

Population Genetics of Klamath Basin Suckers
Phase II Report:
Development and Analysis of Species-Specific Markers
Development of Microsatellite Markers

Submitted to: U. S. Bureau of Reclamation

*In partial fulfillment of contracts from the Bureau of Reclamation, Agreement
No. 99-FG-20-1779.*

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31 December 1999

Summary

We used an innovative AFLP-SSCP technique to develop four species diagnostic loci. Attempts at developing species diagnostic loci (based on findings from Phase I) using inverse PCR techniques were confounded by the duplicated sucker genome. These new loci have proven useful for identifying hybridized individuals and determining the identity of morphologically ambiguous samples. We have documented two potential 'hybrid hotspots' (Klamath and Sprague Rivers) and may find more sites with more extensive sampling throughout the Klamath Basin. In addition, the development of highly polymorphic microsatellite loci for population analysis is nearly complete.

Introduction

The Klamath River Basin is home to four species of suckers; the shortnose sucker (*Chasmistes brevirostris*), the Lost River sucker (*Deltistes luxatus*), the Klamath largescale sucker (*Catostomus snyderi*) and the Klamath smallscale sucker (*Catostomus rimiculus*). The shortnose and Lost River suckers were once among the most abundant lake-dwelling fish in the Klamath River Basin and supported large fisheries earlier in the century. Overexploitation and the large-scale degradation of the Klamath River ecosystem caused the rapid decline of shortnose and Lost River suckers in the 1960's and led to their 1988 listing as endangered species under the Endangered Species Act (Department of the Interior, U.S.F.W.S. 1988). Federal, state, and academic groups are conducting research to understand the biology of these endangered species and their habitats in order to manage them for recovery. In many cases, however, morphometric and meristic differences confuse the field identification of individuals from several populations. Earlier morphologic and genetic studies were unable to resolve questions regarding reproductive isolation, classification, and the systematic relationships among these and other sucker taxa

(Miller and Smith 1981; Harris 1991; Harris and Markle 1993). Additionally, these investigations suggest that recent or historical introgressive hybridization has occurred among Klamath Basin suckers. In particular, there are concerns that shortnose suckers have hybridized with both smallscale and Lost River suckers. The taxonomic and reproductive status of Klamath Basin suckers must be resolved or it is of little use to have extensive biological data for these groups.

The Shortnose-Lost River Sucker Recovery Plan requires a genetic evaluation of these two species throughout their range. Performing phylogenetic and population genetic analyses in conjunction is a powerful approach for identifying evolutionary significant units and units for management. Knowledge of phylogenetic relationships allows for the consideration of the origins of variation and identification of unique gene pools. Determining the amount of gene flow occurring between populations reveals the degree of population independence and structuring. An understanding of which populations are functionally independent will enhance the success of management plans designed for the conservation and recovery of these species. Moritz (1994) suggests that populations are likely to be demographically independent if so few alleles are exchanged that they are considered genetically isolated. Genetically and, therefore, demographically independent populations require treatment as separate management units.

Hybridization is currently seen as a threat to shortnose and Lost River suckers. If hybrids are fertile it is presumed that genetic swamping may overwhelm locally adapted, genetically 'pure' shortnose and Lost River suckers. Since endangered taxa often occur in low abundance, they may be under the threat of elimination through hybridization with more abundant, closely related taxa (Grant and Grant 1992). Hybridization has been shown to occur more frequently in areas of habitat disturbance (Allendorf and Leary 1988). The occurrence of natural hybrids could potentially be used as an indicator for determining priority recovery and management sites.

Hybrids may also compete with endangered taxa for limited spawning habitat and resources. Additionally, under the 'Hybrid Policy' of the Endangered Species Act, natural and artificial hybrids do not receive protection.

Several different laboratories are using independent strategies to find genetic markers to resolve questions regarding reproductive isolation, classification, systematic relationships, and extent of hybridization among Klamath Basin suckers. Dr. Markle's laboratory at Oregon State University is pursuing morphometrics and single copy nuclear DNA (scnDNA) sequence variation and Dr. Dowling at Arizona State University is examining mitochondrial DNA (mtDNA) sequence variation. We were funded in 1998 by the US Fish and Wildlife Service and the Bureau of Reclamation to test both allozymes and Amplified Fragment Length Polymorphisms (AFLP) as possible sources of species diagnostic markers.

During Phase I we screened 66 allozyme loci, 54 of which were monomorphic and showed no variation among the four species. Of the 12 polymorphic loci, nine were not sufficiently diagnostic at the species or population level. We determined that the lack of sufficient diagnostic variation and the need for lethal sampling did not justify continuing this approach. Representatives from two populations of each taxon were also screened for variation with 64 AFLP combinations, seeking taxon specific markers. A number of taxon specific markers were found for Lost River and smallscale suckers including several population specific markers. One marker specific to shortnose suckers was detected while no bands specific to largescale suckers were found. Interspecific comparisons demonstrated that Shortnose and largescale suckers, although distinct, are genetically very similar. The close genetic relationship of these taxa suggests either recent introgressive hybridization or recent speciation between these groups. Both populations of Lost River sucker are very similar and form a distinct group that is more closely related to the shortnose-largescale cluster than to the smallscale group. The Rogue

River and Klamath populations of smallscale sucker form the most distinct group and could easily be classified as separate subspecies.

For Phase II we proposed the following objectives:

- 1) Complete the screening of 64 additional AFLP primer combinations for shortnose and Klamath largescale populations to find additional taxon specific markers for these two closely related taxa.**
- 2) Develop specific primers for at least ten of the best AFLP diagnostic markers.**
- 3) Examine all individuals in the archived 1993 collection with newly developed locus-specific primers.**
- 4) Determine the genetic relationships of the individuals and populations in the archived 1993 collection.**
- 5) Examine all unknown individuals sampled in 1997 by BRD personnel.**

Methods

In addition to the methods outlined in the Phase I report, we used the following procedures:

Inverse PCR

Inverse PCR is a method used to sequence regions of the genome flanking a sequence of interest (*e.g.*, an AFLP band). Taxon-specific markers were selected for continued development from the original AFLP gels. Each band was extracted from the gel matrix, reamplified and sequenced. SCAR (sequence characterized amplified regions) primers used in the inverse-PCR process were designed for each locus. The primers are designed to amplify out from the original band, targeting the flanking sequence. Inverse-PCR first involves cutting whole genomic DNA with restriction enzymes (other than those used in the AFLP process). We used *NlaI* and *SauI* restriction enzymes for the first step. The resulting DNA fragments are then inserted into a vector or ligated with small adapter sequences provided in the Vectorette II System from

Genosys Biotechnologies Inc. PCR primers specific to the vector or ligated adapter sequences are used along with the SCAR primers to amplify the region outside of the original band. These fragments are then sequenced and primers designed to amplify the entire locus, including the original AFLP restriction sites. Once amplified, this single-locus PCR product is cut with the initial AFLP restriction enzymes (EcoRI and MseI). The resulting patterns should produce patterns specific to the taxon that originally possessed the band.

AFLP - SSCP

Rather than continue with the inverse PCR technique, a different strategy was employed. The AFLP combinations that amplified similarly sized bands in all species were targeted. The polyacrylamide gels initially used to visualize the AFLP's separate band fragments based on sequence length. By re-running the original AFLP reactions on single strand conformation polymorphism gels (SSCP), we would then screen the AFLP bands for sequence polymorphism. SSCP gels detect single base substitutions in DNA fragments and have been estimated to have a 99% accuracy rate for detecting polymorphisms in segments 100-300 base pairs in length (Lessa and Applebaum 1993). An SSCP screen of the original AFLP reactions would allow for a relatively simple and rapid survey of sequence divergence across a large number of loci. The SSCP technique would allow us to screen for far more genetic variation than we would have otherwise detected with 64 additional AFLP combinations. Due to the high resolving power of SSCP gels, we could screen for single nucleotide polymorphisms and target variant alleles that are species-specific. Species-specific loci would also be easier to develop from SSCP gels since inverse PCR would no longer be necessary. After sequencing a variant band and designing PCR primers specific to the allele, the locus would then be amplified in all species. The alternate allele present in the remaining species would be sequenced and aligned with the original variant

in order to detect the sequence difference. Depending on the nature of the polymorphism, a restriction enzyme may be used to cut a restriction site present at the polymorphic site. If a restriction site were not present at the site of the polymorphism or if numerous polymorphic sites were present, the alleles would be detected on SSCP gels.

AFLP reactions containing multiple loci (same sized bands) on a single gel (Fig. 1) were selected for continued development with the AFLP-SSCP procedure. Amplified products were run on a 0.5X - SSCP (Molecular Dynamics) gel and visualized with a Molecular Dynamics 595 fluorimager. The amplification products were fluorescently detected using single-primer labeling with fluorescein or staining with an agarose and TMVistra Green overlay (Rodzen *et al.* 1998).

Variant alleles (Fig. 2) fixed for a particular species were excised from the gel and reamplified with the single-base pair extension primers. The resulting PCR amplification was quality tested on a 5% denaturing acrylamide gel and, if a single product were present, was purified with a QIAquick PCR Purification Kit (QIAGEN) and sequenced. PCR primers were designed for the sequenced product with *PrimerSelect* computer software (Lasergene 5.1, DNASTAR Inc.). The remaining representative individuals from each species were amplified and the product was visualized on an SSCP gel (Fig. 3). The alternate allele was excised from the gel, reamplified, purified, and sequenced. The sequence variants were aligned with *MegAlign* computer software (Lasergene 5.1, DNASTAR Inc.), revealing the sequence polymorphisms. The sequences were analyzed for the presence of restriction sites using *MapDraw* computer software (Lasergene 5.1, DNASTAR Inc.). If a restriction site was present at the polymorphism, a restriction fragment length polymorphism (RFLP) analysis was carried out on the archived individuals (Fig. 4). If no restriction site was present at the polymorphic site, or if numerous polymorphic sites were present, all archived individuals were analyzed with SSCP gels (Fig. 6).

Microsatellites

Nuclear microsatellite markers provide many advantages over allozyme loci and complement mtDNA techniques for investigating genetic structure of species (*e.g.*, Estoup *et al.* 1993, Paetkau and Strobeck 1994, and Pope *et al.* 1996). Microsatellite loci usually have more polymorphic loci and more alleles per polymorphic locus than allozyme loci. Relatively high rates of mutation with regard to number of repeat motifs make this a useful technique for fine-scale population structure studies. Microsatellite loci, in contrast to mtDNA, are inherited biparentally and represent multiple markers. In addition, microsatellite loci, in contrast to allozymes, can be scored from tissues sampled non-destructively (*e.g.*, muscle, fin, hair, blood, feces, scale, feather) and preserved by freezing, drying, or alcohol storage, and are ideal for endangered species.

Genetic Identification Services (Chatsworth, CA) created a sub-genomic library using DNA samples from *Deltistes luxatus*. Whole genomic DNA was partially digested with a mixture of the following enzymes: *Bsr*BR I, *Eco*R V, *Hae* III, *Pvu* II, *Sca* I, and *Stu* I. An oligonucleotide linker containing a *Hin*D III site was ligated to fragment in the range of 300-700 base pairs. These fragments were enriched by magnetic bead capture to create two separate libraries for the repeat motifs (CA)_n and (GATA)_n. The captured fragments were captured into the *Hin*D III site of the plasmid pUC 19 and the ligation products electroporated into *E. coli* DH5 α . From each library, nine randomly chosen clones were sequenced to determine enrichment efficiency. The (CA)_n and (GATA)_n libraries each had a 90% enrichment efficiency.

PCR primers are being designed with *PrimerSelect* computer software (Lasergene 5.1, DNASTAR Inc.) and tested for amplification quality and level of polymorphism with three representatives from each species. Amplification is done in an M.J. Research PTC-100 96V thermocycler with a “hot bonnet” lid. PCR products are run on a 5% denaturing acrylamide gel

and visualized with a Molecular Dynamics 595 fluorimager. The amplification products are fluorescently detected using an agarose and TMVistra Green overlay (Rodzen *et al.* 1998).

Results and Discussion of Phase I Objectives

1) Complete the screening of 64 additional AFLP primer combinations for shortnose and Klamath largescale populations to find additional taxon specific markers for these two closely related taxa.

Inverse PCR

Prior to screening the additional loci, we initiated the development of procedures used to convert taxon-specific AFLP bands into locus-specific sequence characterized amplified regions (SCARs). In order to develop PCR primers that would amplify the band of interest in all species and allow us to enzyme restrict the species-specific polymorphic sequence, it was necessary to sequence the DNA regions flanking the restriction sites producing the original AFLP band. Sequencing of the flanking regions was attempted using inverse PCR. Initial attempts at the inverse PCR process proved unsuccessful. Although numerous flanking region sequences were obtained and PCR primers designed to amplify the locus of interest all species, we were unable to locate the polymorphic restriction sequences that provided the original species-specific AFLP bands. Based on the number of AFLP loci attempted and various inverse PCR strategies employed (vector ligation, self-ligation, and a Vectorsite kit), we have likely ruled out many of the technical reasons as to why the original polymorphisms were not encountered. Instead, it is likely that the difficulties experienced were confounded by the polyploid nature of the sucker genome. Suckers have an allotetraploid genetic heritage, meaning each cell contains two homeologous (not identical) sets of chromosomes (Ferris and Whitt 1980). When targeting the region that flanks the species-specific AFLP band, we had an equally likely chance of cross-

amplifying the alternate, homeologous locus rather than the original polymorphic locus due to the overall sequence similarity between the loci. If the homeologous flanking region did not contain the original AFLP restriction polymorphism, we would completely miss the species-specific polymorphism.

Due to the difficulties encountered with inverse PCR, we did not screen the additional 64 AFLP loci. Instead, using SSCP, we screened for sequence polymorphisms within our previous AFLP combinations (see objective 2 below).

2) Develop specific primers for at least ten of the best AFLP diagnostic markers.

AFLP - SSCP

Based on the AFLP-SSCP screening technique, we initially developed nine of the best potential loci. Further screening with more representatives from all taxa along with testing reaction optima led us to finish development of four loci. Two of the loci, Cri1 and Cri2 (for *Catostomus rimiculus*), were diagnostic in Klamath smallscale suckers. The third locus, Csn1 (for *Catostomus snyderi*), was diagnostic for Klamath largescale suckers. The fourth locus, Dlu1 (for *Deltistes luxatus*), was diagnostic for Lost River suckers.

The development of Cri1 is described in Figs. 1-4. This locus is an RFLP that only cuts smallscale suckers. The scoring should be interpreted as 22 (cut) for smallscales, 11 (uncut) for Lost River, Shortnose, and largescale suckers, and 12 for putative hybrids.

Locus Cri2 is a codominant marker with two alleles (Fig. 5). One allele appears to be fixed in smallscales (scored 22), is polymorphic in Shortnose and largescale suckers (scored 11, 12 or 22), and is absent from Lost River suckers (scored 11).

Locus Csn1 is a codominant SSCP marker with two alleles (Fig. 6). One allele has a high frequency in largescales (scored 22 or 12) especially in the Upper Williamson and is absent in Lost River, Shortnose, and smallscale suckers.

Locus Dlu1 is a dominant marker that is very reliable for diagnosing Lost River suckers (Fig. 7). The scoring is 2* (band presence - can't determine homozygotes/ heterozygotes) for Lost Rivers, and 11 (band absence) for Shortnose, smallscale, and largescale suckers.

Microsatellites

Dimeric and tetrameric microsatellite libraries have been developed, and we are currently screening 60 (CA)_n and 160 (GATA)_n clones. All clones have been sequenced, and primers designed and received for 50 of the loci. Another 100 primer sets have been designed for the remaining loci. We will test all of the primers sets and choose the 20 best loci for use in this study. We are currently testing 220 loci for utility in Klamath Basin suckers. Figures 8 and 9 demonstrate two representative highly polymorphic tetra-repeat and di-repeat microsatellite loci, respectively.

3) Examine all individuals in the archived 1993 collection with newly developed locus-specific primers.

The distributions of genotypes for Cri1, Cri2, Csn1, and Dlu1 for the archived collection are listed in Table 1.

4) Determine the genetic relationships of the individuals and populations in the archived 1993 collection.

The distributions of genotypes for each locus have been plotted for each species by location throughout the Klamath Basin (Figs. 10 – 13).

For locus Cri1 (Fig. 10), all smallscale suckers from the Rogue River are (22) homozygotes. All smallscale suckers from the Klamath River are also (22) homozygotes, except for a single (12) heterozygous individual (#106) which is possibly of hybrid origin. A single individual from Upper Klamath Lake identified as a smallscale sucker has the diagnostic (22)

homozygous genotype. All Shortnose suckers from the Lost River and Sprague River basins and Upper Klamath Lake are (11) homozygotes. All Shortnose suckers from the Klamath River are (11) homozygotes except for a single (12) heterozygous individual (#226) which is possibly of hybrid origin. All Lost River suckers from the Lost River and Sprague River basins and Upper Klamath Lake are (11) homozygotes. The two samples from the Klamath River identified as Lost River suckers (#237 and #267) are (12) heterozygotes. These individuals will be discussed later. All largescale suckers from the Upper Williamson and Lost River Basins and Upper Klamath Lake are (11) homozygotes. Of the 26 Sprague River basin largescale suckers, five are (12) heterozygotes and the remaining samples are (11) homozygotes. Largescale suckers from the Sprague River basin will be discussed in detail later.

For locus Cri2 (Fig. 11), all smallscale suckers from the Rogue River are (22) homozygotes. All smallscale suckers from the Klamath River are also (22) homozygotes, except for a single (12) heterozygous individual (#266) which is possibly of hybrid origin. The Upper Klamath Lake individual identified as a smallscale sucker has the diagnostic (22) homozygous genotype. All populations of Shortnose suckers are polymorphic at this locus having 11, 12, and 22 genotypes represented. All Lost River suckers from the Lost River and Sprague River basins and Upper Klamath Lake are (11) homozygotes. The two samples from the Klamath River identified as Lost River suckers (#237 and #267) are (12) heterozygotes. Largescale suckers from the Lost River Basins are (11) homozygotes. Each of the Upper Williamson and Upper Klamath Lake populations have a single (12) heterozygote sample while all remaining individuals are (11) homozygotes. The Sprague River basin largescale suckers are polymorphic at this locus having 11, 12, and 22 genotypes represented.

At locus Csn1 (Fig. 12) all smallscale and Lost River suckers are (11) homozygotes. All Shortnose suckers from the Klamath and Sprague Rivers and Upper Klamath Lake are (11)

homozygotes. In addition, all Shortnose suckers from the Lost River are (11) homozygotes except for a single (12) heterozygous individual (#191). Largescale suckers from the Lost River and Upper Klamath Lake are (11) homozygotes. All Sprague River largescale suckers are (11) homozygotes except for a single (12) heterozygous individual (#320). The Upper Williamson largescale suckers are polymorphic at this locus having 11, 12, and 22 genotypes represented.

At locus Dlu 1(Fig. 13) all smallscale suckers are (11) homozygotes. Shortnose suckers from the Klamath river are (11) homozygotes. There are a few (2*) Shortnose suckers from the Lost River, Sprague River, and Upper Klamath Lake, although most individuals are (11) homozygotes. Lost River suckers from the Klamath River are (11) homozygotes. Most of the Lost River sucker samples from the Lost River, Sprague River and Upper Klamath Lake have the (2*) genotype, although a few (11) homozygotes are present in these populations. In addition, a rare (3) allele is present in two Lost River suckers (#247 and #256) from the Lost River, each having a (23) genotype. Largescale suckers from the Lost River, Upper Klamath Lake and upper Williamson are (11) homozygotes. Three largescale individuals from the Sprague River have the (2*) genotype while the remaining samples are (11) homozygotes.

Of particular interest are individuals 237 and 267, both described as Lost River suckers from the Klamath River. Composite genotypes suggest that these samples are either misidentified or of hybrid origin. Both individuals are heterozygous for both Cri loci, 11 homozygous for the Csn locus, and are 11 homozygotes for the Dlu locus. These results suggest that these individual are misidentified and not Lost River suckers (based on the absence of the diagnostic Dlu1 genotype) or hybrid backcrossed with smallscale suckers (based on both Cri loci). In addition, the occurrence of numerous heterozygous Shortnose and smallscale suckers at Cri1 and Cri2 suggests that hybridization may be occurring among all taxa in the Klamath River.

Collecting a larger number of individuals from the Klamath River is crucial for understanding the status of hybridization that is likely occurring in this area.

The occurrence of heterozygous largescale suckers (at both Cri loci) in the Sprague River was surprising. Locus Cri1, in particular, is an effective diagnostic marker for identifying smallscale suckers. The occurrence of the diagnostic smallscale allele in Sprague River largescale suckers is surprising for several reasons. The occurrence of the diagnostic smallscale allele implies hybrid origin and is surprising since smallscale suckers have not been identified from the Sprague River. In fact, the occurrence of a single smallscale sucker in Upper Klamath Lake was itself surprising since smallscale suckers have only been described from the lower Klamath River. The single specimen from Upper Klamath Lake is the closest known occurrence to the Sprague River. Additionally, largescale suckers are mostly stream and river residents and are not known to occur in larger bodies of water (*e.g.*, Upper Klamath Lake). With these factors combined, the occurrence of putative hybrids in the Sprague River is an intriguing phenomenon. A larger number of samples for the Sprague River would further assist in diagnosing the status of potential hybridization in the Sprague River.

With this suite of four loci we can identify hybridized individuals and likely determine the identity of morphologically ambiguous samples. In many locations, sample sizes are inadequate for rigorous analysis. We have documented two potential ‘hybrid hotspots’ (Klamath and Sprague Rivers) and may find more with more extensive sampling throughout the Klamath Basin.

5) Examine all unknown individuals sampled in 1997 by BRD personnel.

Due to the difficulties encountered with the development of the species-specific loci, we focused our resources on developing microsatellite loci rather than analyzing the remaining samples. We will analyze the 1997 and 1998 samples during phase three.

Research Direction for Phase III

We will analyze all individuals sampled in 1997 and 1998 with the four diagnostic loci (Cri1, Cri2, Csn1, Csn2) developed in Phase II. We will develop at least 20 polymorphic, highly informative microsatellite loci during the first quarter of Phase III. Once developed, these loci will be used to analyze all individuals in the archived collection. We will also examine the 1997 and 1998 collection of samples during phase III.

Objectives for Phase III (2000)

- 1) Examine all unknown individuals sampled in 1997 and 1998 by BRD personnel with Cri1, Cri2, Csn1, and Dlu 1.**
- 2) Examine all archived individuals with 20 newly developed microsatellite loci.**
- 3) Examine all unknown individuals sampled in 1997 and 1998 by BRD personnel with newly developed microsatellite loci.**

Objectives for Phase IV (2001)

Merge the data sets from ASU, OSU, and UC Davis.

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Table 1. The distributions of genotypes for Cri1, Cri2, Csn1, and Dlu1 for the archived collection. