

1 The phylogeography of westslope cutthroat trout

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23 Abstract.-Identifying units of conservation of aquatic species is fundamental to informed natural
24 resources science and management. We used a combination of mitochondrial and nuclear
25 molecular methods to identify potential units of conservation of westslope cutthroat trout, a
26 taxon native to montane river basins of the northwestern U.S. and southwestern Canada.
27 Mitogenomic sequencing identified two major lineages composed of nine monophyletic clades,
28 and a well-supported subclade within one of these, largely delineated by river basins. Analyses
29 of microsatellites and single nucleotide polymorphisms corroborated most of these groupings,
30 sometimes with less resolution but demonstrating more complex connections among clades. The
31 mitochondrial and nuclear analyses revealed that Pleistocene glacial cycles profoundly
32 influenced the distribution and divergence of westslope cutthroat trout, that this taxon crossed the
33 Continental Divide in two separate events, and that genetically pure but nonindigenous fish were
34 widely distributed. Herein, we recognize nine geographically discrete, cytonuclear lineages
35 largely circumscribed by major river basins as potential units of conservation: 1) John Day; 2)
36 Coeur d'Alene; 3) St. Joe; 4) North Fork Clearwater; 5) Salmon; 6) Clearwater headwaters; 7)
37 Clearwater-eastern Cascades; 8) neoboreal, consisting of most of the Columbia upstream from
38 central Washington, the Fraser in British Columbia, and the South Saskatchewan in Alberta; and
39 9) Missouri.

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42 If the biota, in the course of aeons, has built something we like but do not understand, then who
43 but a fool would discard seemingly useless parts? To keep every cog and wheel is the first
44 precaution of intelligent tinkering.

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—Aldo Leopold (1953)

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49 Leopold's (1953) admonition has long served as a core paradigm of conservation biology. In
50 his time, the focus was on preserving species, but species often comprise distinct lineages that go
51 by many names—subspecies, distinct population segments, evolutionarily significant units,
52 operational taxonomic units, or stocks—and constitute potential units of conservation (Avice
53 2000) that can be accorded taxonomic or legal standing, e.g., under the U.S. Endangered Species
54 Act (Waples 1991) or Canada's Species at Risk Act (Mee et al. 2015). Historically, evidence for
55 the presence of these lineages was largely based on variation in morphology, behavior, life
56 history, or ecology, usually associated with a geographically discrete distribution, but
57 increasingly sophisticated molecular tools have revolutionized their identification and spawned a
58 branch of science dedicated to their study, phylogeography (Avice 2000). There is particular
59 urgency to identify units of conservation for aquatic ectotherms such as fishes, mussels, crayfish,
60 and amphibians. These aquatic groups are disproportionately represented among at-risk taxa
61 (Williams et al. 2011), and shifts in climate are beginning to alter their distributions (Comte and
62 Grenouillet 2013; Eby et al. 2014). Correctly inferring the existence and extent of lineages within
63 a taxon also relies on an understanding of the geological and climatic histories of current and
64 former habitats. For aquatic species, especially fishes in western North America, this means
65 appreciating the variation in hydrological connections over evolutionary timescales (McPhail and
66 Lindsey 1986; Minckley et al. 1986), particularly with respect to the influence of Pleistocene
67 glaciations on hydrologic networks (Bernatchez and Wilson 1998). A robust intraspecific
68 phylogeny also demands that a taxon be sampled across its entire range with sufficient intensity
69 to represent all potential units of conservation, and be examined with tools of sufficient
70 sensitivity to recognize them (Heath et al. 2008). It also requires awareness of the potential for
71 human-assisted relocation of nonlocal fish populations (Metcalf et al. 2012; Pritchard et al.
72 2015).

73 The systematics and distribution of cutthroat trout (*Oncorhynchus clarkii*) have been a source
74 of contention since the species was first formally described in the 19th century (Richardson
75 1836). Various forms have been described as full species or as subspecies with little regard to
76 previous work (Thorgaard et al., this volume), and the origin and affiliation of some type
77 specimens has been obscure (Metcalf et al. 2012). Moreover, our understanding of the historical
78 distributions of putative taxa is far from perfect, in part because of the broad-scale husbandry and
79 translocation of some forms of cutthroat trout (Wiltzius 1985) and the rapid extirpation of others

80 (Young and Harig 2001). A valiant attempt to resolve this ambiguity was made by the
81 preeminent taxonomist of the 20th century on western North American trout, Robert Behnke,
82 who divided cutthroat trout into 14 extant and 2 extinct subspecies (Behnke 1992). Among these
83 subspecies was the westslope cutthroat trout *O. c. lewisi*, first observed by Lewis and Clark in
84 1805 at the eventual type location, the Great Falls of the Missouri River (Girard 1856). Primarily
85 relying on morphological evidence, Behnke (1992) asserted that the cutthroat trout of the interior
86 Columbia River basin also represented this subspecies, an observation broadly corroborated by
87 phylogenetic work (Leary et al. 1987; Wilson and Turner 2009; Loxterman and Keeley 2012;
88 Houston et al. 2012). Whether there is substantial phylogenetic diversity within this taxon across
89 its range, however, has not been resolved. Because of the absence of substantial morphological
90 differences among westslope cutthroat trout in different river basins, the U.S. Fish and Wildlife
91 Service concluded that the taxon constituted a single unit of conservation in response to a
92 petition for listing under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service 2003).
93 Yet subsequent molecular studies indicated the presence of marked geographic divergence
94 within westslope cutthroat trout (Drinan et al. 2011; Loxterman and Keeley 2012) that was likely
95 driven by the complex geological and climatic history of the region (McPhail and Lindsey 1986).

96 Although Behnke (1992) was uncertain about the ultimate source of the lineage that led to
97 westslope cutthroat trout, he posited that most of its present distribution could be attributed to
98 events during the Wisconsinan glaciation 100–15 ka ago. He proposed that major waterfalls
99 developed during this time in the Pend Oreille, Spokane, and Kootenai River basins that may
100 have isolated westslope cutthroat trout upstream. Thereafter, the Clark Fork River basin served
101 as the source for fish dispersing—across divides via stream capture—to the east and north to the
102 upper Missouri River and South Saskatchewan River basins in Montana, Wyoming, and Alberta
103 and to the south to the Clearwater and Salmon River basins in Idaho. He postulated that outwash
104 floods from Glacial Lake Missoula carried westslope cutthroat trout to disjunct river basins along
105 the eastern face of the Cascade Range in Washington and to the John Day River basin in Oregon,
106 and may have been an alternate route for populations that colonized the Clearwater and Salmon
107 River basins. Recession of continental glaciers, along with the temporary proglacial lakes at their
108 trailing edges, presumably permitted westslope cutthroat trout to colonize waters farther north in
109 western Montana, northern Idaho, southern British Columbia, and southern Alberta.

110 Our goal was to test this phylogeographic hypothesis (cf. Crisp et al. 2011). We did so by
111 assessing the phylogenetic structure of westslope cutthroat trout across the bulk of its historical
112 distribution, with an additional aim to identify and geographically delineate potential units of
113 conservation, using a combination of mitochondrial and nuclear molecular information on
114 different samples of westslope cutthroat trout, including those used to establish hatchery
115 broodstocks.

116

117 **Methods**

118 *The historical range and its geological history*

119 The historical range of westslope cutthroat trout is thought to constitute portions of the Fraser,
120 South Saskatchewan, Missouri, and Columbia River basins (Shepard et al. 2005; McPhail 2007;
121 Figure 1). Its presence in the Fraser River basin in British Columbia is limited to a few
122 headwater streams (McPhail 2007), whereas it once occupied most of the tributaries in the Bow
123 River and Oldman River basins, part of the South Saskatchewan River basin in Alberta (Yau and
124 Taylor 2013; Fisheries and Oceans Canada 2014). In the Missouri River basin, its range was
125 thought to include the main stem and most tributaries from the Judith River to the headwaters,
126 excluding those blocked by waterfalls (e.g., the Sun River basin above present-day Gibson
127 Dam). In the upper Columbia River basin, the historical range includes 1) the entire Pend Oreille
128 and Kootenai River basins in British Columbia, Idaho, Montana, and Washington; 2) scattered
129 populations in the Kettle River basin in British Columbia (McPhail 2007) and presumably
130 Washington; 3) the Spokane River basin above Spokane Falls, primarily in Idaho, and perhaps
131 the Sanpoil River basin in Washington (Trotter et al. 2001b). Along the eastern Cascades,
132 westslope cutthroat trout are found from the Methow River basin in the north to the Yakima
133 River basin in the south, although there is uncertainty with respect to whether it is indigenous to
134 these and all intervening basins (Trotter et al. 1999, 2001a). In Oregon, westslope cutthroat trout
135 are present in tributaries of the main-stem and North Fork John Day River, but believed to be
136 introduced to the latter (Gunckel 2002). Westslope cutthroat trout are also native to the bulk of
137 two large river basins in the Snake River in Idaho, the Clearwater and Salmon, but are curiously
138 absent from all other basins tributary to the Snake River in Oregon, Washington, and Idaho
139 (except where introduced; Neville and Dunham 2011). Although the four major basins are
140 presently hydrologically isolated, they were presumably connected in the distant or recent past.

141 For example, the upper Columbia and Missouri Rivers were connected by the dual outlets of a
142 lake at Marias Pass in Montana until 1913, when the Union Pacific Railroad diverted all flow to
143 a tributary of the Two Medicine River of the Missouri River basin (Schultz 1941).

144 The modern distribution has been substantially reduced because of habitat alteration and
145 introductions of nonnative species (Shepard et al. 2005). Moreover, hatchery strains of westslope
146 cutthroat trout have been widely introduced throughout the 20th century and could affect both the
147 phylogenetic interpretation and evolutionary integrity of wild populations. Three broodstocks are
148 commonly used at present. In Montana, the primary broodstock was developed from fish in the
149 upper Flathead and middle Clark Fork River basins (Drinan 2010). In Idaho, westslope cutthroat
150 trout were domesticated from populations in the Priest River basin, as was the King's Lake
151 broodstock in Washington (Crawford 1979; M. Campbell, Idaho Department of Fish and Game,
152 personal communication). A second broodstock in Washington, Twin Lakes, was presumably
153 developed from fish collected in the Lake Chelan basin or Methow or Wenatchee River basins
154 (Crawford 1979).

155 The interior Columbia River basin constitutes the western portion of the distribution of
156 westslope cutthroat trout and has been present in some form for at least 15 Ma, roughly the age
157 of the lineage representing cutthroat trout and rainbow trout *O. mykiss* (Stearley and Smith
158 2016). The eruption and deposition of flood basalts from the mid-Miocene to the Pliocene led to
159 repeated re-routing and re-entrenchment of the major tributaries (Fecht et al. 1985). This
160 coincided with the orogeny of the Cascade Range, whereas the topography of the headwaters
161 was set by the orogeny of the Rocky Mountains 60 Ma earlier (Alt and Hyndman 1995). The
162 Pleistocene was characterized by the cyclic advance and retreat of the Cordilleran and Laurentide
163 ice sheets, which buried northerly portions of the current range of westslope cutthroat trout under
164 hundreds of meters of ice. The repeated advance of the Purcell lobe of the Cordilleran ice sheet
165 near Sandpoint, Idaho also led to the damming of the Clark Fork River and filling of Glacial
166 Lake Missoula (Waitt 1985). The repeated failure of this ice dam led to catastrophic jökulhlaups
167 lasting 1–2 weeks in which the discharge from the draining glacial lake temporarily exceeded
168 that of all rivers on Earth combined. These glacial outburst floods, perhaps up to 100 at multi-
169 decadal intervals during the Wisconsinan glaciation (Booth et al. 2003) and an unknown number
170 from earlier Pleistocene glacial intervals (Bader et al. 2016), produced the Columbia Basin
171 scablands. The scablands are characterized by floodwater-scoured surfaces, deep sedimentary

172 deposits from temporary ponding as water backed up at topographic chokepoints, e.g., Wallula
173 Gap in Washington, and huge nickpoints in many river channels that resulted in waterfalls likely
174 to constitute migration barriers to aquatic taxa, e.g., Spokane Falls on the Spokane River,
175 Palouse Falls on the Palouse River, Metalline Falls on the Pend Oreille River, and falls on many
176 lesser basins (Waite 1985). The Okanagan and Columbia River lobes of this ice sheet also led to
177 the formation of Glacial Lake Columbia, which at its highstand extended upstream to the Purcell
178 lobe and portions of the Coeur d'Alene and St. Joe River valleys (Atwater 1987; Hanson and
179 Clague 2016). Contemporaneous mountain glaciers (Pierce 2003) would have forced headwater
180 taxa to lower portions of many watersheds to escape habitats that were cold, turbid, and
181 unproductive. Similar conditions—rerouted and glacially derived streams and temporary glacial
182 lakes—prevailed in the upper Missouri River basin. Until the Pleistocene (and perhaps during
183 portions of it), the Missouri River drained northward to Hudson Bay (Howard 1958). By the Last
184 Glacial Maximum 23–19 ka ago (Hughes et al. 2013), the Laurentide ice sheet had forced the
185 Missouri River south to near its present course within the Mississippi River basin. As that ice
186 sheet retreated, it created a series of proglacial lakes (Alden 1932; Colton et al. 1961) that
187 persisted as recently as 11.5 ka ago (Davis et al. 2006) and may have served as springboards for
188 colonization by aquatic taxa farther north. The retreating Cordilleran ice sheet also left a trail of
189 temporary lakes that could have served as stepping stones for aquatic species to colonize basins
190 farther north, such as the Fraser River (McPhail and Lindsey 1986).

191 Two other major events in the history of the Columbia River basin merit attention. About 3
192 Ma ago, the Columbia River captured the Snake River, which previously drained farther south to
193 the Klamath or Sacramento River basins (Wood and Clemens 2002; Stearley and Smith 2016).
194 Although the connection between the Snake River and lower Columbia River did not promote
195 extensive faunal transfer (at least of salmonids), it did provide a route for another Pleistocene
196 flood: the draining of Lake Bonneville. Unlike the ice dam failures of Glacial Lake Missoula,
197 Lake Bonneville drained by overtopping and rapidly downcutting at Red Rock Pass 17.5 ka ago
198 (Amidon and Clark 2015; Oviatt 2015), delivering 4,750 km³ of water in what represents the
199 largest (by volume) known freshwater flood (O'Connor 1993). Like those of Glacial Lake
200 Missoula, this flood backed up many of the tributary rivers and may have conveyed aquatic
201 species across large distances. Because it was less violent and longer lasting—a couple months
202 to one year (Malde 1968; Jarrett and Malde 1987)—the draining of Lake Bonneville may been

203 more conducive to fish survival and translocation. Interpreting its possible phylogeographic
204 effect is difficult because it was both preceded and followed by outwash floods from Glacial
205 Lake Missoula, the last of which was 14.7 ka ago (Waitt 1985; McDonald et al. 2012; Balbas et
206 al. 2017), with additional floods still later from smaller proglacial lakes that formed as the
207 Purcell lobe of the Cordilleran ice sheet retreated (Waitt et al. 2009; Peters 2012) and perhaps
208 from the longer-persisting Glacial Lake Columbia (Balbas et al. 2017).

209

210 *Conservation units*

211 We define units of conservation (sensu distinct population segments; Waples 1991) as
212 populations or groups thereof that are substantially reproductively isolated and that constitute an
213 important component of the evolutionary legacy of a species. Ideally, units of conservation
214 would be identified based on an array of phylogenetically informative ecological, life history,
215 morphological, and genetic characteristics (Fraser and Bernatchez 2001). Variation in
216 morphology has been related to ecotypic differences in westslope cutthroat trout (Seiler and
217 Keeley 2009), and such differences have been shown to have a genetic basis and contribute to
218 identifying units of conservation in some salmonids (Keeley et al. 2007). To our knowledge,
219 however, there has been no comparison of morphological or ecological traits across the entire
220 range of westslope cutthroat trout (cf. Bestgen et al. 2013). In addition, relative to other Pacific
221 salmon and trout, cutthroat trout are ecological generalists capable of expressing a wide array of
222 life history strategies (Young 1995; Waples et al. 2001), rendering these characteristics less
223 definitive for delineating units of conservation. Given this background of uncertainty, we used
224 well-supported, reciprocally monophyletic mitochondrial clades (Moritz et al. 1995) as working
225 hypotheses about the units of conservation within westslope cutthroat trout, particularly when
226 geographic structuring reinforced those distinctions. We also sought support from analyses of
227 two suites of nuclear markers to corroborate the mitochondrial results.

228

229 *Laboratory analyses*

230 We obtained three sets of genetic information from two groups of samples of westslope
231 cutthroat trout: 1) mitogenomic sequences of specimens from most of the historical range in the
232 U.S. and Canada ($n = 96$ fish); 2) allele frequencies of 10 microsatellite loci (Vu and Kalinowski
233 2009) in the aforementioned specimens (save one from Buck Creek, WA, sample 44); and 3)

234 allele frequencies of 52 variable nuclear single nucleotide polymorphisms (SNPs) from
235 specimens collected across a large portion of the U.S. range ($n = 524$ fish from 55 sites; Table 1;
236 Figure 1). Specimens from wild populations that were representative of those used to establish
237 each of the hatchery broodstocks were included in one or both sample groups. Some of the sites
238 in the first two analyses also had additional individuals analyzed using SNPs ($n = 29$), but spatial
239 overlap in these datasets was not complete.

240 Whole genomic DNA was extracted from tissue samples using the QIAGEN DNeasy Tissue
241 Kit (Qiagen, Valencia, CA, USA). Extracts were converted into indexed genomic libraries using
242 the Illumina TruSeq DNA HT kit (version 2). To enrich samples for mitochondrial DNA targets,
243 we developed hybridization-enrichment baits based on the complete mitochondrial genome
244 sequence for Lahontan cutthroat trout *O. c. henshawi* (NCBI accession AY886762). Baits and
245 blocking probes were designed and synthesized by MYcroarray, LLC (version 2; Ann Arbor,
246 MI), and hybridization-enrichment reactions followed their protocol with a 12-cycle final
247 amplification. Enriched libraries were pooled to 48-plex and sequenced using two lanes of 101-
248 base-pair, single-end reads on the Illumina HiSeq2000 at the Center for Genome Research and
249 Biocomputing at Oregon State University (<http://cgrb.oregonstate.edu/>). Indices were
250 demultiplexed using CASAVA v. 1.8.2, and individual sample mitochondrial genomes were
251 assembled using the *O. c. henshawi* reference and the CLC-Bio v. 7.0 assembler, with the
252 following quality and alignment parameters: quality score limit of 0.05, maximum number of
253 ambiguities per read = 2; mismatch cost = 3, indel cost = 3, indel open cost = 6, indel extend cost
254 = 1, length and similarity fraction = 0.95, and non-specific matches mapped randomly.
255 Individual samples were represented by an average of 3.674 million microreads (minimum =
256 281,755; maximum = 13,176,436); for assembly, we used a maximum of 4 million reads per
257 sample. Short read sequences from this study are available under BioProject ID PRJNA389467
258 from National Center for Biotechnology Information.

259 In the microsatellite analysis, we conducted analyses of 10 loci used in previous studies
260 of westslope cutthroat trout: OclMSU14, OclMSU15, OclMSU17, OclMSU21, OclMSU23,
261 OclMSU24, OclMSU25, OclMSU27, OclMSU30, OclMSU33 (Vu and Kalinowski 2009; Drinan
262 et al. 2011). The reaction volume (10 μ l) contained 1.0 μ l DNA, 1 \times reaction buffer (Applied
263 Biosystems), 2.0 mM MgCl₂, 200 μ M of each dNTP, 1 μ M reverse primer, 1 μ M dye-labeled
264 forward primer, 1.5 mg/ml BSA, and 1U Taq polymerase (Applied Biosystems). The PCR

265 profile was 94 °C/5 min, [94 °C/1 min, 55 °C/1 min, 72 °C/30 s] × 40 cycles. The resultant
266 products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology).

267 For the SNP analyses, we used Competitive Allele Specific PCR (KASPar) assays
268 (KBiosciences, Hoddesdon, Herts, England) to amplify 52 variable nuclear loci (Harwood and
269 Phillips 2011; Kalinowski et al. 2011; Amish et al. 2012; Campbell et al. 2012; Pritchard et al.
270 2012). We used a suite of 44 diagnostic SNP alleles to ensure no fish introgressed with rainbow
271 trout or Yellowstone cutthroat trout *O. c. bowieri* alleles were included in the analysis
272 (McKelvey et al. 2016). The PCR touchdown profile contained an initial annealing temperature
273 of 65 °C and decreased by 0.80 °C per cycle until most cycles ran at 57 °C. We visualized PCR
274 products on an EP1 Reader (Fluidigm) and determined individual genotypes using Fluidigm SNP
275 Genotyping Software.

276

277 *Phylogenetic analyses*

278 We conducted phylogenetic analyses on the three aforementioned sources of data, plus a
279 fourth, to assess intraspecific diversity in westslope cutthroat trout.

280 Mitogenomic sequences were aligned in MAFFT version 7 (Katoh and Standley 2013),
281 followed by minor manual adjustments. We used MitoAnnotator (Iwasaki et al. 2013) to order
282 and identify gene regions. We followed Satoh et al. (2016) and the mitotRNAdb database
283 (<http://mtrna.bioinf.uni-leipzig.de/mtDataOutput/>) to identify paired and unpaired regions in
284 rRNAs and tRNAs and evaluate whether nucleotide insertions or deletions were likely to
285 represent genotyping errors; we subsequently made 12 adjustments to the 1.6 million nucleotides
286 in the dataset. Unique haplotypes were identified using DAMBE version 6 (Xia 2017) and used
287 to construct neighbor-joining and maximum-likelihood phylogenetic trees. To portray
288 evolutionary distances among and between clades, we used MEGA 7.0 (Kumar et al. 2016) to
289 construct a neighbor-joining tree based on the MCL model with gamma-distributed evolutionary
290 rates and some invariant positions, bootstrapped 1,000 times. We included sequences from
291 Lahontan cutthroat trout, greenback cutthroat trout *O. c. stomias*, and rainbow trout as outgroups
292 (GenBank accessions NC006897, KP013107, KP013117, KP013084, DQ288268–71,
293 KP085590). Pairwise genetic distances (as a percentage) between samples and groups were
294 based on the number of nucleotide differences. For the maximum-likelihood analysis, we used
295 PartitionFinder 2.0 (Lanfear et al. 2016) to select the best-fitting partitioning scheme as

296 measured by AICc, constrained to the suite of evolutionary models considered by RAxML
297 (Stamatakis 2014) and excluding all outgroups. Data subsets included 1) codon position by gene
298 for the protein-coding genes (39 subsets); 2) paired and unpaired regions among tRNAs and the
299 origin of replication for the light strand (2 subsets) and between rRNAs (4 subsets); 3) intergenic
300 spacers and the non-coding portions of the control region (1 subset); and 4) conserved sequence
301 blocks of the control region, nucleotides coding for more than one gene, and tRNA anticodons (1
302 subset). Because RAxML will only consider a single evolutionary model for the entire suite of
303 partitions, we then compared AICc scores among maximum likelihood models using the GTR
304 and GTRGAMMA evolutionary models, and chose the model with the best score. We then ran
305 RAxML version 8.1.21 implemented through the RAxMLGUI (Silvestro and Michalak 2012)
306 and set for rapid bootstrapping (1,000 bootstraps) and a thorough ML search. We recognized
307 potential units of conservation as reciprocally monophyletic clades with > 70% bootstrap support
308 in the mitogenomic maximum-likelihood phylogeny. We did not single out subclades therein
309 with high levels of support unless: 1) they differed from all other subclades by more than the
310 average intra-clade divergence (0.04%) or 2) they had been previously recognized as a potential
311 unit of conservation, i.e., fish in the Missouri River basin (Drinan et al. 2011).

312 Further evaluation of these clades as potential units of conservations was undertaken by
313 analyzing a dataset combining all ND2 sequences of westslope cutthroat trout from Loxterman
314 and Keeley (2012) and one additional specimen in GenBank ($n = 93$, accessions EU186799,
315 JQ747580–596; Table 1) with those examined in the mitogenomic analyses (including the
316 outgroups). Some of these specimens ($n = 26$) were used in both analyses, reducing the number
317 of unique specimens ($n = 163$). We built a neighbor-joining tree using unpartitioned data
318 bootstrapped 1,000 times in MEGA 7.0, for which the Tamura-Nei model with gamma-
319 distributed rates was the best-fitting evolutionary model based on AICc scores.

