral series scales	0.54415	-0.3597
Anterior gill rakers	0.51429	0.22194
Basibranchial teeth	0.65823	0.01593
Pyloric caeca	0.07845	0.90616
% variation explained	70	
8 trait models		
Lateral series scales	0.13636	0.38394
Anterior gill rakers	0.26692	0.48646
Basibranchial teeth	0.24683	0.5175
Pyloric caeca	-0.0153	0.08951
Trunk spots	0.44809	-0.2191
Fore-trunk spot ratio	0.55324	-0.2358
Mid-trunk spot ratio	0.58471	-0.1496
Mean spot size	0.01614	0.46281
% variation explained	53	

Table 10. The *F*-statistics and significance probabilities for taxonomic traits used in discriminant function analyses (linear portrayed here not quadratic) to classify various lineages/taxa of cutthroat trout from the Southern Rocky Mountains. Specimens from all four lineages (49 streams and n = 744 specimens) were used.

Trait	*F*-value	Pr > *F*
Lateral series scales	117.43	<0.0001
Anterior gill rakers	73.88	<0.0001
Basibranchial teeth	28.66	<0.0001
Pyloric caeca	17.63	<0.0001
Trunk spots	98.32	<0.0001
Fore-trunk spot ratio	58.26	<0.0001
Mid-trunk spot ratio	156.73	<0.0001
Mean largest spot size	71.19	<0.0001

Table 11. Discriminant function analyses results using the jackknife resubstitution procedure that describes % correct classification of individual fish and populations under the Geographic Model or the Molecular Model. Number of individual fish and number of streams used in each classification group are in parentheses. BC is Bear Creek. East S. is East Slope of the Rocky Mountains populations, West S. is West Slope of the Rocky Mountains populations, and RG is Rio Grande basin populations. The numbers on the diagonal of each matrix depict the % of individuals or populations that were correctly classified while off-diagonal numbers depict the % of individuals or populations misclassified to various taxa or lineages. Total is the % of individuals or populations correctly classified in all taxa or lineages (Total), or for just East and West Slope (E&W, Geographic Model) or Blue and Green lineages (B&G, Molecular Model).

Model, grouping		Drainage alignment				Total % correct
Geographic classification		Bear Creek (n = 36, 2)	East Slope (n = 164, 10)	West Slope (n = 388, 25)	Rio Grande (n = 156, 12)	
Individuals						
	BC	86	3	3	8	
	East S.	3	68	19	10	
	West S.	1	28	64	7	
	RG	1	6	4	89	Total, 71
						E&W, 65
Populations						
	BC	100	0	0	0	
	East S.	0	100	0	0	
	West S.	0	44	56	0	
	RG	0	0	0	100	Total, 78
						E&W, 69
Molecular classification		Bear Creek (n = 36, 2)	Blue lineage (n = 334, 21)	Green lineage (n = 218, 14)	Rio Grande (n = 156, 12)	
Individuals						
	BC	89	0	3	8	
	Blue	1	86	10	3	
	Green	2	17	68	13	
	RG	1	1	9	89	Total, 81
						B&G, 79
Populations						
	BC	100	0	0	0	
	Blue	0	100	0	0	
	Green	0	29	64	7	
	RG	0	0	0	100	Total, 90
						B&G, 86

Table 12. Variation among morphological traits (as % CVs, [(standard deviation/ mean)*100]) of cutthroat trout from Bear Creek, Blue, Green, and Rio Grande taxa/lineages from the Southern Rocky Mountains. Samples sizes are portrayed below group designations.

Variable	Bear Creek (n =36)	Blue Lineage (n = 334)	Green Lineage (n = 218)	Rio Grande Lineage (n = 156)
Lateral series scales	5.9	6.7	6.4	7.0
Anterior gill rakers, total	6.4	6.3	7.8	6.5
Posterior gill rakers, total	45.4	37.9	43.2	46.6
Basibranchial tooth count	165.2	75.7	86.8	88.9
Pyloric caeca	13.9	14.1	15.7	16.5
Trunk spots, total	44.7	34.2	63.5	43.3
Fore-trunk spot ratio	45.2	40.4	67.6	59.0
Mid-trunk spot ratio	26.6	23.6	39.5	36.3
Mean largest spot size	27.3	14.3	15.9	14.9
Presence of spots, top of head	107.2	90.2	125.8	190.0

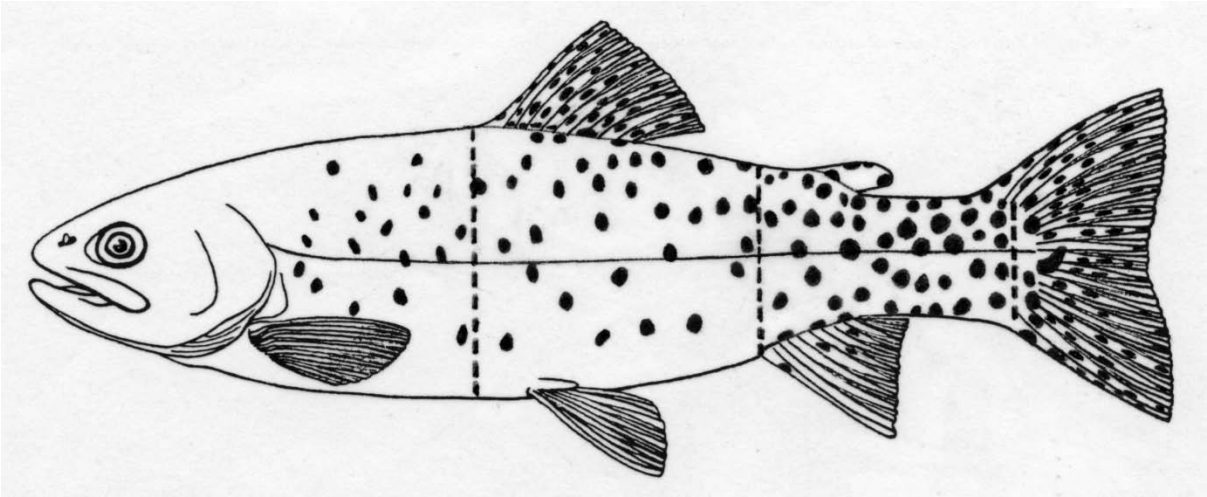


Figure 1. Zone demarcation used to count cutthroat trout spots on the trunk of the body. Dashed lines separate anterior, middle and posterior thirds of the trunk; spots posterior to the dashed line on the caudal peduncle were not counted. Head spots (those on operculum and bony skull) were counted separately from the trunk. The thirds of the trunk were separated into upper and lower zones by the lateral line. Spots that intersected lines for zones were assigned to a zone based on a majority rule. Spots more than half way onto a fin or the right-side body axis were not counted.

