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**Population structure and genetic divergence of rainbow and redband trout
(*Oncorhynchus mykiss*) in the upper Klamath Basin**

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Abstract

35 The salmonid fish species *Oncorhynchus mykiss* is present throughout the lower
Klamath-Trinity River system as anadromous summer- and winter-run steelhead as well
as freshwater resident rainbow trout, all of which are forms of the coastal subspecies *O.*
m. irideus. Although steelhead and other anadromous salmonids historically migrated
40 into the Upper Klamath Basin and associated tributaries, the construction of Copco Dam
in 1918 and Iron Gate Dam in 1962 stopped all upstream migration of fishes past these
barriers. In the Upper Klamath Lake Basin, native redband trout (*O. m. newberrii*) occur
along with coastal rainbow trout trapped above the dams or stocked from hatchery
sources. However relatively little is known about the genetic relationships among *O.*
45 *mykiss* populations within the Klamath Basin. We present a population genetic analysis
of *O. mykiss* samples collected in the Upper Klamath Basin using data from 17 variable
microsatellite loci. Samples included rainbow and redband trout presumably
representative of ancestral coastal and inland lineages, as well as samples of *O. mykiss*
from neighboring inland lake basins. In addition, the Upper Klamath samples were
50 compared with data from *O. mykiss* populations below Iron Gate Dam. Results
demonstrate the presence of distinct inland and coastal genetic lineages, as well as
divergent lineages represented by samples from the inland lake basins, and have
significant implications for future restoration of *O. mykiss* in the greater Klamath-Trinity
system.

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Introduction

Compared with most other salmonid species, rainbow trout, *Oncorhynchus mykiss*, displays an enormous amount of variation in life-history pattern throughout its range (Busby et al. 1994) and even within small individual watersheds (Shapovalov and Taft 1954; Pearse et al. 2009). In addition, their extensive morphological diversity led early researchers to describe as many as 50 distinct species of trout in western North America, almost all of which have now been combined into subspecies of *O. mykiss* (Behnke 1992). Because of its widespread distribution and complex evolutionary history, phylogeographic relationships among many of these forms remain poorly understood. The species' significance to tribal cultural heritage and popularity in recreational fisheries demands an understanding of the ecological and genetic differences among *O. mykiss* populations and subspecies. This knowledge is critical for conservation and management in the many human-impacted watersheds it inhabits.

One enigmatic group of subspecies are the redband trouts, a group of morphologically similar subspecies including *O. m. newberrii* in Upper Klamath Lake, *O. m. gairdneri* in the Columbia and Fraser River basins, and *O. m. stonei* of the Sacramento River system (Behnke 1992; Busby et al. 1994; Currens et al. 2009). Redband trout also occur in the isolated desert basins of southeast Oregon, although their taxonomic association have long been debated (Behnke 1992; Currens et al. 2009). Several genetic studies have examined the relationships among redband trout lineages, as well as compared native redband trout populations with introduced hatchery rainbow trout to detect the presence of introgressive hybridization (Wishard et al. 1984; Berg 1987; Matala et al. 2008; Currens et al. 2009; DeHaan & Adams 2009;

Simmons et al. 2010). Although they share some morphological similarities, genetic data indicate that these subspecies are not monophyletic with respect to other *O. mykiss* lineages (Currens et al. 2009), and the relationships among them and to the coastal lineage *O. mykiss irideus* remain unclear (Behnke 1992; Currens et al. 2009).

90 In the lower Klamath River, anadromous and resident forms of coastal *O. m. irideus* are abundant, including summer- and winter-run steelhead (Busby et al. 1994; Busby et al. 1996) and freshwater resident rainbow trout. However, compared with the Columbia River to the north (Brannon et al. 2004; Knudsen et al. 2002; Kostow 2003; Winans et al. 2004), relatively little is known about the relationships among *O. mykiss* 95 populations in the Klamath River (Buchanan et al. 1994; Papa et al. 2007; Pearse et al. 2007), or between them and other populations in the Klamath Mountain Province - Evolutionary Significant Unit (Busby et al. 1994; Riesenbichler et al. 1992). Although steelhead and other anadromous salmonids historically migrated into the upper Klamath Basin and associated tributaries (Hamilton et al. 2005), the construction of Copco Dam 100 #1 in 1918 and Iron Gate Dam in 1962 stopped upstream migration of fishes past these barriers, effectively separating the upper and lower Klamath basins. In addition, non-native stocks of *O. mykiss* have been widely planted in the upper basin (ODFW, unpublished records), and the extent of their genetic impact on naturally spawning *O. mykiss* populations is not known. Recent efforts to mitigate or remove the dams 105 blocking anadromous access to the upper Klamath have highlighted the importance of understanding the genetic relationships among fish populations throughout the basin to guide recovery planning efforts (Klamath Hydroelectric Settlement Agreement, Public Draft January 8, 2010).

Redband trout in Upper Klamath Lake (*O. m. newberrii*; Behnke 1992, 2002) are
110 presumed to co-occur with both coastal *O. m. irideus* trapped above the dams and
stocked hatchery rainbow trout. Recently, an allozyme study published by Currens et al.
(2009) set the stage for a more detailed analysis of *O. mykiss* within the Upper Klamath
Basin by surveying *O. mykiss* populations over a broad geographic range and
developing hypotheses about the evolution of coastal and inland trout lineages. Here we
115 use data from 18 microsatellite loci that had previously been shown to be variable and
informative for population genetic analysis of coastal *O. mykiss* (Garza et al. 2004;
Pearse et al. 2007) to describe fine-scale patterns of genetic variation and population
structure observed in samples of *O. mykiss* from throughout the Upper Klamath Basin.
We use these data to test the hypothesis that 1) divergent genetic lineages of *O. mykiss*
120 occupy the Upper Klamath Basin, 2) coastal *O. mykiss* found above Iron Gate Dam and
the other mainstem Klamath River dams are derived from stocks below the dams, and
3) redband trout associated with Upper Klamath Lake are distinct from those occupying
the headwaters, and are more closely related to coastal *O. m. irideus*. We place these
results in context through comparison with samples from three neighboring inland
125 basins (Goose Lake, Chewaucan Basin, and Fort Rock Basin; Figure 1) and previously
published data from *O. mykiss* populations in the lower Klamath River (Pearse et al.
2007) to examine the relationships of Upper Klamath Basin populations to other
redband trout and coastal rainbow trout lineages.

Methods

Samples

Samples were collected by Oregon Department of Fish and Wildlife staff in the
135 summer of 2000 from locations throughout the Upper Klamath Basin (Figure 1) using
standard electrofishing procedures. Following an initial analysis, supplemental samples
were collected in the summer of 2007 to expand the sample set. Within the Upper
Klamath Basin, the samples in this study can be divided into Upper Klamath River
140 populations, consisting of samples collected between Link River Dam and Iron Gate
Dam, and Upper Klamath Lake populations, consisting of samples from all tributaries
above Link River Dam (See map, Figure 1). In addition, samples were collected from
stream tributaries to Goose Lake, Chewaucan Basin, and Fort Rock Basin, three
neighboring isolated inland lake basins in southeast Oregon. For comparison, samples
from three anadromous steelhead populations in the Lower Klamath watershed were
145 included in the analysis: Blue Creek, Methodist Creek (a Salmon River tributary), and
Horse Linto Creek (a Trinity River tributary)(LKBL, MSME, and TRHL, respectively;
Pearse et al. 2007).

DNA Extraction and Genetic Data Collection

150 DNA was extracted from all samples using DNeasy 96 tissue extraction kits on a
BioRobot 3000 (Qiagen, Inc.), following the manufacturers protocols. Extracted DNA
was diluted ~10:1 and used for PCR amplification of 18 microsatellite loci previously

optimized for use in *O. mykiss* (*Omy77*, *OtsG401*, *OtsG243*, *OtsG253b*, *One11b*,
Omy1011, *Omy27*, *OtsG249b*, *OtsG409*, *OtsG103*, *OtsG85*, *Oki23*, *Ots1b*, *Ssa85*,
155 *One13b*, *Ssa289*, *OtsG3*, *OtsG43*; Garza et al. 2004; Pearse et al. 2007). Each locus
was amplified individually, and PCR products were pooled prior to electrophoreses on
ABI 377 sequencers (Applied Biosystems, Inc.). Microsatellite genotypes were scored
using Genescan 3.0 and Genotyper 2.1 software (Applied Biosystems, Inc.). All
genotypes were independently verified by two people to ensure correct and consistent
160 scoring. Discrepancies between the two scores were resolved either by consensus, by
re-genotyping, or by deletion of that genotype from the data set.

Data Analysis

The data were analyzed with a suite of complementary analytical methods in
165 order to identify concordant patterns in the distribution of genetic variation within and
among individuals and populations (Pearse & Crandall 2004). The computer programs
GENETIX (Belkhir et al. 1996-2004) and GENEPOP (Raymond and Rousset 1995)
were used to estimate basic population genetic statistics, test for Hardy-Weinberg and
linkage equilibrium, and estimate the distribution of population genetic variation using F-
170 statistics (Wright 1931). To correct for differences in sample size among populations
when estimating allelic diversity, allelic richness, A_r , was calculated using rarefaction
sampling based on a minimum of eight genes (HP_RARE; Kalinowski 2005).

Genetic similarities and relationships among populations were visualized using
two approaches. First, the principal components analysis program PCA-GEN
175 (<http://www2.unil.ch/popgen/softwares/>) was used to graphically represent the genetic

variation among populations and individuals in two- and three-dimensional space. Second, the software package PHYLIP (Felsenstein 2004) was used to calculate Cavalli-Sforza and Edwards (1967) chord distances and Nei's genetic distance (Nei et al. 1983) and generate neighbor-joining networks based on these distances. Statistical support for population relationships was evaluated for both distance networks using 10,000 bootstrap samples from the data set, and the resulting trees was visualized using TREEVIEW (Page 1996).

To complement the analyses based on population allele frequencies, genotype data were used to cluster individuals using the Bayesian program STRUCTURE (Pritchard et al. 2000). This method is useful in the evaluation of genetic similarities among groups of individuals, and shared genetic clustering among individuals can be interpreted as a signal of migration and/or common ancestry. Conversely, strong population structure is indicated when individuals have high values of Q assigning to different genetic clusters. The approach used by STRUCTURE is especially informative as an exploratory tool for data analysis because it does not require *a priori* designation of discrete populations. Instead, the program partitions the genotypes into a specified number of clusters, K , and assigns each individual proportionally to one or more of the clusters. Consistent patterns of individual and population level division over a range of values of K provide an indication of the true population genetic relationships. For STRUCTURE, default parameters were used, including assumption of correlated allele frequencies, with 10,000 burn-in simulations followed by 90,000 data collection simulations with three separate runs for each value of K ranging from two to 12.

Results

200 *Genetic Data*

A total of 1,006 *O. mykiss* individuals sampled from 33 sites in the upper Klamath Basin during the summer of 2000 were initially genotyped at 18 polymorphic microsatellite loci. However, in some Upper Klamath Basin populations, one locus, *OtsG43*, appeared to be amplifying a pseudo-locus in addition to the expected locus, presumably due to the ancestral tetraploidy common to salmonids. Because these
205 pseudo-alleles overlapped in size with the distribution of expected alleles, the genotype was impossible to determine for many individuals and this locus was dropped from the analysis.

Due to the poor quality of DNA recovered from the tissue samples of some
210 populations, 298 individuals failed to produce amplified genotypes for the minimum of nine loci, which was used as a criterion for inclusion in the final data set. The remaining individuals generally had high data quality; 95% of the retained individuals have data for 10 or more microsatellite loci. To replace the individuals from sites with poor initial DNA quality as well as obtain samples from more locations, additional samples were
215 collected in the summer of 2007. This effort provided 186 individuals from 8 sample sites, including new sites in the Fort Rock and Chewaucan River basins (Figure 1; Table 1), all of which were successfully genotyped. The final combined data set thus consisted of multilocus genotypes for 894 individuals from 37 populations (Table 1). These data were combined with previously collected microsatellite data from three sites in the lower
220 Klamath River (Pearse et al. 2007) for the final analysis.

Population Genetic Analysis

Summary statistics of genetic variation for all populations are shown in Table 1. Tests for deviations from Hardy-Weinberg and linkage equilibrium did not uncover any systematic patterns of disequilibrium, and no population was significantly out of equilibrium for more than two loci after Bonferroni correction for multiple comparisons. Such sporadic disequilibria are common for microsatellite loci in salmonid populations, particularly when juvenile fish are sampled from relatively small populations.

All analyzed populations were significantly genetically differentiated, as measured by pairwise F_{ST} values, except for the group of populations in the Upper Klamath River (populations 2-6, Keno reach, JC Boyle reservoir and bypass, and Spencer Creek; data not shown). Pairwise F_{ST} values among all populations were generally high relative to those typically seen in coastal anadromous steelhead populations (mean pairwise F_{ST} 0.1-0.28), a pattern expected in populations of resident trout and in the possible presence of barriers to fish movement. The most strongly differentiated populations within the Upper Klamath Basin based on mean pairwise F_{ST} values were Moss Creek (0.28; population 7) and Rock Creek, Sprague R. (0.28; population 16). However, F_{ST} values are strongly influenced by factors that increase genetic drift, so these results may reflect their small population sizes rather than greater isolation or divergence time for these populations. This conclusion is supported by the low allelic richness seen in both of these populations (Table 1). In contrast, the high mean pairwise F_{ST} values seen in the three Goose Lake populations and the Chewaucan River (populations 34-37) likely do reflect evolutionary divergence since these populations do not display such reduced genetic diversity (Table 1).

245 Qualitative examination of the allele frequency distributions across populations
revealed a distinction between “coastal” and “inland” trout in the Upper Klamath Basin.
Considering these groups of populations, many alleles present at substantial
frequencies (greater than 10%) in Upper Klamath Lake and/or Goose Lake populations
were absent from the Upper Klamath River populations. In contrast, alleles present in
250 the Upper Klamath River tended to be shared with most or all populations in the Upper
Klamath and Goose Lake basins. For example, allele 96 at locus *Omy77* was observed
in more than half of the Upper Klamath Lake headwaters populations at frequencies up
to 34%, as well as in all three Goose Lake populations at frequencies greater than 50%,
but was absent below Link River Dam. Similarly, no allele larger than 240 basepairs
255 was observed at locus *Omy1011* in the Klamath River or Goose Lake populations, but
an additional 25 alleles were observed in the Klamath Lake headwaters and Fort Rock
Basin populations, ranging in size up to 344 basepairs and at frequencies as high as
24%. This latter example, combined with the observed presence of novel pseudo-alleles
at the excluded locus (*OtsG43*), suggests that isolation between inland and coastal
260 forms persisted for a sufficient time period for substantial accumulation of *de novo*
mutations in addition to changes in allele frequencies and supports the hypothesis that
little ongoing gene flow is maintained among these populations.

Statistical evaluation of population genetic similarity using principle components
analysis (PCA) also supported a division between the inland and coastal lineages, as
265 well as clearly differentiating the Goose Lake and Chewaucan Basin populations (34-
37) from all other samples included in the study. Overall patterns of genetic similarity
among populations can be seen in the PCA, with the primary division between coastal

and inland populations indicated by the diagonal line (Figure 2). Above and to the left of this line, the Upper Klamath River populations (2-6) cluster tightly together along with the three lower Klamath River populations and the sample from Jenny Creek (population 1). Samples from the streams tributary to the west side of Upper Klamath Lake (7, 8, and 9), as well as a sample from the lower Sprague River (site 16) also clustered above the diagonal. Below and to the right of the diagonal, the Upper Klamath Lake headwaters populations are defined by a much looser grouping, indicative of the increased genetic divergence among these populations, as expected of resident trout with reduced migratory behaviors. The Fort Rock Basin populations (31-33) are associated with these populations, while Goose Lake and the Chewaucan Basin form a distinct cluster (Figure 2). Notably, samples from the Wood River, lower Williamson, and lower Sprague River (10-12, 15) cluster together in the far right of Figure 2, separately from the main group of Upper Klamath Lake headwaters populations. Trout Creek (population 14) occupies a central position, intermediate between the inland and coastal population groups.

The population relationships identified by PHYLIP in unrooted neighbor-joining networks (Figures 3 and 4) were largely concordant with the patterns seen in the PCA. This included strong bootstrap support for a group of populations associated with Upper Klamath Lake redband trout (10-12, 15), and for the separation of the Chewaucan River and three Goose Lake Basin populations and from all other samples (34-37; Figures 3 and 4). More inclusive groups, such as the populations representing the Upper Klamath River populations with Cherry and Rock Creeks (8 and 9), were only weakly supported, and relatively little support is found for substructure within the upper Klamath

headwaters populations, with the exception of the two upper Sycan River populations (Sites 19 and 20). Interestingly, in the network based on Nei's genetic distance, the population sample from trout creek (14) is unstable in its position in the network. This population appears with the upper headwaters populations in the neighbor-joining
295 network (Figure 4), but clusters with the Upper Klamath River populations in the bootstrap consensus tree, as it does in the networks base on Cavalli-Sforza and Edwards' chord distances.

Individual Genotypic Analysis

300 The program STRUCTURE proportionally divides each individual among a specified number of genetic clusters, K , and estimates the likelihood of each value of K . The distribution of proportional assignments, Q , for all individuals in a population then provides an indication of genetic associations among populations and the amount of admixture present. When large numbers of populations are analyzed, however, the
305 method may fail to provide consistent results over multiple runs with a single K value and can have a tendency to overestimate K (Waples & Gaggioti 2006). This effect can be seen in the variation among the three runs at each value of K (Figure 5). Thus, the most informative interpretation is based on the identification of consistent, biologically reasonable, patterns across a range of values of K (Pritchard et al. 2000).

310 Examination of the results from STRUCTURE for the present data set shows considerable variation both across the range of values of K from two to 9, and among different simulation runs at the same K . Nonetheless, some clear and consistent patterns are seen across runs. First, population genetic differentiation is clear, with high

Q-values for almost all individuals leading to high population Q-values seen at K=2 for
315 almost all samples ($Q < 0.2$ or $Q > 0.8$; Table 1). The only exceptions to this were Trout
Creek (population 14) and Pothole Creek (28), both of which consisted of individuals
with a range of intermediate individual q-values, suggestive of ongoing hybridization
between divergent lineages in these populations. Second, the same clear separation
between Upper Klamath Lake and headwaters populations and all other samples was
320 identified with the individual-based STRUCTURE analysis (cluster B at K=2; Table 2).
As in the population analyses, the west side lake tributaries and lower Sprague River
sites (7,8,9, and 16) were an exception to this general result and clustered with the
populations below Link River Dam. This division appeared in three identical runs at K=2,
was supported across the full range of K, and was evident from both the individual and
325 population q-values.

At higher values of K, variation in specific genetic clusters among runs was
common, but several consistent groupings were evident in multiple runs over a range of
K values up to nine. Table 2 shows representative results for K=2, 4, 6, and 9; above
K=9 the patterns were much less clear, and high variance in $\ln P(D)$ (Figure 5) and low
330 population q-values predominated. As at K=2, a primary grouping of all upper Klamath
headwaters populations was consistent at all higher values of K. Similarly, a consistent
“upper Klamath River” cluster predominated in the populations below Link River Dam
(2-6), as well as in the small tributaries on the west side Klamath Lake (7, 8, 9; Moss,
Rock and Cherry Creeks) and a single site in the lower Sprague River (cluster B at K=4;
335 Table 2). Interestingly, in most runs with $K > 5$, the lower Williamson River (Site 12)
clustered with the Wood River (Sites 10 & 11) and lower Sprague River (Ridgeway

Ranch, Site 15) as well as $q \sim 0.4$ of Anderson Ranch (Site 17), also in the lower Sprague River. This pattern parallels that seen in the PCA and neighbor-joining analyses and may indicate genetic differentiation of the lacustrine upper Klamath Lake redband trout, *O. m. newberrii*, noted by Behnke (1992) as distinct from the stream-dwelling redband trout in the headwaters. Finally, in all runs with $K > 4$ the Goose Lake samples were identified as distinct from the Klamath Basin populations with high q -values and consistently clustered together with the Chewaucan Basin. Similarly, the Fort Rock Basin populations were identified as unique and did not cluster with either the upper Klamath Basin or the other inland lake basin populations (Table 2).

Discussion

350 With the exception of the group of populations sampled in the Klamath River
between Iron Gate and Link River dams, every population sampled for the present study
was genetically distinct as measured by pairwise F_{ST} values. This result was also
supported by the clustering analysis with STRUCTURE, which assigned high q-values
associating most individuals and populations with single genetic clusters. The lack of
355 significant differentiation among upper Klamath River populations is consistent with the
extensive fish movements reported in these areas, despite constraints imposed on fish
migrations by the construction of dams and regulation of flow in the upper Klamath
River (Jacobs et al. 2008).

 The patterns observed with the microsatellite data show strong similarities to the
360 allozyme findings of Buchanan et al. (1994) and Currens et al. (2009), including the
primary division between inland and coastal trout lineages. Analysis of the data
presented here also identified a secondary division that separated *O. mykiss*
populations in the lower reaches of the Upper Klamath Lake system from populations
farther up in the headwaters of the Williamson and Sprague Rivers. This result is
365 evident in the concordant patterns observed in multiple complementary methods of
population genetic analysis and also closely parallels the allozyme allele frequency
patterns noted by Currens (2009).

 Behnke (1992, 2002) identified redband trout found in Upper Klamath Lake,
which was the original type source of *O. m. newberrii*, as typical of the subspecies and
370 noted morphological differences between these trout and the stream-dwelling redband
trout in the headwaters of upper Klamath Basin. Long term isolation of the headwaters

populations is supported, at least in the case of the Williamson River, by the presence of barrier waterfalls that prevent fish movement into the upper reaches of that system. Neither of these genetic groups is closely associated with the coastal *O. m. irideus* populations below Link River dam, and our data suggest that there is substantial divergence between the redband trout present in the headwaters of the Williamson and Sprague rivers and those found in Upper Klamath Lake. The latter are known to be migratory adfluvial populations that primarily spawn in the highly spring-influenced tributaries of Upper Klamath Lake. It is worth noting, that extensive stocking took place in some of these tributaries, including the Williamson and Wood Rivers, beginning in 1925 and continuing as late as 1990 (ODFW, unpublished records).

The presence of distinct inland and coastal genetic groups of *O. mykiss* in Upper Klamath Basin supports the hypothesis that an ancestral lineage of redband trout, isolated in the upper basin, was secondarily invaded by the coastal lineage when the lower Klamath River connected with Upper Klamath Lake and provided an outlet to the Pacific Ocean (Currens 1997; Currens et al. 2009). However, the distribution of populations associated with the two lineages is not clearly divided at Link River dam, but rather is a mosaic pattern centered around Upper Klamath Lake. Historically, anadromous salmonids including steelhead trout had access to the Upper Klamath basin via the Link River (Hamilton et al. 2005). Today, Link River dam is not a complete physical barrier between Upper Klamath Lake and River populations since a fish ladder allows passage. Although the extent to which migratory *O. mykiss* use this facility is not clear, the small creek populations along the West shore of Upper Klamath Lake (Cherry, Moss, and Rock Creeks; sites 7-9) consistently group with the coastal-lineage upper

395 Klamath River populations, as does Rock Creek in the lower Sprague River (Site 16),
and do not show evidence of significant genetic exchange with the redband trout
populations in the headwaters or Upper Klamath Lake.

The samples from Trout Creek (population 14), a tributary of the lower Sprague
River, also showed an association with the coastal Klamath River genetic lineage rather
400 than the Upper Klamath Lake or headwaters populations. However, the individuals in
Trout Creek were clearly identified as a mixture of both coastal and inland lineages
based on the analysis with STRUCTURE (results not shown). The Trout Creek
population also appears in an intermediate position in the principal components analysis
(Figure 2) and is unstable in its position in the phylogenetic networks. These results,
405 and the close proximity of populations 14, 15, and 16 despite their divergent genetic
structure, suggest that the lower Sprague River is an area of active hybridization. More
detailed sampling and analysis of sites in the vicinity of Trout Creek in the Sprague
River is needed to resolve the fine-scale genetic structure in this watershed.

The single site sample analyzed from Jenny Creek in the present study (Site 1)
410 did not show a strong genetic similarity to either the upper Klamath Lake or River
groups, but instead appeared most similar to the lower Klamath River populations. The
population sample from Jenny Creek was consistently pure at all values of K ($q > 0.9$),
consistent with its isolation. At higher values of K it was identified as either a unique
population or clustered with the upper Klamath River sites and/or the three lower
415 Klamath River populations (Table 2). In previous analyses based on allozymes
(Buchanan et al. 1994; Currens et al. 2009), genetic associations to both the coastal
and inland lineages were observed in populations sampled from different tributaries and

reaches of Jenny Creek. However, because only a single site in Jenny Creek was
sampled for the present study, it is difficult to relate the current results to these earlier
420 studies, especially given the history of stocking coastal origin hatchery *O. mykiss* in this
watershed and the presence of waterfall barriers which isolate it from the main stem of
the Klamath River.

Overall, the results of our detailed microsatellite analysis of *O. mykiss* in the
upper Klamath Basin are largely concordant with the hypothesized broad-scale
425 relationships among coastal rainbow trout/steelhead and inland redband trout lineages.
Based on morphological characteristics (Behnke 1992, 2002) and allozyme data
(Currens et al. 2009), Upper Klamath redband trout, *O. m. newberrii*, appear to be a
distinct native form of redband trout, while populations of coastal-lineage *O. m. irideus*
predominate below Link River Dam and inhabit some tributaries of Upper Klamath Lake.
430 Furthermore, as suggested by Currens et al. (2009), the redband trout in the
headwaters of the Williamson and Sprague Rivers are genetically distinct from those
associated with lower tributaries of Upper Klamath Lake. Finally, the separation of
Goose Lake and Chewaucan Basin trout from Fort Rock Basin samples with our genetic
data is consistent with the suggestion that the former are ancestrally associated with *O.*
435 *m. stonei* from the Sacramento and Pit Rivers, while the latter derive from Columbia
River redband trout, *O. m. gairdneri* (Currens 2009).