320 Because the microsatellites and variable SNPs for westslope cutthroat trout were developed
321 (Campbell et al. 2012) almost entirely from specimens representing the two most northeasterly
322 and least genetically divergent clades identified in the mitogenomic analysis in the Missouri,
323 Kootenai, and Clark Fork River basins, ascertainment bias was more likely to prevent the
324 phylogenetic tree topology from representing the species tree (Lachance and Tishkoff 2013) but
325 less likely to incorrectly assign individuals to groups (Bradbury et al. 2011). Consequently, we
326 evaluated whether putative groups identified in STRUCTURE 2.3.4 (Hubisz et al. 2009), using

327 either the microsatellite or SNP datasets, matched those of the best-supported clades in the
328 mitogenomic analyses. We applied settings recommended by Falush et al. (2003) for detecting
329 subtle population subdivision by using the admixture model, correlated allele frequencies among
330 populations, and an allele frequency distribution parameter (λ) set to 1. We allowed STRUCTURE
331 to infer the value of the model's Dirichlet parameter (α) for the degree of admixture from the
332 data. In the SNP analysis, we used $K = 15$ (the number of major river basins) as the maximum
333 number of potential groups and completed 20 replicate runs for each value of K . In the
334 microsatellite analysis, we used $K = 10$ (the number of mitogenomic clades) as the maximum
335 number. In both analyses, we set the burn-in period to 100,000 MCMC repetitions and the post-
336 burn-in period to 100,000, ignoring the user-defined population-of-origin or sampling location
337 for each individual (i.e., adopting non-informative priors). We attempted to identify an optimal
338 value of K by calculating the maximum likelihood value and ΔK (Evanno et al. 2005) using the
339 program STRUCTURE HARVESTER (Earl and vonHoldt 2012).

340 Stocking of genetically pure westslope cutthroat trout was inferred from the appearance of
341 mitochondrial haplotypes outside their river basin of origin, or of individuals in nuclear analyses
342 that assigned to groups related to those that founded the primary broodstocks in Idaho and
343 Montana. For Montana, we regarded samples from the South Fork Flathead River (sample codes
344 35, 94, and 179; Table 1) as representative of broodstock genotypes. Samples from the Priest
345 River basin (codes 24, 137, and 138) were deemed to represent fish from the Idaho broodstock,
346 as well as the King's Lake broodstock in Washington. The Falls Creek sample (code 60)
347 represented Washington's Twin Lakes broodstock, but these genotypes were not found outside
348 the eastern Cascades river basins.

349

350 **Results**

351 *Mitochondrial analyses*

352 Hybridization-capture probes allowed us to enrich mitochondrial genome sequences to a very
353 high level (mean mitochondrial representation = 57.5% of all sequences), resulting in
354 mitochondrial genome assemblies represented by extremely high mean depth (range: 997—
355 20,208 reads), and complete coverage for all positions in the genome. Complete mitogenomes
356 (16,676 nucleotides) included 13 protein-coding genes, 2 rRNAs, 22 tRNAs, and several
357 noncoding regions (Table 2), and revealed 89 haplotypes among the 96 specimens. The

358 mitogenomic maximum-likelihood phylogeny recovered two main lineages (mean difference
359 between lineages based on number of nucleotide differences, 0.42%; mean difference within
360 lineages, 0.06–0.11%) that contained nine highly supported, monophyletic clades and one
361 subclade (Figure 2; Table 3). The topology and levels of support in the mitogenomic neighbor-
362 joining analysis were effectively identical (results not shown). One lineage (hereafter, the
363 southern lineage) contained six clades (1–5b) largely associated with individual river basins: the
364 John Day River (1) in Oregon; the upper Coeur d’Alene (2) and St. Joe Rivers (3) in the Spokane
365 River basin in Idaho; the North Fork Clearwater River (4) in Idaho; and the lower and middle
366 Salmon River (5a) and upper Salmon River (5b) in Idaho. The latter two clades, however,
367 overlapped geographically within the basin. The four clades (6–9) in the other main lineage (the
368 northern lineage) were less geographically circumscribed: the Clearwater headwaters (6), which
369 included specimens from the upper Selway and South Fork Clearwater Rivers in Idaho; the
370 Clearwater-eastern Cascades (7), with representatives from tributaries to the Middle Fork
371 Clearwater River in Idaho and from the Wenatchee River, Lake Chelan, and Methow River
372 basins on the eastern side of the Cascade Range in Washington; and a neoboreal (8) clade, which
373 included all river basins in Alberta, British Columbia, Montana, northern Idaho, and northeastern
374 Washington. Most of these constitute basins that were covered by continental ice sheets or the
375 proglacial lakes at their margins. Disjunct representatives of this clade, however, were found in
376 the Salmon, Clearwater, and Spokane River basins in Idaho. Also recovered in these analyses
377 was a well-supported subclade within the neoboreal group that represented all specimens (save
378 one) from the Missouri River (9) basin in Montana.

379 The analysis of ND2 sequences from fish genotyped for this study and from public databases
380 had 27 haplotypes, including 8 not observed in the mitogenomic analysis (Table 1). Consistent
381 with the previous analysis, the ND2 neighbor-joining tree resolved the two main lineages and
382 recovered similar geographic structure (Figures 3), but with less resolution and support of
383 additional clades despite greater geographic coverage. This analysis diagnosed the John Day,
384 Coeur d’Alene, and St. Joe River clades, and grouped but did not distinguish among the North
385 Fork Clearwater and Salmon River specimens. It also pooled all Clearwater River and eastern
386 Cascade specimens, and did not resolve Missouri River specimens as a distinct group within the
387 neoboreal clade.

388

389 *Nuclear analyses*

390 In the STRUCTURE analysis of SNPs, measures of the optimal number of groups were not
391 concordant. The log-likelihood estimate peaked at the maximum number of groups ($K = 15$)
392 whereas ΔK identified $K = 2$ as the optimum number of clusters. Given this outcome, we
393 examined how assignment to groups changed across the entire range of values (Figure 4 depicts
394 $K = 4, 8, \text{ and } 15$). The intermediate value ($K = 8$) was equivalent to the number of mitogenomic
395 clades geographically represented in the SNP analysis (samples equivalent to the North Fork
396 Clearwater River and Clearwater River headwater samples were not available). Although these
397 results broadly corroborated the mitogenomic classification, there were important differences.
398 Specimens from the John Day River were the first group to be segregated as distinct (at $K = 3$).
399 Specimens from the Salmon River formed a distinct group at intermediate and higher levels of K ,
400 as did those from the Missouri River. The Clearwater River and eastern Cascades clade grouped
401 at the lower levels of K , but split into distinct clusters at the highest levels of resolution. More
402 complex were patterns among the Coeur d'Alene River, St. Joe River, and neoboreal clades.
403 Specimens from the Coeur d'Alene and St. Joe Rivers largely assigned to a single group at all
404 levels of K , but this group also included specimens from the neoboreal clade from the lower
405 Clark Fork, Pend Oreille, and lower Kootenai River basins for lower levels of K . The rest of the
406 neoboreal clade tended to split along major watershed boundaries at higher levels of K ,
407 particularly the upper Kootenai River.

408 In the microsatellite analyses, $K = 5$ (Figure 5) had the largest log-likelihood estimate and the
409 third-highest ΔK (which peaked at $K = 2$). The smaller sample sizes in this analysis afforded less
410 resolution but nonetheless highlighted groups consistent with those identified previously: 1) the
411 John Day and Clearwater Rivers and eastern Cascades basins; 2) the Salmon River; 3) the Coeur
412 d'Alene and St. Joe Rivers, some samples from the Pend Oreille and lower Kootenai Rivers, one
413 from downstream on the Columbia River, and one from the Fraser River; 4) the Clark Fork and
414 upper Kootenai Rivers in the Columbia basin and the Oldman and Bow Rivers in the South
415 Saskatchewan basin; and 5) the Missouri River.

416

417 *Stocked fish*

418 The genotypes of one or more fish at as many as 18 sites were suspected of being influenced
419 by introductions of nonintrogressed but nonindigenous westslope cutthroat trout (Table 4). The

420 bulk of these observations were from Idaho, and involved the appearance of mitochondrial
421 haplotypes typical of the neoboreal group in the Salmon and Clearwater River basins. Although
422 we regarded specimens with neoboreal haplotypes in the St. Joe and Coeur d'Alene River basins,
423 primarily from the lowermost portions of both basins, as being nonindigenous, this interpretation
424 in uncertain. Fish with either the neoboreal or southern haplotypes tended to share a nuclear
425 genotype that was also typical of fish found farther north in the Pend Oreille, lower Clark Fork,
426 lower Kootenai, middle Columbia, and Fraser River basins. Thus, the St. Joe or Coeur d'Alene
427 River specimens may have been indigenous representatives of fish that colonized basins to the
428 north, including that from which Idaho's broodstock was derived, rather than reflect recent
429 introductions of that hatchery stock. In Montana, the two likely examples of nonindigenous
430 specimens were from disparate parts of the Missouri River basin that harbored fish having
431 mitochondrial or nuclear genotypes akin to those from west of the Continental Divide.

432

433 **Discussion**

434 The phylogeographic structure of westslope cutthroat trout is consistent with unglaciated
435 basins serving as refugia during glacial cycles, followed by extensive colonization from some of
436 those refugia during interglacial intervals (Bernatchez and Wilson 1998; Hampe and Petit 2005).
437 The deepest divergence among populations involved the John Day, Salmon, Clearwater, and
438 Spokane River basins, which were south of the maximum advance of the Cordilleran ice sheet.
439 Much lower levels of divergence characterized areas formerly occupied by the ice sheets or the
440 glacial lakes at their margins, collectively represented by the neoboreal mitogenomic clade (and
441 including the Missouri River basin). Although we have foregone estimating dates of divergence
442 among clades, much of the diversity seems to be associated with recent climatic events. The
443 radiation following the Last Glacial Maximum likely reflects the divergence within most
444 mitogenomic clades (mean, 0.04%), whereas previous interglacials over 100 ka ago may have
445 led to divergence between the Coeur d'Alene and St. Joe River clades, the neoboreal and the
446 Clearwater-eastern Cascade clades, and the North Fork Clearwater and Salmon River clades, and
447 still older events to the split between the southern and northern major lineages. Continental
448 glaciation during previous glacial maxima is likely to have extirpated groups that advanced
449 northward during earlier interglacial intervals. That all samples from the previously glaciated
450 area form a single mitogenomic clade suggests that no individuals representing earlier colonizers

451 are present in this area. Below, we consider the phylogeographic relevance of these river basins
452 to westslope cutthroat trout in more detail.

453 The clade in the John Day River basin, though clearly a member of the southern major
454 lineage, exhibited the greatest divergence from all other groups. This watershed constitutes the
455 downstreammost extent of westslope cutthroat trout in the Columbia River basin and is separated
456 from the next nearest populations in the eastern Cascade Range in Washington by hundreds of
457 kilometers, and by hundreds more from the nearest members of the southern lineage in the
458 Clearwater and Salmon Rivers in Idaho. There has been uncertainty with regard to whether these
459 fish were indigenous or introduced from Montana, Idaho, or Washington (Gunckel 2002), but the
460 evidence indicates that these fish constitute a distinct, relictual population of westslope cutthroat
461 trout that did not arrive via glacial outwash floods from upstream. Whether their distribution
462 extends to the North Fork John Day River basin, which also supports westslope cutthroat trout
463 but for which there are extensive records of stocking of this taxon (Gunckel 2002), has not been
464 evaluated. As alluded to earlier, the absence of westslope cutthroat trout from nearby basins in
465 southeastern Washington and northeastern Oregon with abundant suitable habitat is somewhat
466 surprising. That westslope cutthroat trout may have been present, however, is hinted at by the
467 presence of their mitochondrial haplotype among anadromous rainbow trout in at least one area,
468 the Tucannon River in Washington (Brown et al. 2004), although unrecorded stocking of
469 westslope cutthroat trout is a plausible alternative explanation.

470 The Salmon River basin constitutes a modern stronghold for westslope cutthroat trout
471 (Shepard et al. 2005). It also hosts two relatively divergent mitogenomic clades of the southern
472 lineage which are found exclusively in this basin. Nevertheless, the clades are geographically
473 interspersed and showed little differentiation in the nuclear analyses. Their origin may have
474 reflected isolation in different parts of the Salmon River basin during a previous glacial interval
475 followed by subsequent mixing. Their nearest relatives appear to be fish found in the North Fork
476 Clearwater River to the north, but whether these groups represent descendants of one another or
477 of simultaneous divergence in these different locations is uncertain.

478 The Clearwater River basin in Idaho constitutes a biodiversity hotspot for westslope cutthroat
479 trout. It hosts a geographically complex mixture of members of both primary mitogenomic
480 lineages and four of the 10 mitogenomic clades of westslope cutthroat trout, two of which are
481 endemic (and a third may be introduced). Its position beyond the southerly extent of the

482 Cordilleran ice sheet, combined with its relatively low elevation and high precipitation, seems to
483 have contributed to the basin being a refugium for disjunct or locally endemic populations of an
484 array of plant and animal taxa during the Last Glacial Maximum (summarized in Shafer et al.
485 2010). That does not suggest that all portions of the basin were amenable to westslope cutthroat
486 trout throughout this or earlier glacial cycles. Pollen core records from near the headwaters
487 indicate a cold, arid climate with vegetation dominated by sagebrush, and that western red cedar
488 *Thuja plicata*, currently a dominant forest overstory species typical of mesic, montane
489 environments, did not colonize the basin until the last 5 ka (Herring and Gavin 2015). Moreover,
490 mountain glaciers would have been prevalent in the higher ranges, constraining westslope
491 cutthroat trout to the lower portions of many watersheds until conditions permitted them to
492 colonize these areas, perhaps from multiple locations within the basin.

493 A remarkably close relationship between the populations in the eastern Cascade Range in
494 Washington and those in Clearwater River basin—especially the Lochsa River basin—in Idaho
495 is supported in all analyses. This enigmatic pattern raises questions about how westslope
496 cutthroat trout crossed the majority of the lower Snake and middle Columbia River basins in
497 recent geological time and in which direction. The simplest explanation would be stocking of the
498 Twin Lakes broodstock from Washington in the Clearwater River in Idaho, but there are no
499 records to support this (Evan Brown, Idaho Department of Fish and Game, personal
500 communication). There is, however, evidence of the disjunct distribution of undescribed species
501 of sculpins in central Idaho basins and along the eastern Cascade Range (M. Young, unpublished
502 data). Given that the modern distribution of these sculpins does not include lacustrine or large,
503 warm riverine environments, it is more likely that they, and thus westslope cutthroat trout, were
504 transported downstream from central Idaho to their present distribution in central Washington.
505 The limited divergence in westslope cutthroat trout haplotypes between these locations also
506 indicates that this transfer was recent, and may have involved the emptying of Lake Bonneville
507 or the last of the Glacial Lake Missoula outburst floods.

508 The major forks of the Spokane River basin are the Coeur d'Alene and St. Joe Rivers. In their
509 headwaters, these basins harbor distinct clades that are nonetheless sister to one another and
510 represent the southern lineage of westslope cutthroat trout. A comparable pattern of relatedness
511 and divergence was evident in the cedar sculpin *Cottus schitsuumsh*, a recently described species
512 that is largely confined to the Spokane River basin (LeMoine et al. 2014). The lower portions of

513 both the Coeur d'Alene and St. Joe Rivers, however, also hosted members of the mitogenomic
514 neoboreal clade. Fish from throughout both basins shared a nuclear genotype that also appeared
515 in fish farther north in the middle Columbia, Pend Oreille, lower Kootenai, lower Clark Fork
516 River, and Fraser River basins, all presently unconnected to the Spokane River basin. The pattern
517 could have arisen from 1) stocking of fish from the Priest River basin, the source of Idaho's
518 broodstock, throughout the upper Spokane River basin, for which there is some evidence (Table
519 4); or 2) ephemeral connections associated with fluctuating levels of proglacial lakes that
520 promoted northerly expansion of genotypes from the Spokane River basin. As noted earlier,
521 Glacial Lake Columbia reached tens of kilometers up the Coeur d'Alene and St. Joe River
522 valleys and would have facilitated the movement of fish between these basins. This would also
523 have provided access to the Pend Oreille basin across the low divide in Rathdrum Prairie in
524 northern Idaho. Much of this watershed was subjected to the extreme conditions associated with
525 the repeated, catastrophic drainage of Glacial Lake Missoula, but Glacial Lake Columbia
526 persisted for some time after the last of these floods (Balbas et al. 2017). As the Purcell trench
527 lobe of the Cordilleran ice sheet receded, it would have exposed the lower Kootenai River valley
528 but blocked drainage to the north, diverting streams from that area through the trench cut by the
529 Purcell lobe and connecting the lower Kootenai and Pend Oreille basins (Langer et al. 2011).
530 Another connection between these basins appears to have existed at the low divide between the
531 Bull River (lower Clark Fork) and Bull Lake (lower Kootenai) in Montana (Langer et al. 2011).
532 Each of these connections would have provided access to fish originating from the Spokane
533 River basin, which may have culminated in their reaching the Fraser River basin via the
534 succession of glacial lakes trailing the receding ice sheet. That a fish from that basin was
535 assigned to a microsatellite group representing fish from the upper Spokane River basin argues
536 for such a connection.

537 Yet it is also clear that these fish—or this particular wave of colonizers—did not found the
538 modern populations of westslope cutthroat trout inhabiting the Kootenai River above Kootenai
539 Falls or the Clark Fork River above present-day Noxon Dam or Thompson Falls. All these fish
540 constitute a single mitogenomic clade with a broadly shared nuclear genotype that suggests a
541 limited number of founding individuals or a limited number of sources. Whatever their origin,
542 this is now the most widely distributed clade of westslope cutthroat trout, and one that also
543 displays substantial internal structure. For example, the nuclear data (e.g., SNP STRUCTURE plots

544 for $K = 2-6$) indicate historical connections between populations in upper Kootenai and Flathead
545 River basins, for which there are a number of plausible pathways that might have existed
546 depending on the timing of recession of particular lobes of the Cordilleran ice sheet, the presence
547 or absence of terminal or lateral moraines that affected drainage patterns, or the existence of
548 multiple outlets of lakes near drainage divides, e.g., between the Little Bitterroot (Flathead), Big
549 Thompson (Clark Fork), and Fisher (upper Kootenai) Rivers, or the Stillwater (Flathead) and
550 Tobacco (upper Kootenai) Rivers.

551 There is also evidence that these fish not only colonized two additional major river basins
552 across the Continental Divide—the Missouri and South Saskatchewan Rivers—but that these
553 appear to be independent events. Based on our results and on earlier studies (Drinan et al. 2011),
554 the Missouri River clade is a recent derivative of the widely distributed neoboreal clade, likely
555 founded from a single source; all individuals share two diagnostic mutations in the mitogenome
556 (one each in tRNA for tyrosine and the control region). The nuclear data imply that their closest
557 relatives are those in the upper Flathead River basin. A likely route is via the Middle Fork
558 Flathead River and Nyack Creek to Summit Creek, thence to the Two Medicine River basin and
559 the rest of the Missouri River basin. This invasion was likely to have coincided with the
560 lingering presence of glacial lakes formed by the Laurentide ice sheet because the Two Medicine
561 River is tributary to the Marias River, which presently flows into the Missouri River well
562 downstream of the Great Falls of the Missouri. Because the falls constitute a barrier to upstream
563 fish migration, this location was likely inundated by a glacial lake (e.g., Glacial Lake Great Falls
564 had a highstand of 1200 m [Calhoun 1906], about 250 m higher than the river elevation at the
565 falls) which would have enabled westslope cutthroat trout to access the Missouri River
566 headwaters. Furthermore, the lack of genetic divergence (mean distance 0.01%, with several
567 geographically distant pairs of samples with identical mitogenomic haplotypes) among westslope
568 cutthroat trout in this basin also indicates that their recent arrival may be their first. The upper
569 Missouri River basin was not covered by the Laurentide Ice Sheet during any portion of the
570 Pleistocene, thus suitable habitat for cutthroat trout should have persisted throughout much of
571 this period and permitted greater divergence. That westslope cutthroat trout did not extend their
572 distribution to the Yellowstone River basin, where Yellowstone cutthroat trout are found, points
573 to the absence of a migration corridor via the lower Missouri and Yellowstone Rivers for either
574 subspecies when they colonized. That a single clade of sculpins *Cottus* sp. (Young et al. 2013;

575 M. Young, unpublished data) and of mountain whitefish *Prosopium williamsoni* (Whiteley et al.
576 2006) co-occur with both subspecies in these areas, however, demonstrates that interbasin
577 exchanges were possible at an earlier time.

578 The westslope cutthroat trout of Alberta in the South Saskatchewan River basin appear to
579 have arrived via a different route. They share mitogenomic affinities with fish of the Clark Fork
580 River basin upstream from Thompson Falls and lack the diagnostic mutations seen in the fish
581 from the Missouri River basin. The microsatellite analyses also group these fish with those in the
582 upper Clark Fork River, not with those from the Missouri River. That the invasions of the South
583 Saskatchewan and upper Missouri River basins were independent events is also supported by the
584 distribution of bull trout *Salvelinus confluentus*, a cold-water salmonid with similar habitat
585 preferences, which are present in the former and absent from the latter (Ardren et al. 2011).
586 Moreover, westslope cutthroat trout may have been constrained to a limited portion of the South
587 Saskatchewan River basin. Their current distribution includes only the Bow and Oldman River
588 basins (Fisheries and Oceans Canada 2014), and Marnell (1988) argued that they were
589 historically absent from Waterton Lakes and the Belly River and crossed the Continental Divide
590 farther north. Based on topography, a plausible route would have been via the Kootenay River to
591 Summit Lake in British Columbia, thence to Crowsnest Lake in Alberta, which is part of the
592 Oldman River basin.

593 Based on our combined analyses, we find evidence for nine units of conservation within this
594 taxon (Table 5). We acknowledge that different interpretations of the importance of modern-day
595 isolation, administrative boundaries, ecoregions, or geographic mixing of genotypes (Mee et al.
596 2015) could alter this total. For example, a reasoned case can be made that fish representing the
597 neoboreal clade in the lower Kootenai, lower Clark Fork, Pend Oreille, middle Columbia, and
598 Fraser River basins should be classified separately from those found elsewhere because their
599 nuclear genotypes are distinct. Similarly, one could contend that fish in the eastern Cascades in
600 Washington are distinct from those in the Clearwater River basin in Idaho based on geographic
601 isolation and different administrative authorities. Such arguments are valid, thus we consider our
602 estimate an initial hypothesis to be revised following additional sampling and more
603 comprehensive analyses, but would be cautious about divisions at much smaller scales. Leary et
604 al. (1988) reported that the bulk of genetic variation in westslope cutthroat trout in Montana was
605 partitioned among individual populations, and consequently advocated for saving as many

606 populations as possible to preserve the genetic legacy of this taxon. The substantial structuring of
607 headwater populations has been observed elsewhere among westslope cutthroat trout (Taylor et
608 al. 2003; Young et al. 2004) and other salmonid species (Ryman 1983; Wenburg and Bentzen
609 2001; Pritchard et al. 2009), though it may be an artifact of the widespread anthropogenic
610 isolation of headwater trout populations (Campbell et al., this volume). In contrast, our data (also
611 see Drinan et al. 2011) representing nearly the entire range of westslope cutthroat trout
612 demonstrates that the primary divisions within this taxon arise at broader spatial scales, roughly
613 conforming to major watershed boundaries and cycles of Pleistocene glaciation, which is
614 consistent with hypotheses about rangewide genetic structure in temperate zone species (Hampe
615 and Petit 2005). Although we concur with the intent to save as many populations as possible, the
616 larger groups we identified are more likely to satisfy criteria for recognizing units of
617 conservation (Waples 1991; Mee et al. 2015).

618 Our data and that of others confirms that the westslope cutthroat trout constitutes a coherent
619 taxon that may warrant recognition as a full species. The level of divergence within westslope
620 cutthroat trout (0.39% in a 640-bp fragment of cytochrome c oxidase subunit I (COI), the
621 barcode region) is comparable to that of other species of fishes (0.36%) worldwide (Ward 2009),
622 whereas its divergence (1.25% in the COI fragment, 1.11% across the mitogenome) from its
623 nearest relative (Lahontan cutthroat trout) attains or exceeds that of many species pairs (April et
624 al. 2011). Nonetheless, substantial uncertainties remain with regard to the placement of
625 westslope cutthroat trout within the overall cutthroat trout lineage. Our mitogenomic analyses
626 affirmed that westslope cutthroat trout haplotypes are highly divergent from those of rainbow
627 trout (4.80% across the mitogenome), and more similar to Lahontan cutthroat trout than to
628 greenback cutthroat trout (1.58%), but there is little consensus among the different genomic
629 regions. Others have suggested that westslope cutthroat trout may be most closely related to
630 rainbow trout based on variation in isozyme alleles (Leary et al. 1987), to coastal cutthroat trout
631 based on karyotypes or morphology (Loudenslager and Gall 1980; Behnke 1992), to Lahontan
632 cutthroat trout based on mitochondrial gene sequences (Wilson and Turner 2009; Loxterman and
633 Keeley 2012), either of the latter two based on analyses of Y chromosomes (Brunelli et al. 2013),
634 and to all three based on a panel of variable nuclear SNP markers (Houston et al. 2012). That
635 each analysis supports a different relationship may be more indicative of the relative strengths
636 and weaknesses of different markers than of an ambiguous phylogeny. For example, allozymes

637 can grossly and unpredictably underestimate haplotype variation (Buth 1984). The karyotype
638 analysis was conducted on a sample of five westslope cutthroat trout from one hatchery
639 broodstock (Loudenslager and Gall 1980) and its results contradict those of an earlier study with
640 specimens from elsewhere (Simon and Dollar 1963). Both studies could be correct because
641 geographical variation in chromosomal characteristics or multiple karyotypes within populations
642 are common (Thorgaard 1983). Mitochondrial sequences form the basis of a multitude of
643 projects to identify animal species (Ratnasingham and Herbert 2013), but introgression and
644 reticulate evolution among lineages weaken relationships based solely on mitochondrial data
645 (Taylor and Harris 2012). And all genetic analyses are vulnerable to incomplete geographic
646 sampling, and the geographic origin and ancestry of specimens used to develop nuclear markers
647 and diagnostic phenotypes can undermine phylogenetic inferences because of ascertainment bias
648 (Lachance and Tishkoff 2013) or environmentally driven selection. Given the relative youth of
649 cutthroat trout lineages, more intensive and representative sampling of the nuclear genome and
650 of populations likely to be indigenous will be required to resolve this issue.

651 Although our work represents the most comprehensive effort to describe the phylogeography
652 of westslope cutthroat trout, many details have yet to be resolved. Locations represented by one
653 or two individuals would benefit from more thorough sampling to reveal the distribution and
654 mixing of presumptive haplotypes and the discovery of new ones, such as in the John Day River
655 (and its presumably stocked North Fork) in Oregon, the eastern Cascade River basins in
656 Washington, and many portions of the Clearwater River in Idaho. Westslope cutthroat trout in
657 the Yakima River basin are of unknown provenance, whether related to lineages in the John Day,
658 the eastern Cascades (as implied in Figure 1), or to one as yet undescribed. Similarly, additional
659 work to delineate hatchery stocks using nuclear markers would clarify the extent to which
660 introgression of introduced forms affects indigenous populations, and would help avoid the use
661 of introduced forms when attempting to establish basin-specific stocks. Despite these
662 uncertainties, the phylogeographic patterns we have observed are concordant with the varying
663 connections among river basins in the intermountain West throughout the late Pleistocene. These
664 patterns can be used as a foundation to understand phylogeographic structure in other freshwater
665 taxa, albeit with an awareness of the nuances associated with the long evolutionary history of
666 cutthroat trout in western North America.

667

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675

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- 992

993 Table 1. Samples used in the phylogeographic analysis of westslope cutthroat trout. Site labels are in Figure 1.

994

Site	4th-code HU ¹	State/ Province	Latitude	Longitude	<i>N</i> ²	MG ³	ND2 ⁴	SNP ⁵	Label
Albert	Middle Clark Fork	MT	46.9213	-114.3678	1	1	A		1
Beaver	Yaak	MT	48.8174	-115.6787	1	2	A		2
Bostwick	Gallatin	MT	45.7995	-110.9981	10	3	A	x	3
Moose	Blackfoot	MT	46.9227	-112.8534	6	4	A	x	4
Deer	Bitterroot	MT	45.5746	-114.4784	13	5	A	x	5
EF Bull	Lower Clark Fork	MT	48.1151	-115.6868	11	6	B	x	6
Ketchikan	North Fork Flathead	MT	48.9771	-114.5071	1	7	A		7
McGuire	Upper Kootenai	MT	48.6896	-115.2728	10	8	A	x	8
Martin	Bitterroot	MT	46.0057	-113.8161	1	9	A		9
McCabe	Blackfoot	MT	47.1324	-113.0840	6	10	A	x	10
Miller	Fisher	MT	48.0827	-115.4642	5	11	A	x	11
Meadow	Lower Selway	ID	45.7154	-115.1953	12	12	C	x	12
Norton	Upper Clark Fork	MT	45.9811	-112.8041	8	13	A	x	13
Ontario	Upper Clark Fork	MT	46.4454	-112.3940	8	14	A	x	14
North	Bitterroot	MT	46.7343	-114.6085	1	15	A		15
Tyler	Flint-Rock	MT	46.6377	-113.5047	1	16	A		16
Wilkes	Lower Clark Fork	MT	47.4962	-115.4883	1	17	A		17
Canuck	Moyie	MT	48.9988	-116.0618	8	18	A	x	18
Ball	Lower Kootenai	ID	48.7712	-116.6069	10	19	A	x	19
Rampike	Upper Coeur d'Alene	ID	47.8197	-115.9619	10	20	D	x	20
Trail	Upper Coeur d'Alene	ID	47.8291	-116.3941	10	21	D	x	21
West Gold	Pend Oreille Lake	ID	47.9259	-116.5078	10	22	A	x	22
Avalanche	Upper Missouri	MT	46.7439	-111.5180	7	23	A	x	23
Beaver	Priest	ID	48.7489	-116.9270	10	24	A	x	24
Mokins	Upper Spokane	ID	47.7826	-116.6663	1	25	A		25

Site	4th-code HU ¹	State/ Province	Latitude	Longitude	<i>N</i> ²	MG ³	ND2 ⁴	SNP ⁵	Label
NF St. Joe	St. Joe	ID	47.4055	-115.8382	1	26	B		26
Skin	Moyie	MT	48.7728	-116.1446	1	27	A		27
Slowey	Middle Clark Fork	MT	47.2808	-114.9731	1	28	A		28
Dry Wolf	Judith	MT	46.9492	-110.5684	1	29	A		29
Fish	Jefferson	MT	45.8032	-112.4725	1	30	A		30
Heller	St. Joe	ID	47.0662	-115.2167	1	31	E		31
Tributary of Armstrong	Coeur d'Alene Lake	ID	47.5543	-116.5993	1	32	A		32
NF Dupuyer	Two Medicine	MT	48.0636	-112.8182	1	33	A		33
Sawmill	Belt	MT	47.1013	-110.7097	1	30	A		34
Youngs	South Fork Flathead	MT	47.2838	-113.3568	1	35	A		35
French	Upper North Fork Clearwater	ID	46.5143	-115.6768	1	36	C		36
Osier	Upper North Fork Clearwater	ID	46.8055	-115.0836	1	37	F		37
Crooked Fork	Lochsa	ID	46.6843	-114.6958	1	38	C		38
Pete King	Lochsa	ID	46.1929	-115.7064	1	39	C		39
Baldy	South Fork Clearwater	ID	45.9415	-115.6750	1	40	C		40
Bad Luck	Upper Selway	ID	45.8956	-114.7553	1	41	B		41
Flat	Upper Selway	ID	45.7213	-114.8575	1	42	C		42
Twisp	Methow	WA	48.3839	-120.4342	5	43	G	x	43
Buck	Wenatchee	WA	47.9163	-120.7132	1	36	C		44
Cedar	Pend Oreille	ID	48.8333	-117.4952	9	45	A	x	45
Ninemile	Sanpoil	WA	48.3596	-118.7420	1	46	A		46
Mill	Upper Salmon	ID	44.4940	-114.4200	10	47	F	x	47
Morse	Pashimeroi	ID	44.6222	-113.7999	9	47	F	x	48
Jacobs Ladder	Lower Middle Fork Salmon	ID	45.0766	-115.3373	10	49	F	x	49
MF Little Timber	Lemhi	ID	44.5867	-113.4618	1	50	F		50
American	South Fork Clearwater	ID	45.7452	-115.9635	1	51	C		51
Scotchman	Flint-Rock	MT	46.3818	-113.5519	1	52	H		52

Site	4th-code HU ¹	State/ Province	Latitude	Longitude	<i>N</i> ²	MG ³	ND2 ⁴	SNP ⁵	Label
Fourmile	Smith	MT	46.5447	-110.7372	8	53	A	x	53
Kraft	Swan	MT	47.4142	-113.7778	10	4	A	x	54
Leiberg	Upper Coeur d'Alene	ID	47.7209	-116.3834	10	55	I	x	55
NF Grouse	Pend Oreille Lake	ID	48.4962	-116.3481	1	56	J		56
NF Teton	Teton	MT	47.9905	-112.8143	1	23	A		57
SF Red	South Fork Clearwater	ID	45.6282	-115.4310	10	58	A	x	58
Yoosa	Clearwater	ID	46.4100	-115.5864	7	45	A	x	59
Falls	Lake Chelan	WA	48.0810	-120.3577	6	60	C	x	60
Thayer	Big Hole	MT	45.2054	-113.2944	1	61	A		61
Hungery	Lochsa	ID	46.3758	-115.5400	1	62	C		62
Ross	Upper Kootenai	MT	48.1853	-115.9790	1	63	K		63
Ditch	South Fork Clearwater	ID	45.7712	-115.3051	10	64	L	x	64
EF Emerald	St. Joe	ID	46.9871	-116.4163	1	65	B		65
Big	Pashimeroi	ID	44.4416	-113.5987	1	66	F		66
Bitter	Kettle HW	BC	49.0153	-118.1243	1	67	B		67
Blackbird	Middle Salmon-Panther	ID	45.0675	-114.3487	1	68	F		68
Blairmore	Oldman	AB	49.7143	-114.4630	1	69	M		69
Bluff	St. Joe	ID	47.1668	-115.5088	1	70	E		70
Boundary	Upper Middle Fork Salmon	ID	44.5276	-115.3004	1	71	F		71
Buffalo	Beaverhead	MT	45.1646	-113.2000	1	61	A		72
Cayuse	Upper North Fork Clearwater	ID	46.5998	-114.8931	1	73	C		73
Colson	Middle Salmon-Panther	ID	45.3247	-114.5365	1	74	N		74
Crazy	Fraser HW	BC	51.0228	-118.6053	1	75	A		75
Duck	Lower Middle Fork Salmon	ID	44.8522	-114.5220	1	76	F		76
Floodwood	Lower North Fork Clearwater	ID	47.0241	-115.9553	1	77	F		77
Hartley	Kootenai HW	BC	49.5761	-115.0255	1	78	A		78
Indian Grave	Lochsa	ID	46.4787	-115.0899	1	79	C		79

Site	4th-code HU ¹	State/ Province	Latitude	Longitude	N^2	MG ³	ND2 ⁴	SNP ⁵	Label
John Day	Upper John Day	OR	44.2978	-118.5501	1	80	O		80
Meadow	South Fork Salmon	ID	44.8950	-115.3404	1	81	P		81
Monk	Priest HW	BC	49.0329	-116.9780	1	82	A		82
NF Elkhorn	Upper Middle Fork Salmon	ID	44.6405	-115.3324	1	83	Q		83
Pack	Pend Oreille Lake	ID	48.6592	-116.6244	1	84	B		84
Sheep	Middle Fork Flathead	MT	48.2321	-113.6183	1	85	A		85
Split	South Fork Salmon	ID	45.0809	-115.7844	1	86	F		86
Toby	Columbia HW	BC	50.3598	-116.5000	1	87	A		87
Truman	Flathead Lake	MT	47.9938	-114.4016	1	88	R		88
Twin	Upper Salmon	ID	44.5792	-114.4275	1	89	B		89
Waiparous	Bow	AB	51.3864	-115.1686	1	90	A		90
Warm Springs	Upper Salmon	ID	44.1029	-114.7472	1	91	F		91
Werner	North Fork Flathead	MT	48.5931	-114.3679	1	92	S		92
Withington	Lenhi	ID	45.0422	-113.7966	1	93	F		93
Wounded Buck	South Fork Flathead	MT	48.2280	-113.9705	1	94	A		94
Gravey	Upper North Fork Clearwater	ID	46.5284	-115.1336	1	95	C		95
Twentyfivemile	Middle Fork Flathead	MT	48.2055	-113.3799	1	96	A		96
WF Bitterroot	Bitterroot	MT	45.5040	-114.3334	1		Z		97
Beaver	Blackfoot	MT	47.0190	-112.7674	1		Z		98
Chimney	Blackfoot	MT	46.7626	-113.0219	1		Z		99
Canyon	Bow	AB	50.8953	-114.7774	1		Z		100
Picklejar 2	Bow	AB	50.5198	-114.7737	1		Z		101
Picklejar 4	Bow	AB	50.5188	-114.7841	1		Z		102
Prairie	Bow	AB	50.8783	-114.8885	1		W		103
Quirk	Bow	AB	50.8068	-114.7533	1		W		104
Running Rain	Bow	AB	50.5045	-114.9452	1		Z		105
Silvester	Bow	AB	50.8614	-114.7257	1		Z		106

Site	4th-code HU ¹	State/ Province	Latitude	Longitude	N ²	MG ³	ND2 ⁴	SNP ⁵	Label
Akolkolex	Columbia HW	BC	50.9151	-117.8787	1		Z		107
Botts	Columbia HW	BC	50.7986	-116.3615	1		Z		108
Cedar	Columbia HW	BC	51.2685	-116.9912	1		K		109
Driftwood	Columbia HW	BC	50.8700	-116.4734	1		Z		110
Ladybird	Columbia HW	BC	49.4514	-117.8259	1		V		111
Neale	Columbia HW	BC	51.4593	-117.0917	1		Z		112
West Fisher	Fisher	MT	48.0537	-115.5182	1		Z		113
Doctor	Kootenai HW	BC	50.0911	-116.0867	1		Z		114
Lodgepole	Kootenai HW	BC	49.2819	-114.8143	1		X		115
Lussier	Kootenai HW	BC	50.0686	-115.5088	1		Z		116
Tributary to Albert	Kootenai HW	BC	50.5663	-115.5596	1		Z		117
Little Eightmile	Lemhi	ID	44.7685	-113.4424	1		F		118
Reservoir	Lemhi	ID	44.7202	-113.1261	1		F		119
Big	Lower Middle Fork Salmon	ID	45.0732	-115.3535	1		Z		120
Flume	Lower Middle Fork Salmon	ID	44.8181	-114.5326	1		F		121
MF Salmon	Lower Middle Fork Salmon	ID	44.9995	-114.7292	1		F		122
NF Smith	Lower Middle Fork Salmon	ID	45.1895	-115.3464	1		F		123
WF Cedar	Lower North Fork Clearwater	ID	46.9419	-115.8490	1		T		124
Albert	Middle Clark Fork	MT	46.9743	-114.2704	1		Z		125
Second	Middle Clark Fork	MT	47.1279	-114.7446	1		Z		126
Siegel	Middle Clark Fork	MT	47.2967	-114.7817	1		Z		127
Beaver	Middle Salmon-Panther	ID	45.2825	-114.2774	1		F		128
Beaver	Middle Salmon-Panther	ID	45.2837	-114.2854	1		F		129
Salmon	Middle Salmon-Panther	ID	45.3463	-113.9195	1		Z		130
Weaver	Moyie HW	BC	49.4034	-116.0229	1		Z		131
Harvey	NF Flathead HW	BC	49.2491	-114.5677	1		Z		132
Lynx	Oldman	AB	49.4961	-114.4960	1		Z		133

Site	4th-code HU ¹	State/ Province	Latitude	Longitude	<i>N</i> ²	MG ³	ND2 ⁴	SNP ⁵	Label
Oldman	Oldman	AB	50.0570	-114.5917	1		Z		134
Twin	Oldman	AB	50.1885	-114.4420	1		Z		135
Vicary	Oldman	AB	49.8014	-114.4648	1		Z		136
Hunt	Priest	ID	48.5809	-116.7722	1		AA		137
Lime	Priest	ID	48.8966	-116.9642	1		Z		138
Bear	South Fork Salmon	ID	44.8332	-115.5125	1		F		139
Halfway	South Fork Salmon	ID	44.7791	-115.5419	1		F		140
NF Coeur d'Alene	Upper Coeur d'Alene	ID	47.9586	-116.0987	1		V		141
Ross	Upper Kootenai	MT	48.1831	-115.9788	1		K		142
WF Quartz	Upper Kootenai	MT	48.5255	-115.7228	1		Z		143
Dagger	Upper Middle Fork Salmon	ID	44.4676	-115.3303	1		F		144
EF Sulphur	Upper Middle Fork Salmon	ID	44.5492	-115.4636	1		F		145
Garden	Upper Middle Fork Salmon	ID	44.7427	-115.1376	1		F		146
MF Salmon	Upper Middle Fork Salmon	ID	44.7304	-115.0185	1		F		147
MF Salmon	Upper Middle Fork Salmon	ID	44.5670	-115.3007	1		N		148
Alpine	Upper Salmon	ID	43.9046	-114.9227	1		F		149
Alpine	Upper Salmon	ID	43.9068	-114.9322	1		Z		150
Cinnibar	Upper Salmon	ID	44.3564	-114.4992	1		F		151
Island	Upper Salmon	ID	44.0960	-114.5929	1		Y		152
Jordan	Upper Salmon	ID	44.4303	-114.7272	1		F		153
Morgan	Upper Salmon	ID	44.6517	-113.9324	1		F		154
Road	Upper Salmon	ID	44.1569	-114.1735	1		F		155
Rough	Upper Salmon	ID	44.2413	-114.8006	1		F		156
Salmon	Upper Salmon	ID	44.2538	-114.5629	1		F		157
Salmon	Upper Salmon	ID	44.5447	-114.1767	1		F		158
Salmon	Upper Salmon	ID	44.6874	-114.0524	1		F		159
Salmon	Upper Salmon	ID	44.4258	-114.2225	1		F		160

Site	4th-code HU ¹	State/ Province	Latitude	Longitude	<i>N</i> ²	MG ³	ND2 ⁴	SNP ⁵	Label
Salmon	Upper Salmon	ID	44.2482	-114.5303	1		U		161
Tributary to Challis	Upper Salmon	ID	44.5037	-114.4571	1		F		162
Magruder	Upper Selway	ID	45.6854	-114.7750	1		V		163
Trapper	Big Hole	MT	45.6425	-112.8711	9			x	164
Little Boulder	Bitterroot	MT	45.7288	-114.2451	10			x	165
Overwhich	Bitterroot	MT	45.7176	-114.0799	8			x	166
WF Bitterroot	Bitterroot	MT	45.5040	-114.3334	9			x	167
Morrell	Blackfoot	MT	47.2992	-113.4639	8			x	168
Nevada	Blackfoot	MT	46.8177	-112.5681	9			x	169
Snowbank	Blackfoot	MT	47.0561	-112.6494	6			x	170
Musselshell	Clearwater	ID	46.3524	-115.7558	7			x	171
4th of July	Coeur d'Alene Lake	ID	47.6106	-116.5178	10			x	172
Big Cherry	Fisher	MT	48.1990	-115.5969	9			x	173
Cow	Fisher	MT	48.2257	-115.2143	9			x	174
West Fisher	Fisher	MT	48.0427	-115.4746	9			x	175
Schwartz	Flint-Rock	MT	46.6941	-113.8228	10			x	176
MF Little Timber	Lemhi	ID	44.5867	-113.4618	6			x	177
Big	North Fork Flathead	MT	48.5269	-114.3391	10			x	178
Black Bear	South Fork Flathead	MT	47.7446	-113.3834	10			x	179
Beaver	St. Joe	ID	47.0458	-115.4580	9			x	180
Bird	St. Joe	ID	47.2889	-115.5919	10			x	181
Snowshoe	Upper Clark Fork	MT	46.6860	-112.4654	10			x	182
Spotted Dog	Upper Clark Fork	MT	46.4483	-112.5164	6			x	183
Twin Lakes	Upper Clark Fork	MT	46.0631	-113.2235	8			x	184
Independence	Upper Coeur d'Alene	ID	47.8806	-116.4098	10			x	185
Laverne	Upper Coeur d'Alene	ID	47.7075	-116.3756	9			x	186
WF Eagle	Upper Coeur d'Alene	ID	47.7367	-115.8078	10			x	187

Site	4th-code HU ¹	State/ Province	Latitude	Longitude	<i>N</i> ²	MG ³	ND2 ⁴	SNP ⁵	Label
Rail	Upper John Day	OR	44.3462	-118.5664	30			x	188
Roberts	Upper John Day	OR	44.3332	-118.5781	22			x	189

995 ¹HU, hydrologic unit. For Canadian sites, this refers to the basin.

996 ²*N*, sample size. Values = 1 except sites for which SNP analyses were also performed.

997 ³MG, mitogenomic sequence. Bolded labels denote a haplotype is shared. All individuals were used in microsatellite analyses (except
998 sample 44).

999 ⁴ND2, haplotypes of NADH dehydrogenase 2.

1000 ⁵SNP, single nucleotide polymorphism analysis.

1001 Table 2. Haplotype diversity and variation by gene for 96 westslope cutthroat trout mitogenomic
 1002 sequences.

1003

Gene	Position		Length	H ¹	Genetic difference (%)	V	Pi	Non-syn
	Start	End						
ATPase 6	8113	8795	683	9	0.34 (0.15–0.59)	9	3	3
ATPase 8	7955	8122	168	5	1.19 (0.60–2.38)	4	3	2
COI	5475	7025	1551	13	0.33 (0.06–0.64)	17	12	0
COII	7189	7879	691	6	0.27 (0.14–0.43)	5	1	1
COIII	8796	9580	785	15	0.56 (0.13–1.27)	17	11	1
Cyt b	14382	15522	1141	16	0.26 (0.09–0.44)	16	10	7
ND1	2848	3822	975	17	0.53 (0.10–1.03)	20	10	6
ND2	4035	5084	1050	19	0.50 (0.10–0.95)	22	10	9
ND3	9651	9999	349	6	0.69 (0.29–1.15)	6	4	1
ND4	10360	11740	1381	18	0.39 (0.07–0.80)	22	11	6
ND4L	10070	10366	297	3	0.45 (0.34–0.67)	2	0	1
ND5	11953	13791	1839	38	0.34 (0.05–0.87)	45	17	19
ND6	13788	14309	522	11	0.44 (0.19–0.77)	9	5	2
r12S	69	1015	947	14	0.24 (0.11–0.42)	12	4	
r16S	1088	2772	1685	29	0.25 (0.06–0.53)	30	11	
tRNA-Ala	5159	5227	69	1	0	0	0	
tRNA-Arg	10000	10069	70	2	1.43	1	0	
tRNA-Asn	5229	5301	73	1	0	0	0	
tRNA-Asp	7101	7174	74	2	1.35	0	0	
tRNA-Cys	5336	5402	67	2	1.49	1	0	
tRNA-Gln	3896	3966	71	1	0	0	0	
tRNA-Glu	14310	14378	69	1	0	0	0	
tRNA-Gly	9581	9650	70	2	1.43	1	1	
tRNA-His	11741	11809	69	1	0	0	0	
tRNA-Ile	3827	3898	72	1	0	0	0	
tRNA-Leu	2773	2847	75	2	1.33	1	0	

tRNA-Leu2	11880	11952	73	1	0	0	0
tRNA-Lys	7880	7953	74	2	1.35	1	0
tRNA-Met	3966	4034	69	2	1.45	1	0
tRNA-Phe	1	68	68	5	2.35 (1.47–2.94)	4	2
tRNA-Pro	15594	15663	70	1	0	0	0
tRNA-Ser	7026	7096	71	1	0	0	0
tRNA-Ser2	11810	11878	69	5	2.32 (1.45–2.90)	4	1
tRNA-Thr	15523	15594	72	1	0	0	0
tRNA-Trp	5086	5157	72	2	1.39	1	1
tRNA-Tyr	5403	5473	71	3	1.41	2	1
tRNA-Val	1016	1087	72	2	1.39	1	1
OL	5302	5345	44	4	2.27	2	1
CR	15664	16676	1013	25	0.36 (0.10–0.89)	16	9
Mitogenome	1	16676	16676	84	0.19 (0.01–0.49)	273	130

1004 ¹Gene abbreviations: ATP, ATPase; CO, cytochrome *c* oxidase; Cyt b, cytochrome b; ND,
 1005 NADH dehydrogenase; r, ribosomal RNA; tRNA, transfer RNA; OL, origin of light-strand
 1006 replication; CR, control region.

1007 ²Abbreviations: H, number of haplotypes; V, number of variables sites; Pi, number of
 1008 parsimony-informative sites; Non-syn, number of non-synonymous mutations.

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1012 Table 3. Mean pairwise genetic divergence (%) among clades (below the diagonal) and within clades (on the diagonal).

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	John Day	Coeur d'Alene	St. Joe	North Fork Clearwater	Salmon A	Salmon B	Clearwater headwaters	Clearwater- E. Cascades	Neoboreal	Missouri
John Day	NA									
Coeur d'Alene	0.20	0.05								
St. Joe	0.19	0.10	0.01							
North Fork Clearwater	0.24	0.16	0.16	0.04						
Salmon A	0.21	0.13	0.14	0.11	0.02					
Salmon B	0.21	0.13	0.14	0.11	0.05	0.03				
Clearwater headwaters	0.36	0.41	0.40	0.44	0.42	0.42	0.11			
Clearwater- E. Cascades	0.34	0.39	0.39	0.43	0.40	0.40	0.14	0.04		
Neoboreal	0.36	0.42	0.41	0.46	0.43	0.43	0.16	0.10	0.04	
Missouri	0.37	0.43	0.42	0.46	0.43	0.44	0.17	0.10	0.04	0.01

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1018 Table 4. Stocking records associated with sample sites exhibiting geographically incongruent genotypes. Data are ND2 sequences,
 1019 mitogenomic (MG) sequences, or SNPs. Stocking information is from the online databases for Idaho
 1020 (<https://idfg.idaho.gov/fish/stocking>) and Montana (<http://fwp.mt.gov/fishing/mFish/>).
 1021

Site code	Site	State	Data	Expected genotype	Nearby stocking	Date	Number
25	Mokins	ID	MG	Coeur d'Alene	Hayden Lake	1969–2007	4,853,000
26	NF St. Joe	ID	MG	St. Joe	St. Joe River	1968–1973	235,000
29	Dry Wolf	MT	MG	Missouri	No data		
32	Tributary of Armstrong	ID	MG	Coeur d'Alene	Coeur d'Alene Lake	1967–1969	73,000
41	Bad Luck	ID	MG	Clearwater headwaters	Canyon Creek Lakes, White Cap Creek Lakes	1968–2008	83,000
48	Morse ²	ID	SNP	Salmon	No data		
55	Leiberg	ID	MG	Coeur d'Alene	Coeur d'Alene River	1968–1972	153,000
59	Yoosa ¹	ID	MG	Clearwater-E. Cascades	No data		
65	EF Emerald	ID	MG	St. Joe	No data		
89	Twin	ID	MG	Salmon B	Twin Creek, WF Bear Creek	1990–2008	1,500
120	Big	ID	ND2	NF Clearwater-Salmon	Belvidere Lakes	1971–1989	26,000
130	Salmon	ID	ND2	NF Clearwater-Salmon	No data		
141	NF Coeur d'Alene	ID	ND2	Coeur d'Alene	LNF Coeur d'Alene River, Spruce Creek	1968–1972	264,000
150	Alpine	ID	ND2	NF Clearwater-Salmon	Alpine Creek Lakes	1970–2009	44,000
152	Island	ID	ND2	NF Clearwater-Salmon	Island Lake	1969–1999	14,000
163	Magruder	ID	ND2	Clearwater-E. Cascades	Gold Pan Lake	1968–1994	18,000
164	Trapper	MT	SNP	Missouri	No data		
177	MF Little Timber	ID	SNP	Salmon	NF Little Timber Lakes	1983–1986	500

1022 ¹All fish in the SNP analysis clustered as expected.

1023 ²The fish in the mitogenomic analysis clustered as expected.

1024

1025

1026 Table 5. Proposed units of conservation for westslope cutthroat trout.

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Name	Location	Evidence ¹
John Day	Upper portion of the John Day River basin	G, MG, MS, SNP
Coeur d'Alene	Upper portion of the Coeur d'Alene River basin	G, MG
St. Joe	Upper portion of the St. Joe River basin	G, MG
North Fork Clearwater	Upper portion of the North Fork Clearwater River	G, MG, ND2 (in part)
Salmon	Salmon River basin	G, MG, ND2 (in part), MS, SNP
Clearwater headwaters	Headwaters of the Selway and South Fork Clearwater Rivers	G, MG
Clearwater-eastern Cascades	Lochsa River, lower Selway River, and lower portions of tributaries to the Middle Fork Clearwater River, and the Wenatchee River, Lake Chelan, and Methow River basins	G (in part), MG, MS, SNP
Neoboreal	Columbia River basin from the Sanpoil River upstream (excluding the Spokane River), Fraser River basin, and the South Saskatchewan River basin	G, MG, ND2 (in part), MS, SNP
Missouri	Missouri River basin	G, MG, MS, SNP

1029 ¹Evidence is from geographic discreteness (G), mitogenomic sequences (MG), microsatellites (MS), or single nucleotide
 1030 polymorphisms (SNP).

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1032

1033 Figure captions.

1034

1035 Figure 1. Sample locations, river basins, and units of conservation within the historical range of
1036 westslope cutthroat trout. (A) Sources of westslope cutthroat trout specimens that were
1037 genetically analyzed using mitogenomic sequences and microsatellites (squares), ND2 sequences
1038 (diamonds), or SNPs (circles). Fish from some locations were analyzed using more than one
1039 method. Major river basins are: 1, John Day; 2, Clearwater; 3, North Fork Clearwater; 4,
1040 Salmon; 5, St. Joe; 6, Coeur d'Alene; 7, Wenatchee River, Lake Chelan, and Methow River in
1041 the eastern Cascades; 8, Pend Oreille; 9, Kootenai; 10, Clark Fork; 11, Bitterroot; 12, Blackfoot;
1042 13, Flathead; 14, Bow (north) and Oldman (south) Rivers in the South Saskatchewan; and 15,
1043 Missouri. (B) Extent of proposed units of conservation. Colored polygons correspond to those
1044 identifying mitogenomic clades (Salmon River clades are combined). The dotted red line denotes
1045 the Continental Divide.

1046

1047 Figure 2. Maximum-likelihood phylogeny of individual mitogenomic haplotypes ($n = 89$) of
1048 westslope cutthroat trout from across its historical range. Support (as a percentage, based on
1049 1,000 bootstraps) is given above each node. Only support $> 70\%$ is shown. Mitogenomic clades
1050 discussed in the text are identified by a unique color. Sample labels are in Table 1.

1051

1052 Figure 3. Neighbor-joining phylogeny of individual ND2 haplotypes ($n = 27$) of westslope
1053 cutthroat trout from across its historical range. Support (as a percentage, based on 1,000
1054 bootstraps) is given above each node. Only support $> 70\%$ is shown. Mitogenomic clades
1055 discussed in the text are identified by a unique color. ND2 haplotype labels are in Table 1.

1056

1057 Figure 4. Assignment of westslope cutthroat trout ($n = 524$) to $K = 4, 8,$ or 15 genetic clusters
1058 analyzed at 52 variable SNP loci using STRUCTURE. Each individual (denoted by a single bar) is
1059 represented by the proportion of their genotype that was assigned to each of K clusters. Samples
1060 are sorted geographically by basin, but STRUCTURE was run with no geographic priors. The left
1061 vertical axis identifies major drainage basins of each sample and the right vertical axis the
1062 mitogenomic clades. Clades for the North Fork Clearwater River and Clearwater River

1063 headwaters were not included because no fish from these areas were examined in the SNP
1064 analysis.

1065

1066 Figure 5. Assignment of westslope cutthroat trout ($n = 95$) to $K = 5$ genetic clusters analyzed at
1067 10 microsatellite loci using STRUCTURE. Each individual (denoted by a single bar) is represented
1068 by the proportion of their genotype that was assigned to each of K clusters. Samples are sorted
1069 geographically by basin, but STRUCTURE was run with no geographic priors. The left vertical axis
1070 identifies major drainage basins of each sample.

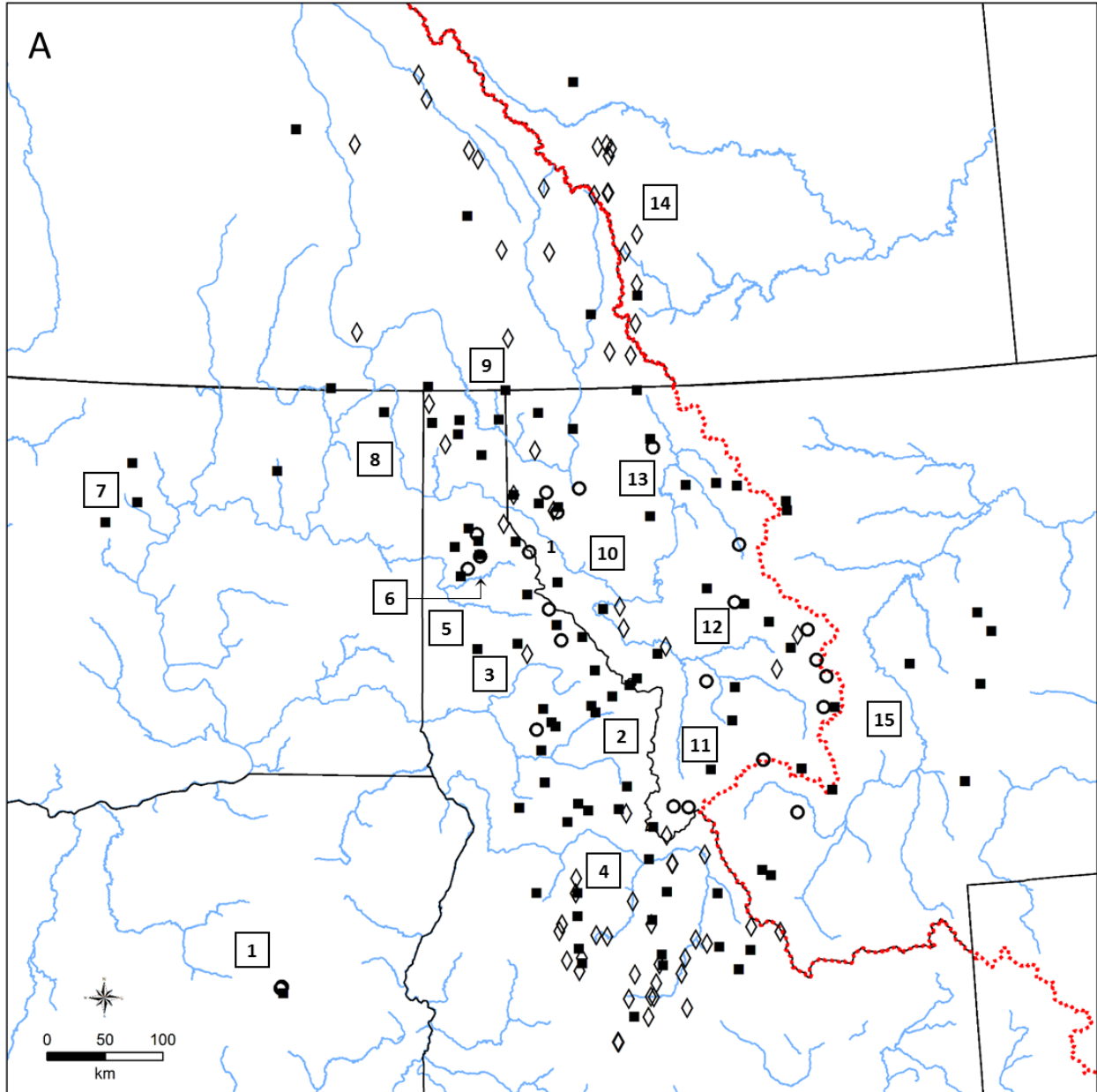
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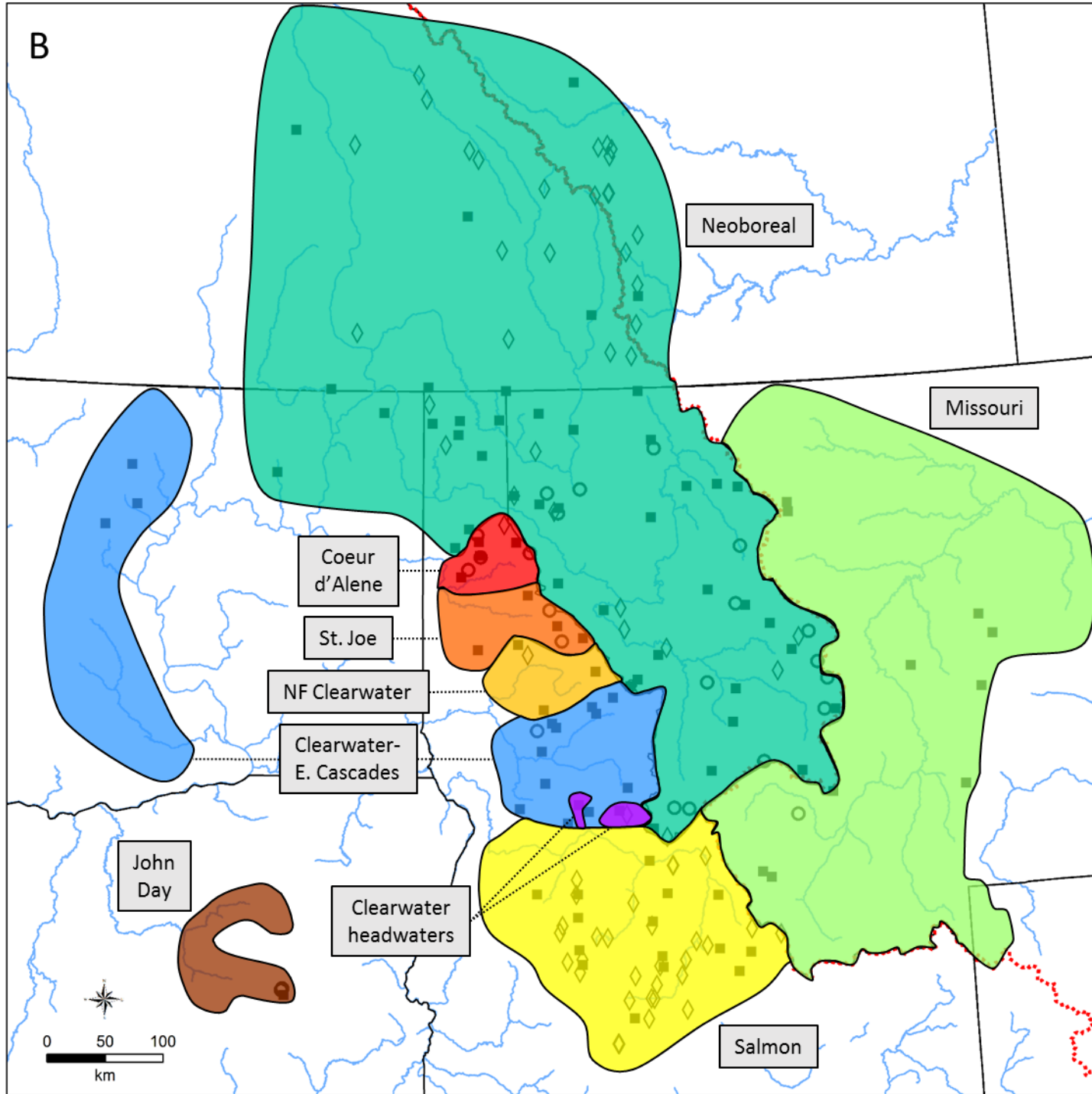
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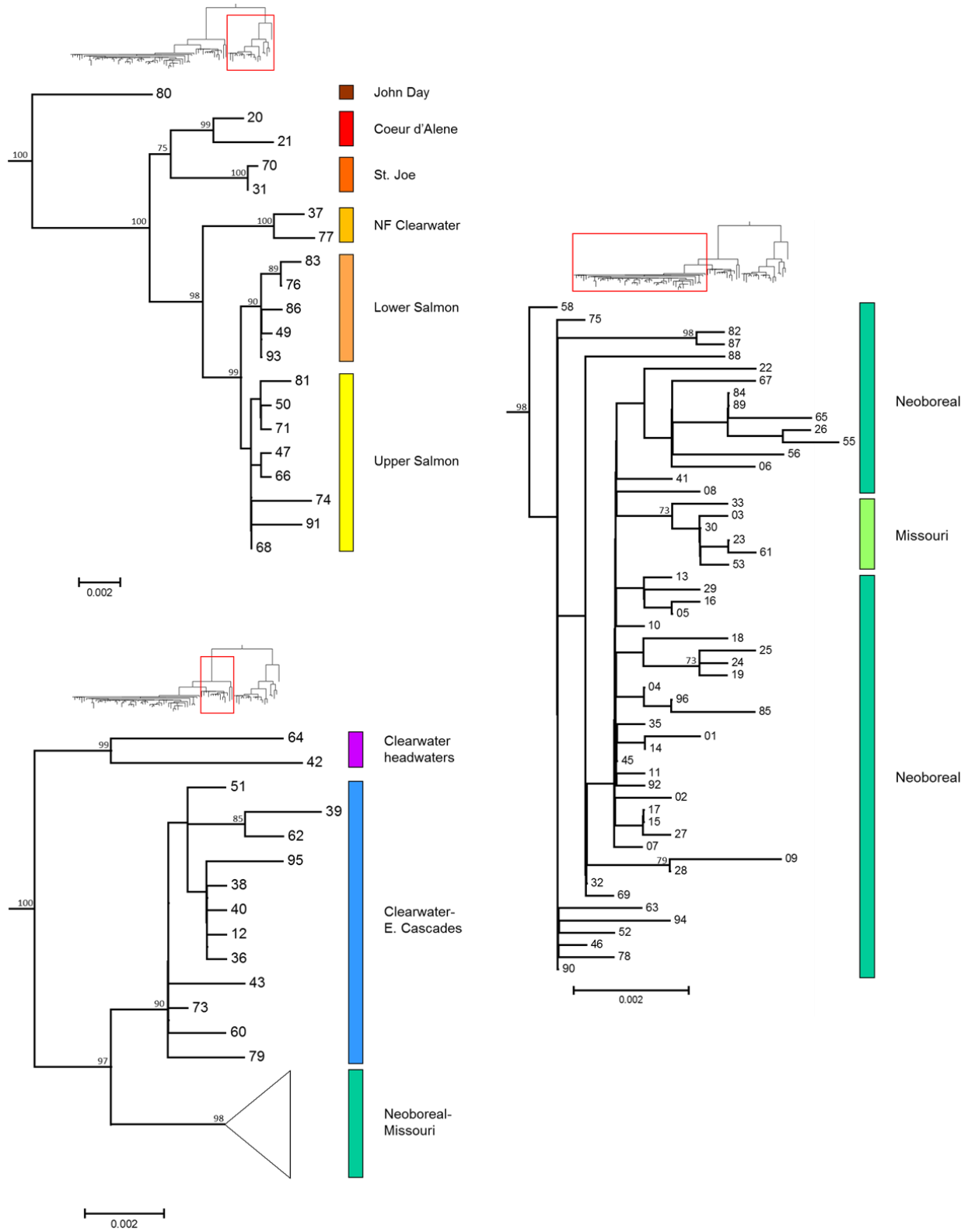


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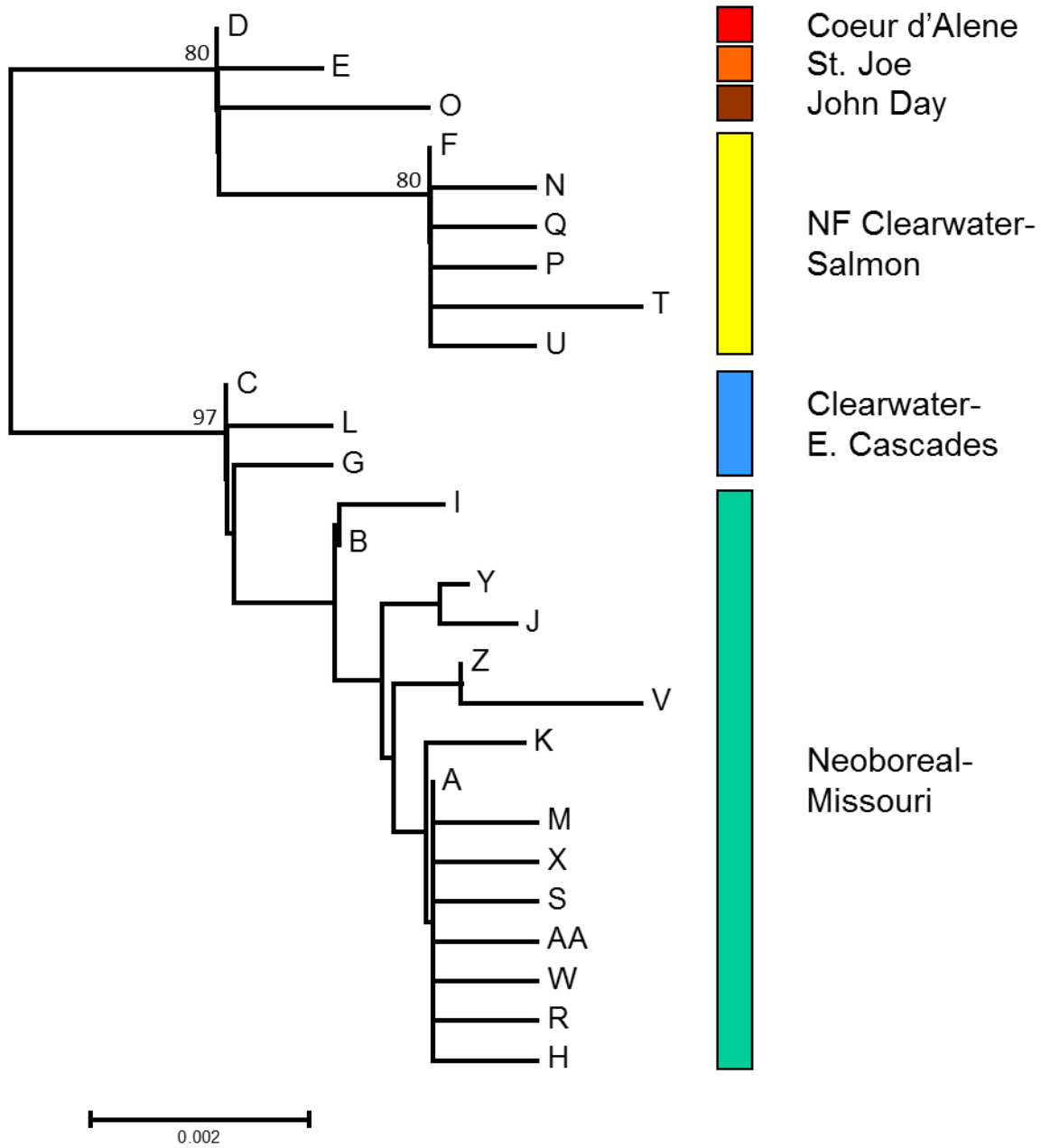
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