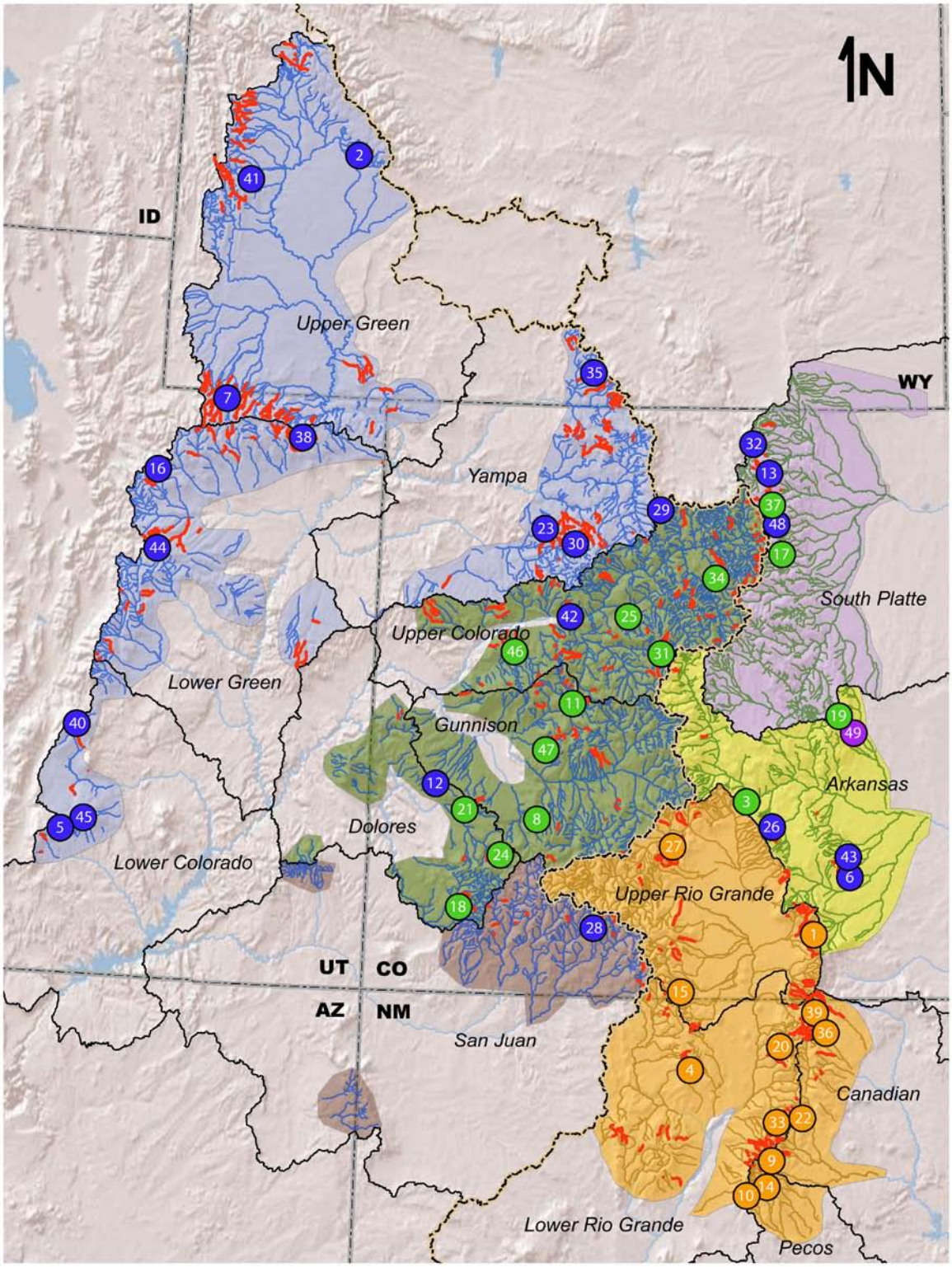
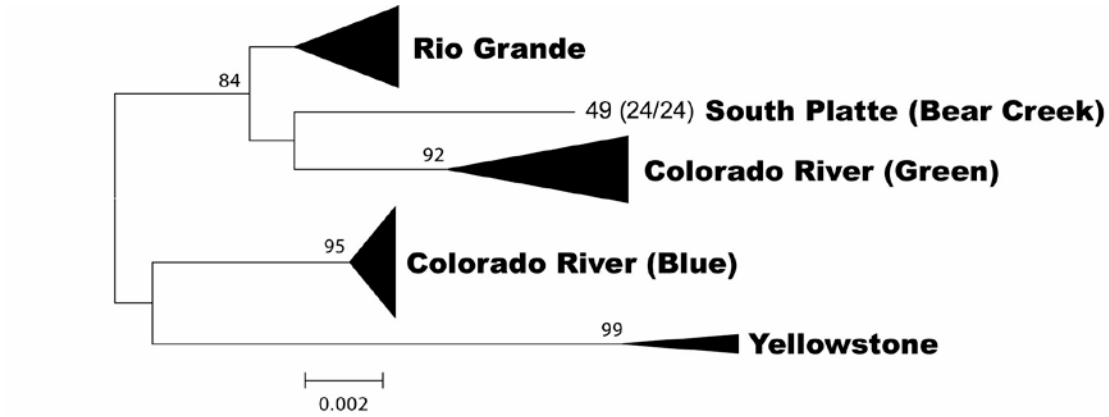
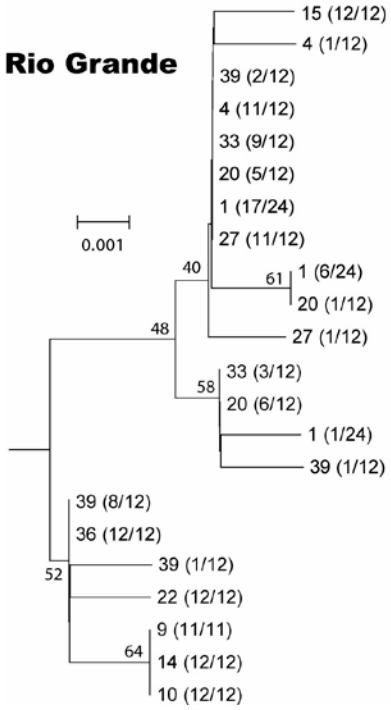
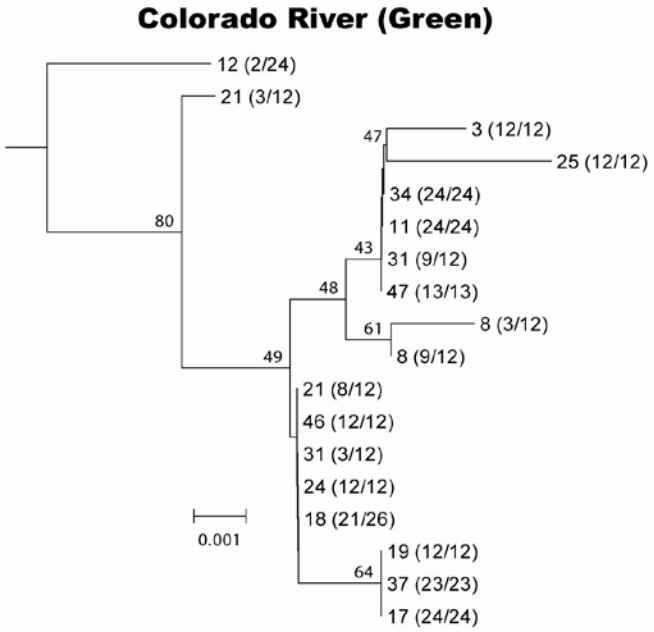


Figure 2. Study area map and sampling sites. Fourteen hydrologic units from five western states that comprise the accepted historical range of Colorado River cutthroat trout (blue labeled

streams), greenback cutthroat trout (green streams), and Rio Grande cutthroat trout (orange streams) are named in italics. Current Conservation Populations from which our study populations were randomly drawn are highlighted in red. The historical ranges of various 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. The lineage of each population sampled in the study (dots) defined by mitochondrial ND2 phylogenies are colored per the lineage ranges, and numbers in each dot represent streams sampled in this study.


Rio Grande
84
49 (24/24) South Platte (Bear Creek)
92
Colorado River (Green)
95
Colorado River (Blue)
99
Yellowstone
0.002


Rio Grande
15 (12/12)
4 (1/12)
39 (2/12)
4 (11/12)
33 (9/12)
20 (5/12)
1 (17/24)
27 (11/12)
0.001
40
61
1 (6/24)
20 (1/12)
27 (1/12)
48
33 (3/12)
58
20 (6/12)
1 (1/24)
39 (1/12)
39 (8/12)
36 (12/12)
52
39 (1/12)
22 (12/12)
9 (11/11)
64
14 (12/12)
10 (12/12)


Colorado River (Green)
12 (2/24)
21 (3/12)
47
3 (12/12)
25 (12/12)
34 (24/24)
11 (24/24)
80
43
31 (9/12)
47 (13/13)
48
61
8 (3/12)
8 (9/12)
49
21 (8/12)
46 (12/12)
31 (3/12)
24 (12/12)
18 (21/26)
0.001
64
19 (12/12)
37 (23/23)
17 (24/24)

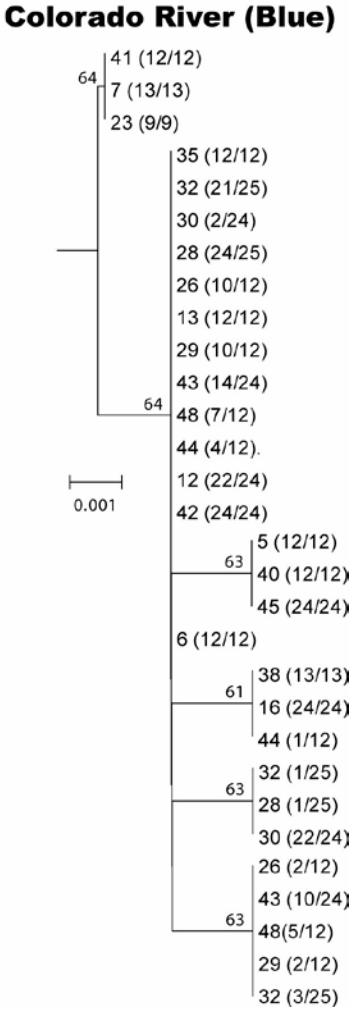

Colorado River (Blue)
64
41 (12/12)
7 (13/13)
23 (9/9)
35 (12/12)
32 (21/25)
30 (2/24)
28 (24/25)
26 (10/12)
13 (12/12)
29 (10/12)
43 (14/24)
64
48 (7/12)
44 (4/12).
12 (22/24)
0.001
42 (24/24)
63
5 (12/12)
40 (12/12)
45 (24/24)
6 (12/12)
61
38 (13/13)
16 (24/24)
44 (1/12)
63
32 (1/25)
28 (1/25)
30 (22/24)
26 (2/12)
43 (10/24)
63
48(5/12)
29 (2/12)
32 (3/25)

Figure 3. 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.

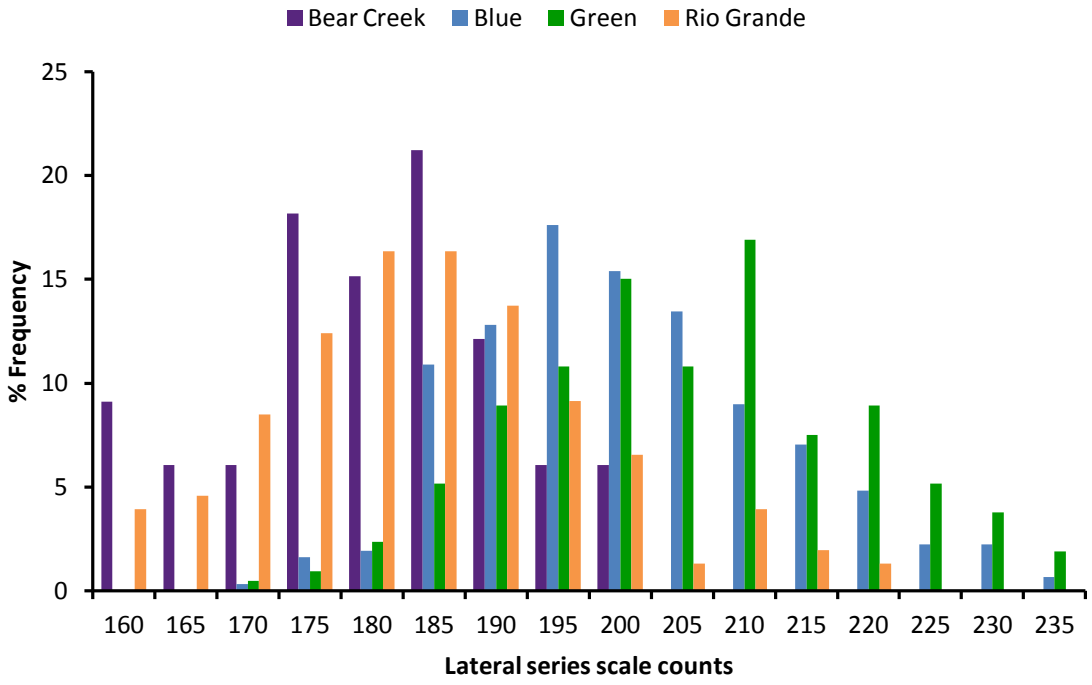


Figure 4. Percent frequency of lateral series scale counts among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012).

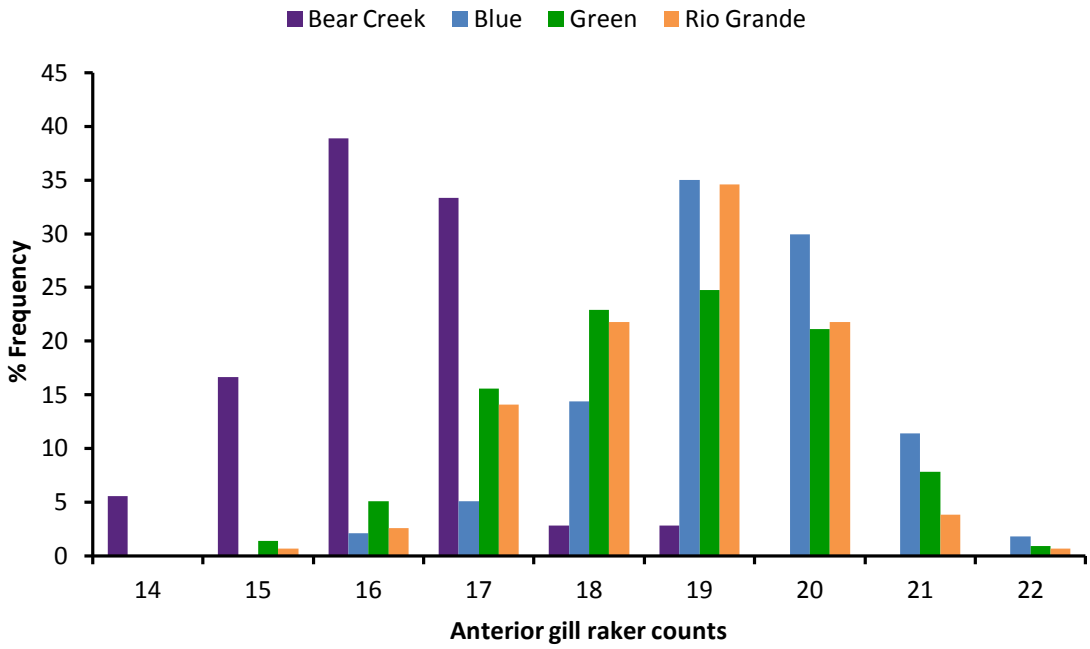


Figure 5. Percent frequency of total anterior gill raker counts among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012).

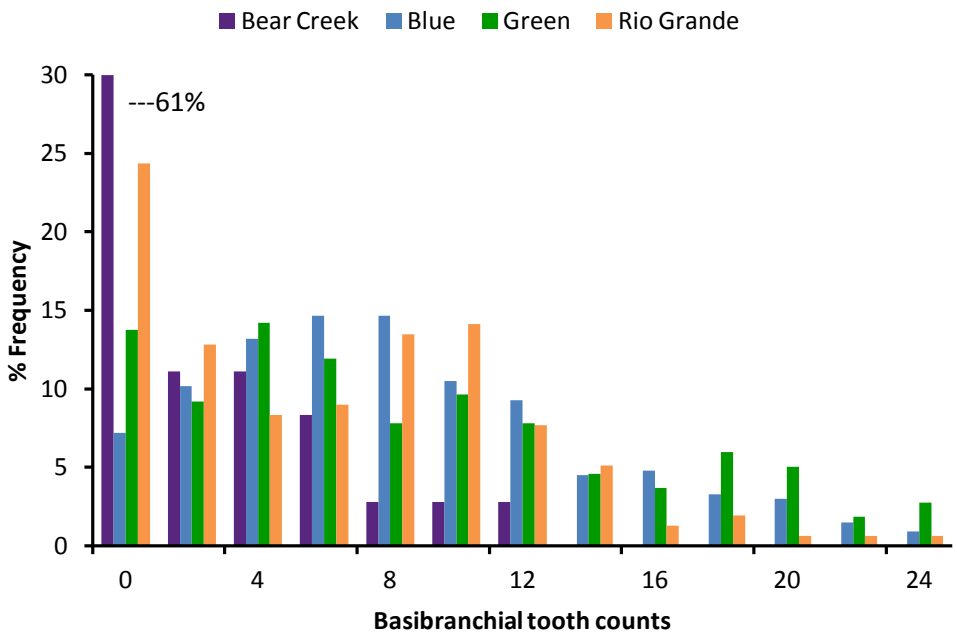


Figure 6. Percent frequency of basibranchial tooth counts among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012) noting that % of Bear Creek fish without teeth is 61%.

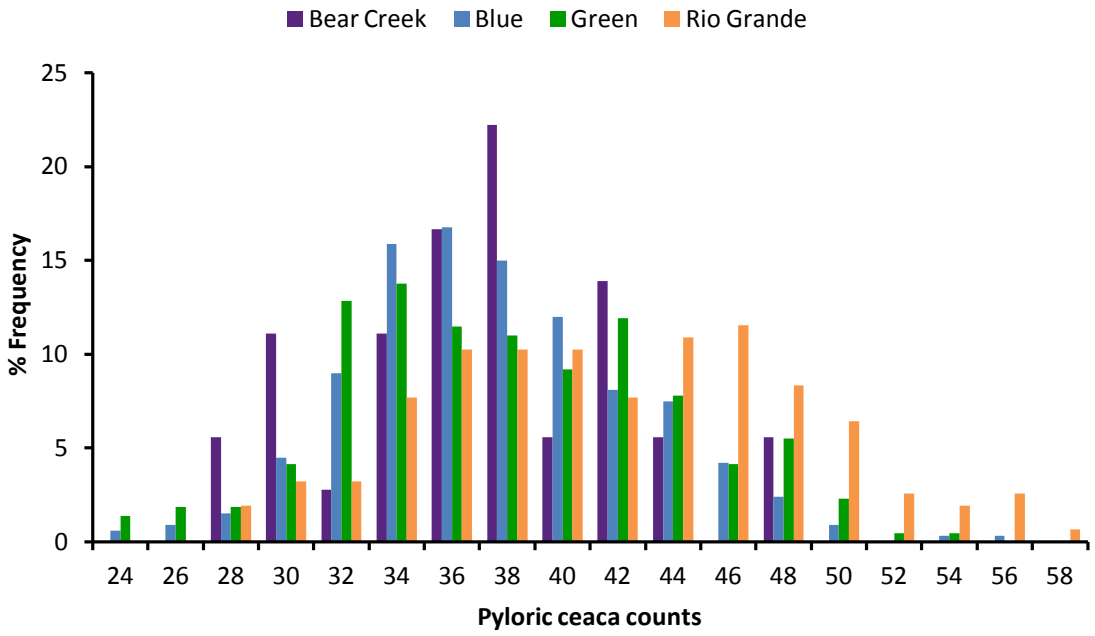


Figure 7. Percent frequency of pyloric caeca counts among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012).

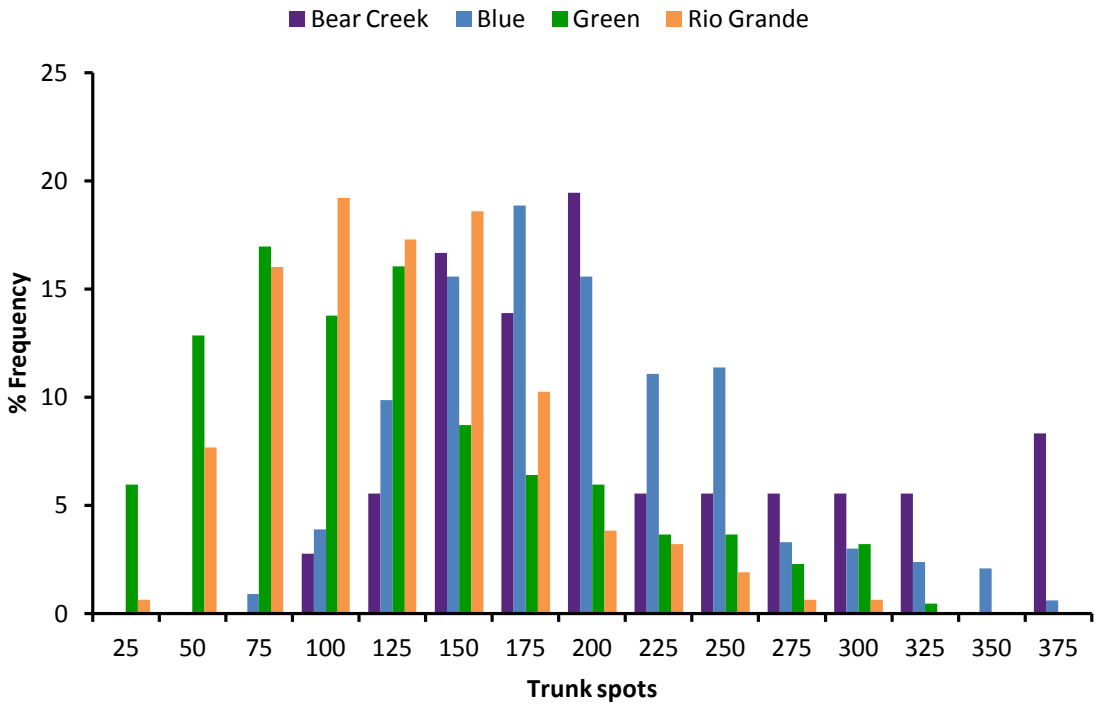


Figure 8. Percent frequency of total trunk spot counts among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012). A few high Bear Creek and Blue Lineage values (≥ 400) were not shown to increase clarity.

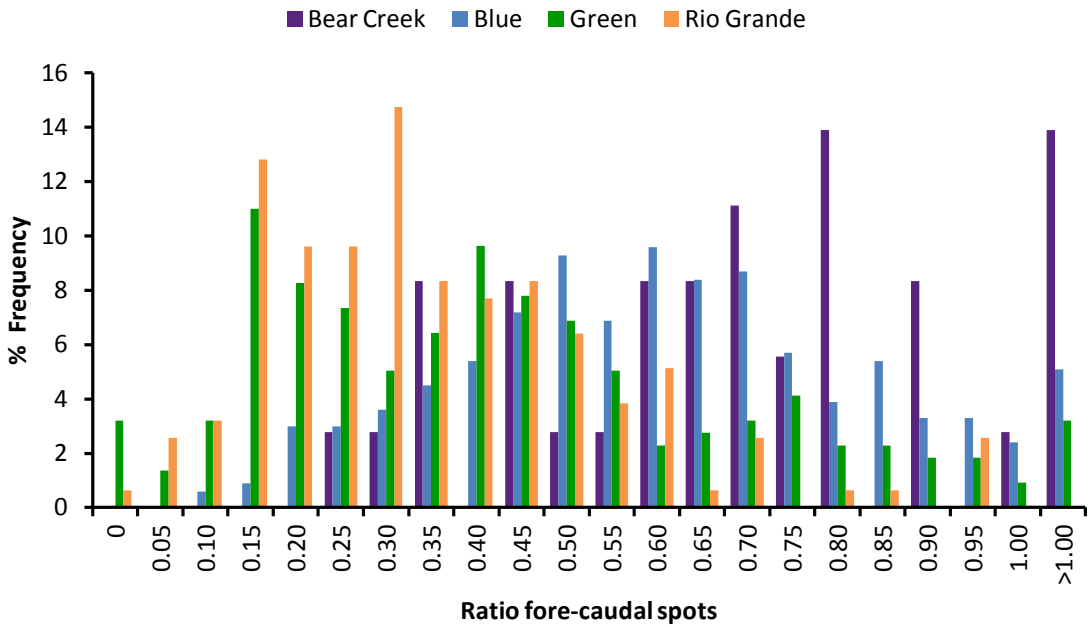


Figure 9. Percent frequency of the ratio of total spot number in the anterior-most quadrants of the trunk from the opercle to the origin of the dorsal fin (not including the head) divided by the total spot counts in the posterior-most quadrants from the origin of the anal fin to the end of the caudal peduncle (Fore-spot ratio) among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012).

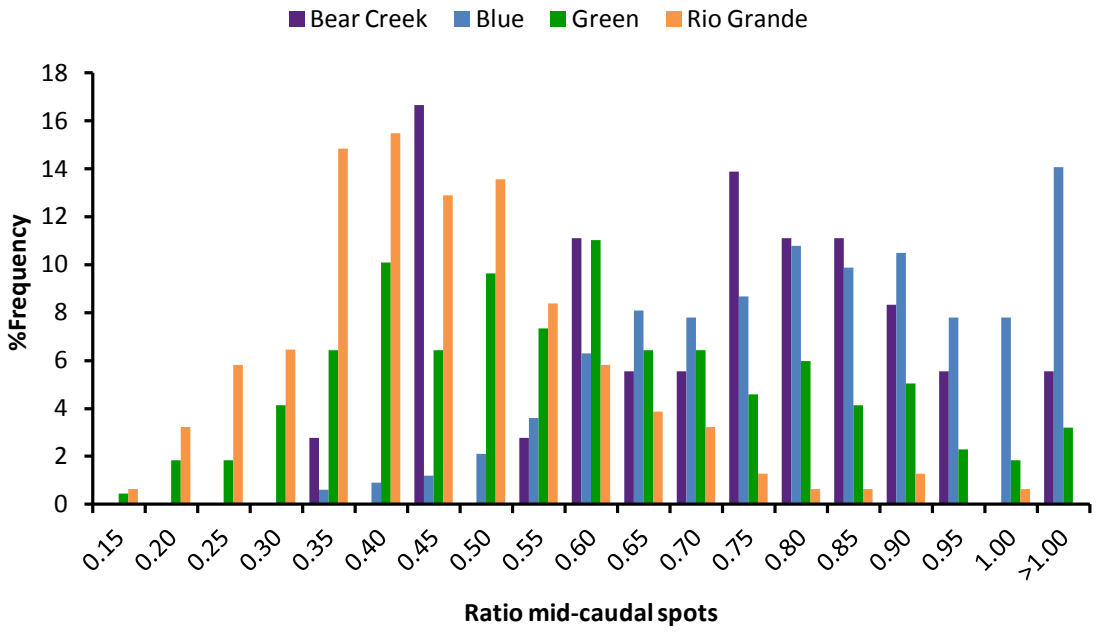


Figure 10. Percent frequency of ratio of total spot number in the middle quadrants of the trunk from the origin of the dorsal fin to the origin of the anal fin divided by the total spot count in the posterior-most quadrants from the origin of the anal fin to the end of the caudal peduncle (Mid-spot ratio) among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012).

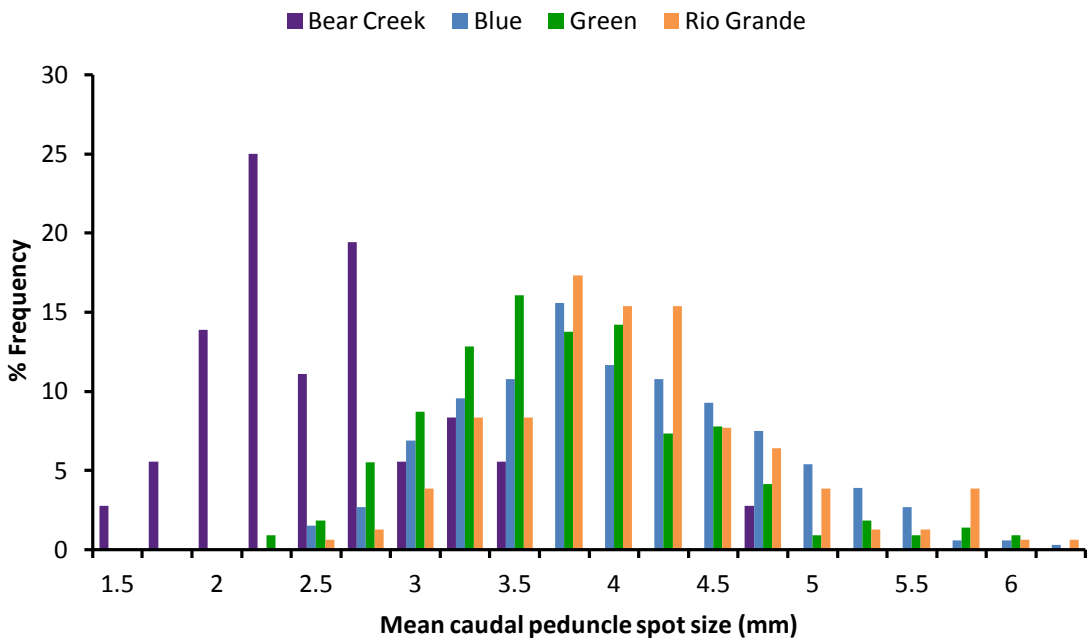


Figure 11. Percent frequency of mean spot size (mm) of the three largest spots on the trunk among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012).

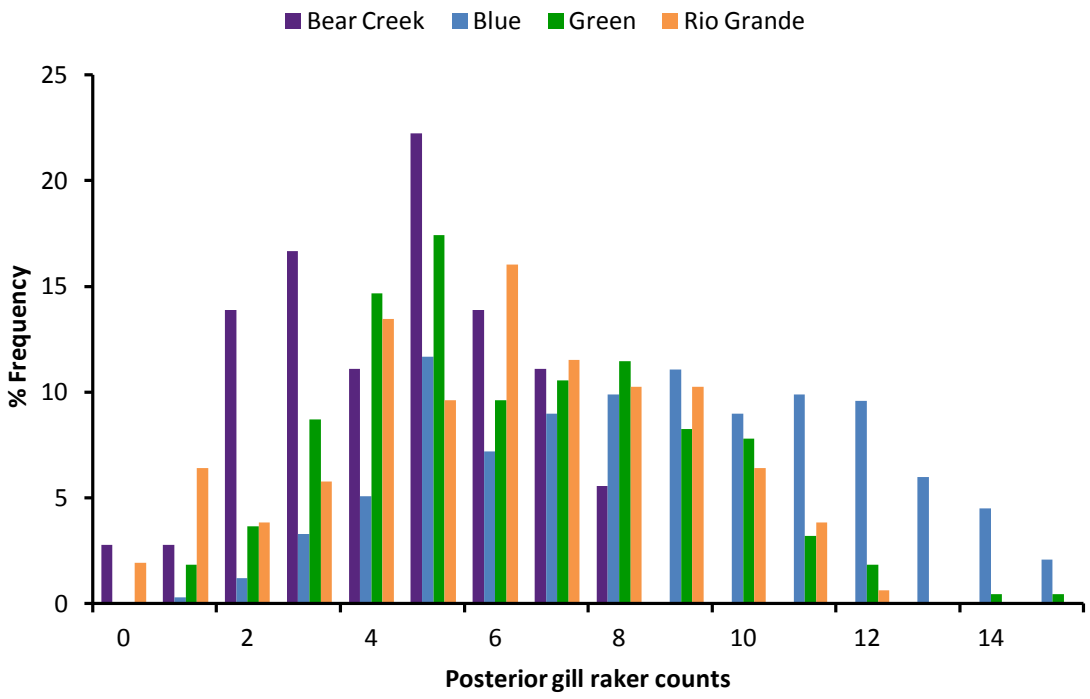


Figure 12. Percent frequency of posterior gill raker counts among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012).

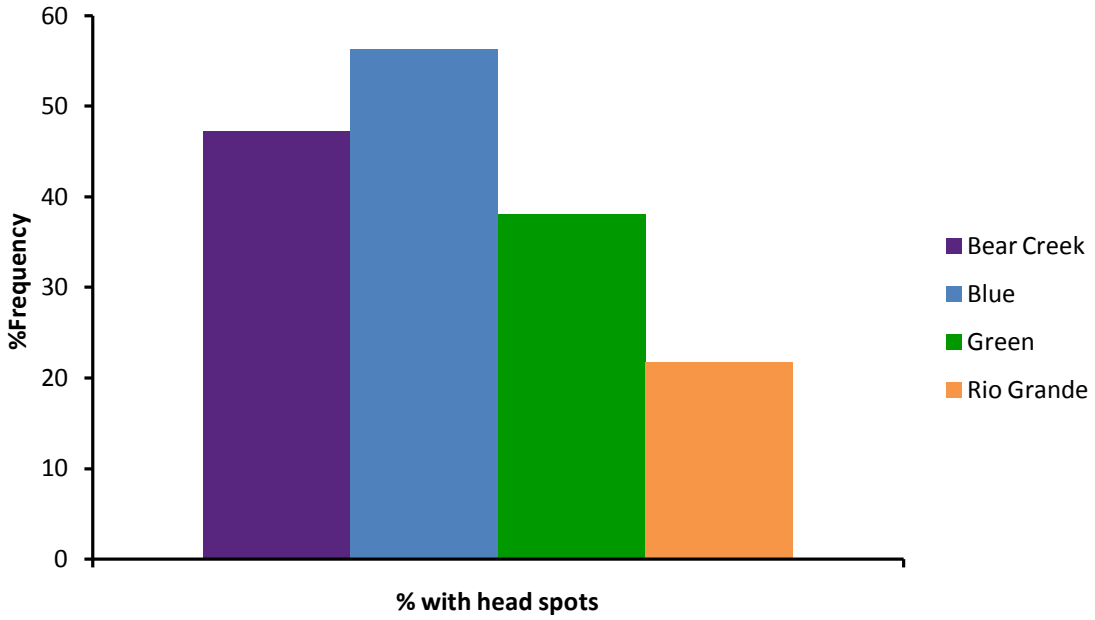


Figure 13. Percent frequency of individual cutthroat trout with spots on top of the head among four lineages of cutthroat trout under the Molecular classification groups (Metcalf et al. 2012).

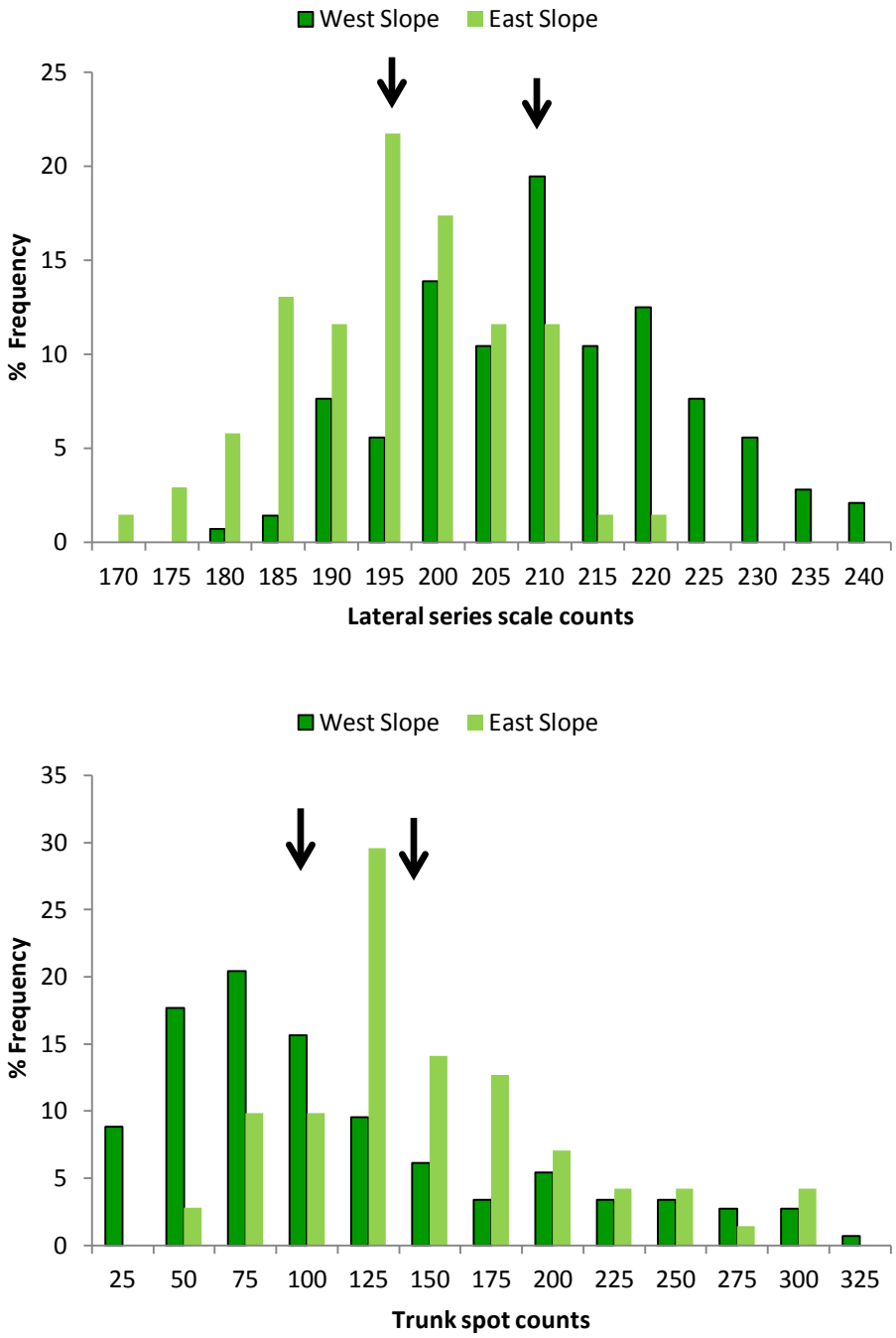


Figure 14. Percent frequency of lateral series scale counts (upper panel) and trunk spot counts (lower panel) for Green Lineage fish from West and East Slope populations. Arrows indicate mean counts.

Geographic Model

Molecular Model

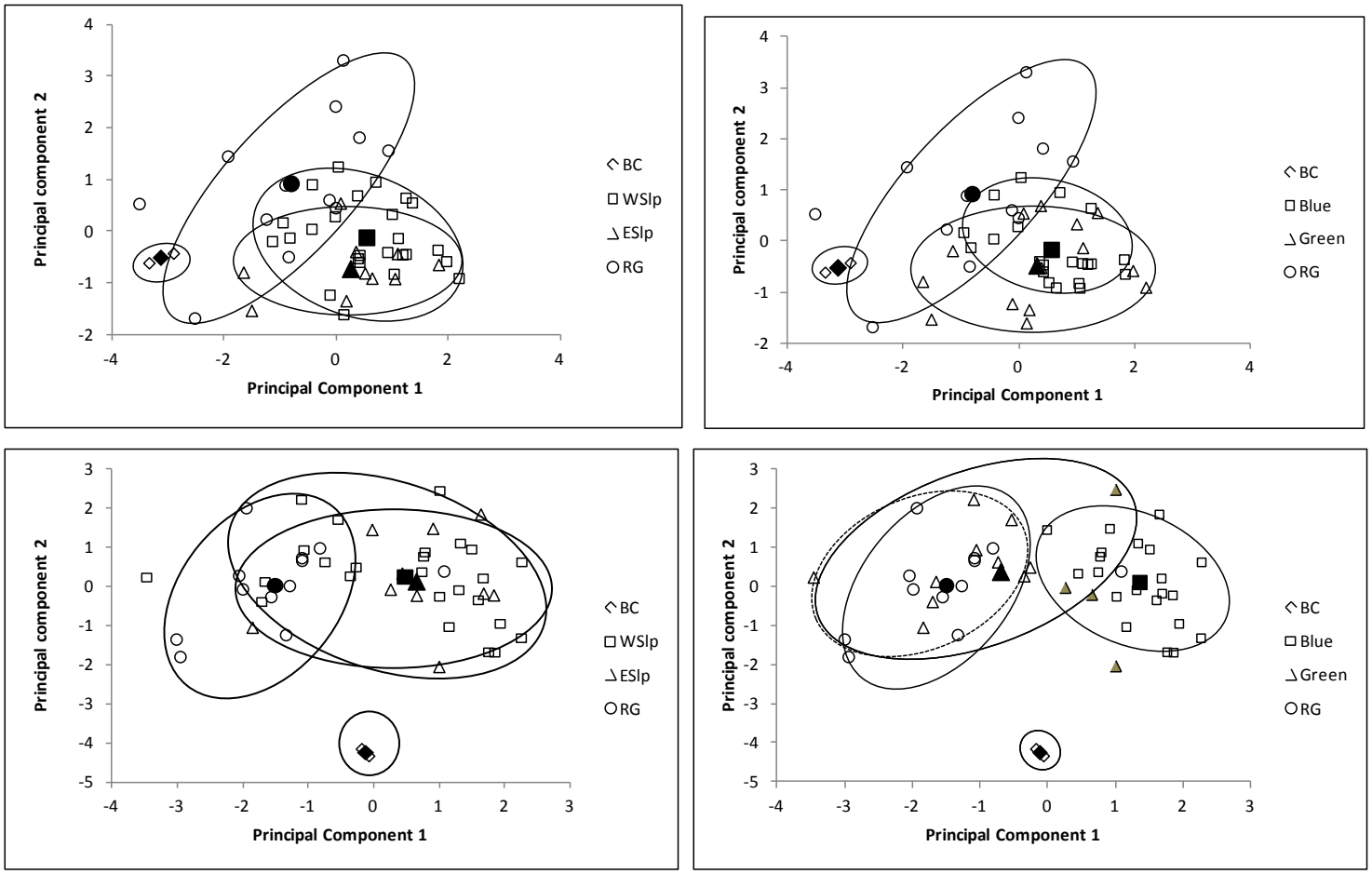


Figure 15. Principal component scatterplots of eigenvectors for taxa (Geographic Model, left panels) or lineages (Molecular Model, right panels) of cutthroat trout from the Southern Rocky Mountains, including Bear Creek. Individual symbols are means for study

populations. Upper scatterplots depict those using only four historically used meristic traits and lower plots depict those using four historically used meristic traits plus four spot traits. Larger solid symbols are centroids calculated from the mean eigenvectors for each taxon or lineage; group ellipses encompass most or all of observations. The four shaded triangles in the lower right box depict Green Lineage cutthroat trout populations residing in Front Range streams in the Arkansas and South Platte River basins; the dashed ellipse encompasses all Green Lineage populations except for those Front Range populations.