The findings of this study have implications for current plans for the removal of
dams in the upper Klamath River (Klamath Basin Restoration Agreement,
<http://klamathrestoration.gov/home>) and the reintroduction of anadromous salmonids
440 into the Upper Klamath Basin (Hooton and Smith 2008). Our results indicate that

multiple lineages of *O. mykiss* are present in the upper basin. Prior to dam construction, anadromous life histories were likely present (Hamilton et al. 2005), possibly from both coastal and inland lineages. However given the productive capacity of Upper Klamath Lake and the upper basin, it is also likely that resident life histories co-occurred with anadromous forms of *O. mykiss* in the Upper Basin. If connectivity of the upper basin with the lower basin and Pacific Ocean is reestablished, anadromous life histories of *O. mykiss* could be naturally re-established in the Upper Basin by two potential mechanisms; resumption of seaward migration by populations occupying the Upper Basin or invasion of the Upper Basin by anadromous forms from the lower Klamath Basin. The degree to which resident and anadromous life histories persist in a reconnected Upper Klamath Basin depends on their long term productive capacity. Anadromous forms may be more productive during periods of favorable seaward migration and marine productivity whereas resident forms may have an advantage during periods when Upper Klamath Lake is productive. Because of the complexity and uncertainty surrounding the relationship between resident and anadromous forms of *O. mykiss* in the Upper Klamath Basin, the Oregon Department of Fish and Wildlife is taking a conservative approach to reestablishing steelhead in the Upper Basin. The reintroduction plan adopted by the Oregon Fish and Wildlife Commission in 2008 (Hooton and Smith 2008) calls for no active intervention in the re-establishment of steelhead in the Upper Klamath basin.

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Figure Legends:

580 Figure 1. Map of Upper Klamath region showing sampling sites and names of major rivers and basins. Inset shows location in southern Oregon and northern California. Numbers indicate samples sites as in Table 1, letters as follows: A, Iron Gate Dam, B, J. C. Boyle Dam, C, Keno Dam, D, Link River Dam, E, barrier waterfall on Williamson River.

585 Figure 2. Principal Component Analysis showing all upper Klamath and interior basin samples, plus lower Klamath River steelhead samples. Numbers correspond to sample sites in Table 1. The group of Goose Lake and Chewaucan River populations is highlighted in the lower left. The diagonal line identifies the division between Upper Klamath River and Upper Klamath Lake populations. See text for details.

590 Figure 3. Unrooted neighbor-joining network showing relationships among populations based on Cavalli-Sforza and Edwards' chord distances. Numbers at branch ends indicate populations listed in Table 1. Numbers on internal branches indicate the percent of resampled data sets in which the indicated clade was present, out of 10,000 bootstrapped resamplings of the data. Values greater than 70 percent are generally interpreted as suggestive of strong support for a grouping.

595 Figure 4. Unrooted neighbor-joining network showing relationships among populations based on Nei's genetic distances. Numbers at branch ends indicate populations listed in Table 1. Numbers on internal branches indicate the percent of resampled data sets in which the indicated clade was present, out of 10,000 bootstrapped resamplings of the data. Values greater than 70 percent are generally interpreted as suggestive of strong support for a grouping.

600 Figure 5. LnP(D) results from three replicate STRUCTURE runs at each value of K from 2 to 12. See text for details.

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Table 1: List of collection locations for all samples in the present study. Site No. refers to Figure 1. Summary statistics for each population are also shown: number of samples (N), observed heterozygosity (H_0), allelic richness (Ar), mean pairwise F_{ST} , and mean Q_1 -value across three runs at $K=2$.

Site No.	Tributary	Stream/Site	N	H_0	Ar	F_{ST}	Q_1
Upper Klamath River							
1	Iron Gate Reservoir	Jenny Creek	39	0.65	3.9	0.15	0.03
2	Klamath River	Below J.C. Boyle dam	14	0.65	4.2	0.10	0.05
3	Klamath River	J.C. Boyle bypass reach	19	0.66	4.1	0.09	0.12
4	Spencer Creek	Above culvert	33	0.66	3.8	0.11	0.05
5	Spencer Creek	Below culvert	35	0.68	3.9	0.11	0.07
6	Klamath River	Keno reach	10	0.74	4.1	0.11	0.02
Upper Klamath Lake							
7 ¹		Moss Creek	33	0.41	2.3	0.28	0.01
8 ¹		Rock Creek	37	0.60	3.0	0.20	0.02
9		Cherry Creek	26	0.58	3.4	0.14	0.06
10 ¹	Wood River	Fort Creek	36	0.54	3.2	0.17	0.96
11		Upper Wood River	32	0.49	2.8	0.19	0.98
12 ¹	Williamson River	Spring Creek	39	0.48	3.1	0.15	0.97
13		At Deep Creek Ranch	20	0.66	4.1	0.12	0.84
14	Sprague River/Trout Creek	South Fork Trout Creek	19	0.64	3.7	0.12	0.44
15	Sprague River	Ridgeway Ranch	17	0.47	3.0	0.17	0.97
16	Sprague River	Rock Creek	10	0.30	2.0	0.28	0.04
17	Sprague River	Anderson Ranch	28	0.57	3.8	0.10	0.86
18	Sprague R./ Sycan River	Long Creek	26	0.62	3.8	0.12	0.93
19	Sprague R./ Sycan River	Paradise Creek	24	0.62	4.0	0.10	0.84
20	Sprague R./ Sycan River	Rock Creek	36	0.65	4.0	0.10	0.83
21	North Fork Sprague R.	Fivemile Creek	16	0.54	3.6	0.12	0.85
22	North Fork Sprague R.	Upper North Fork Sprague R.	31	0.62	3.9	0.11	0.92
23	South Fork Sprague R.	Robinson Spring Creek	31	0.54	3.3	0.15	0.96
24	South Fork Sprague R.	Fishhole Creek	34	0.66	3.8	0.11	0.89
25	South Fork Sprague R.	Demming Creek	14	0.60	3.0	0.18	0.98
26	South Fork Sprague R.	Brownsworth Creek	16	0.60	3.7	0.12	0.83
27	South Fork Sprague R.	Leonard Creek	12	0.61	3.6	0.11	0.96
28	South Fork Sprague R.	Pothole Creek	27	0.69	4.1	0.11	0.49
29	South Fork Sprague R.	Buckboard Creek	29	0.46	3.0	0.16	0.96
30 ²	Lost River	Miller Creek/North Canal	30	0.61	3.3	0.16	0.19
Inland Basins							
31 ¹	Fort Rock Basin	Buck Creek	20	0.67	4.5	0.11	0.20
32 ¹	Fort Rock Basin	Bridge Creek	15	0.66	4.2	0.12	0.28
33 ¹	Fort Rock Basin	Silver Creek	14	0.67	4.6	0.11	0.17
34 ¹	Chewaucan Basin	Chewaucan River	15	0.57	3.7	0.19	0.02
35	Goose Lake Basin	Bauers Creek	22	0.53	3.4	0.19	0.02
36	Goose Lake Basin	Thomas Creek	19	0.48	3.4	0.19	0.03
37	Goose Lake Basin	Drews Creek	16	0.50	3.4	0.20	0.03
Lower Klamath River							
LKBL ³		Blue Creek	48	0.73		0.10	0.02
MSME ³	Salmon River	Methodist Creek	54	0.66		0.09	0.10

TRHL ³ Trinity River	Horse Linto Creek	48	0.71	0.10	0.05
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1. Includes samples collected in 2007.
 2. The Lost River drainage is not a direct tributary of the Klamath River.
 3. Data from Pearse et al. 2007.

Table 2: Results of analysis using STRUCTURE, showing cluster associations for population Q-values >0.5 at K=2, 4, 6, and 9. Populations noted in parentheses did not show consistently high Q-values associated with any group of populations, but were most strongly associated with the indicated group. See text for details.

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K2	K4	K6	K9	populations	population group
A	A	A	A	LKBL, MSME, TRHL	Klamath River below Iron Gate
A	A	A	B	1	Jenny Creek
A	B	A	C	2,(3),4,5,6,8,9,16	Upper Klamath River & Lake; Lower Sprague
A	B	B	D	7	Moss Creek
A	A	C	E	30(28)	Lost River
A	A	C	F	31,32,33	Fort Rock Basin
A	C	D	G	34,35,36,37	Goose Lake and Chewaucan Basin
B	D	E	H	10,11,12,15	Wood River; Lower Williamson, Lower Sprague
B	D	F	I	13,(14),(17),18-27,29	Upper Klamath headwaters

Figure 1:

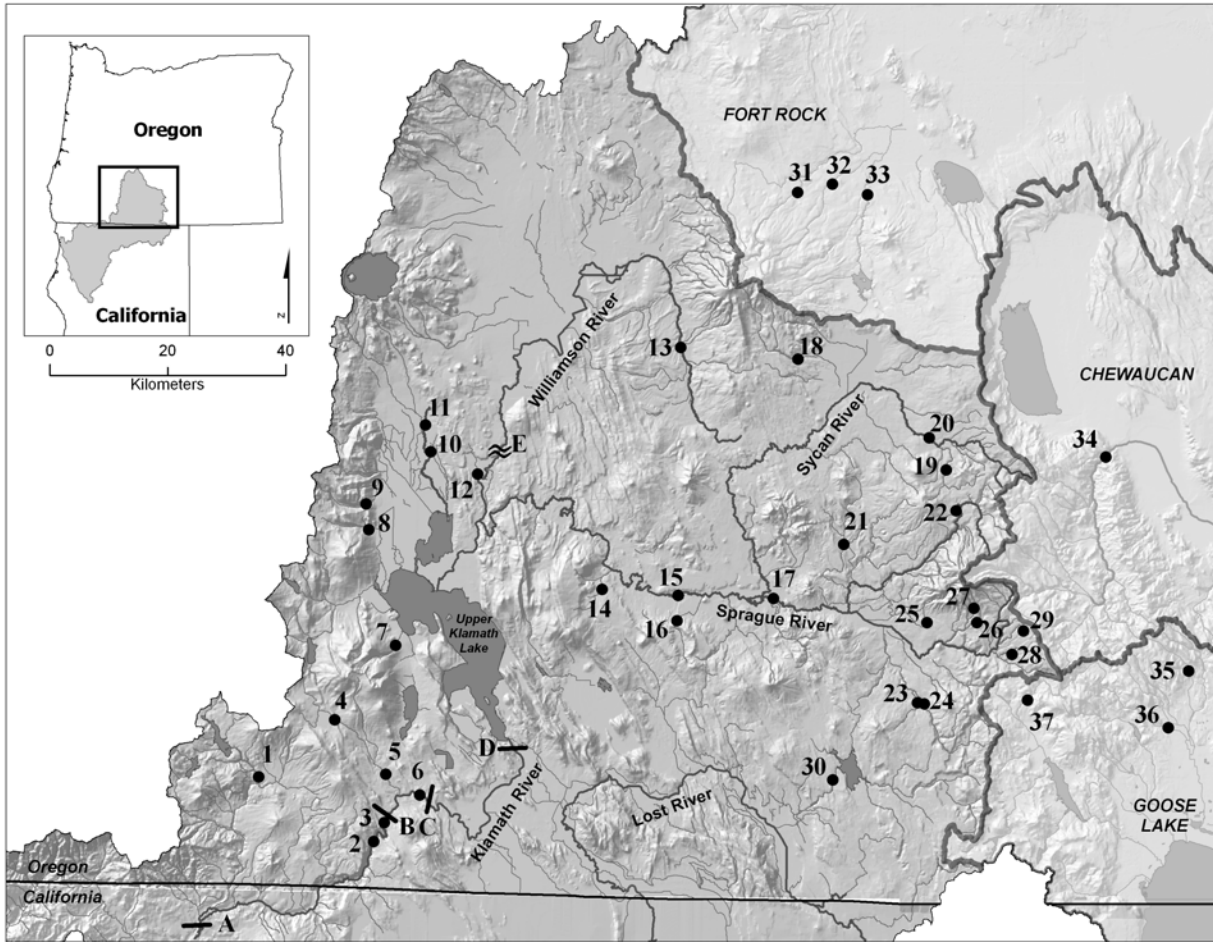
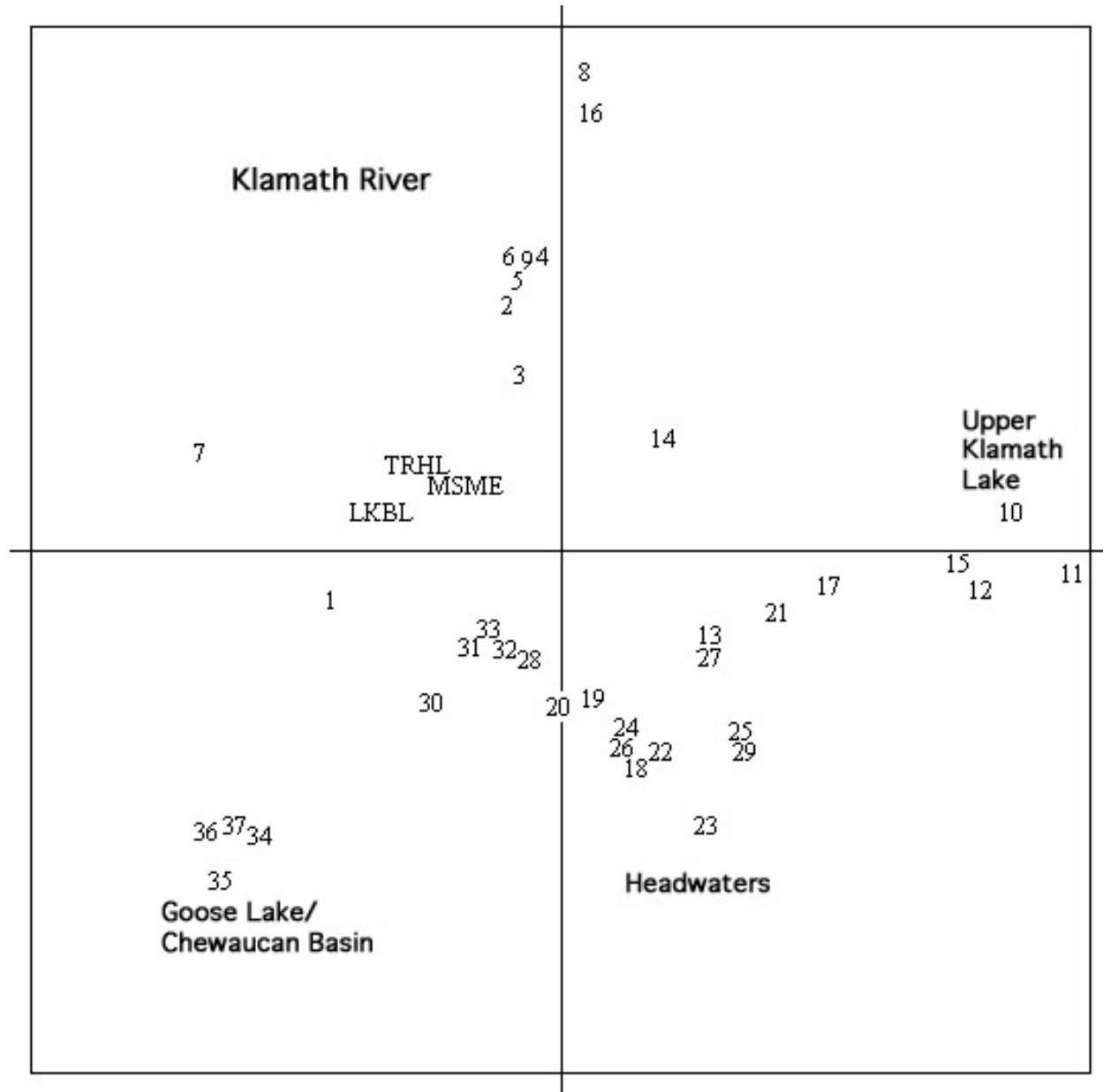
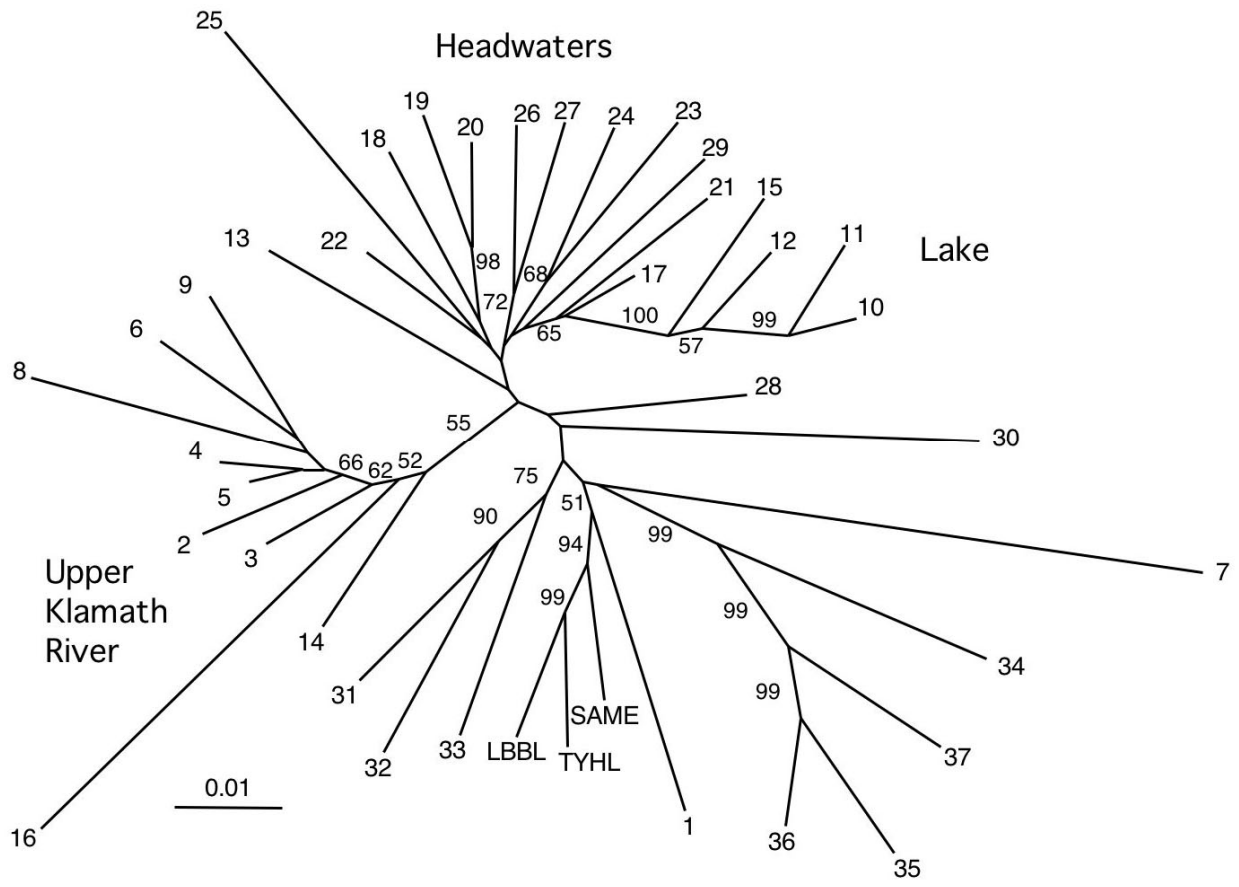


Figure 2:



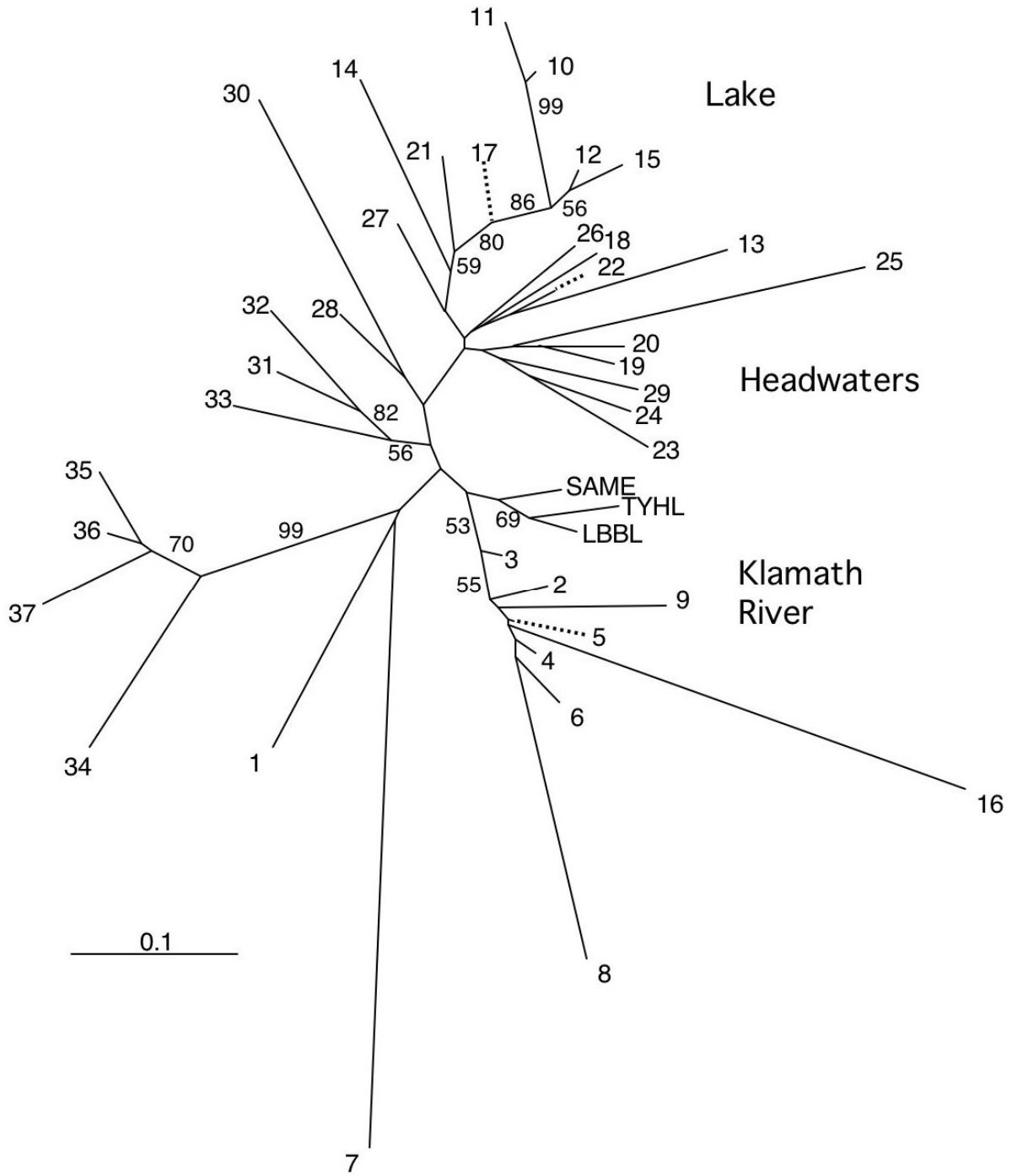
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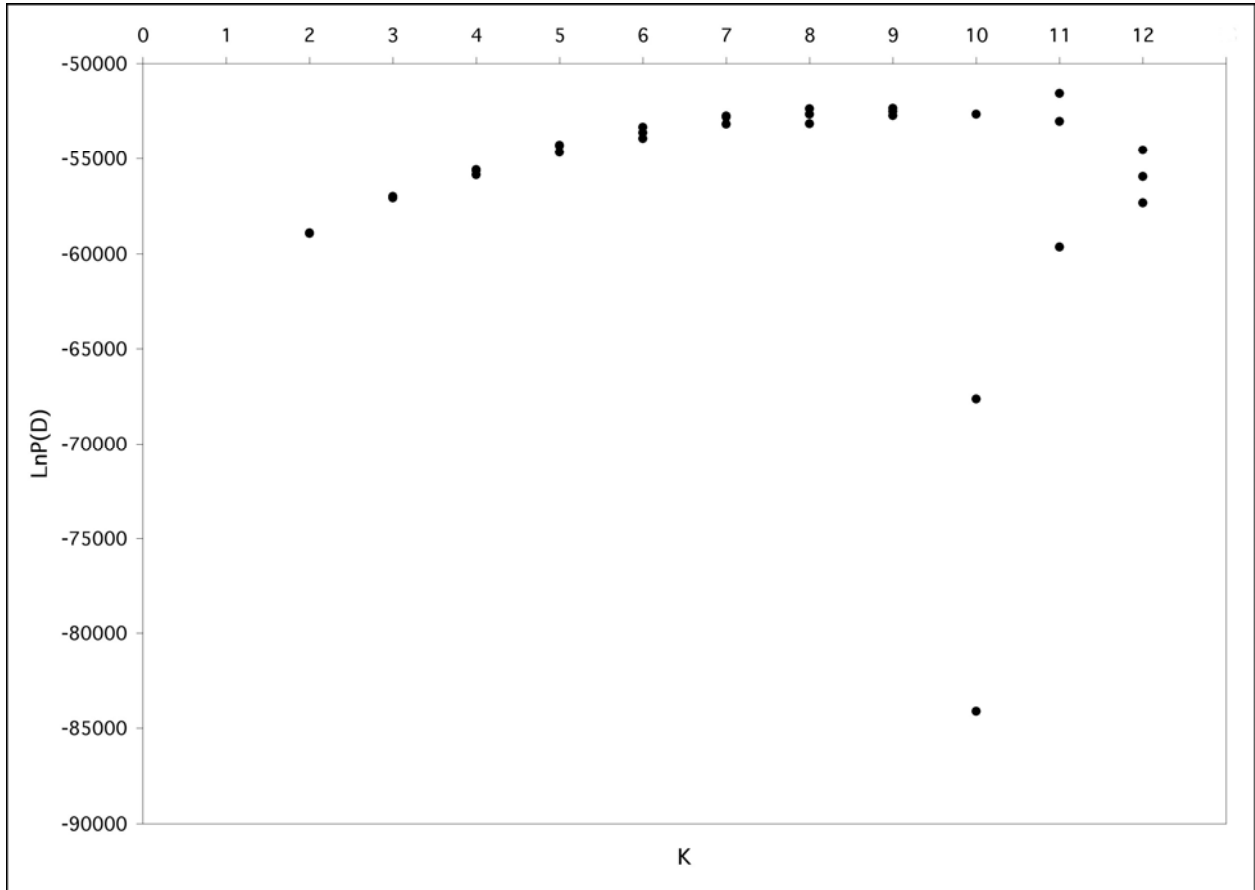
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Figure 4:

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670 Figure 5.



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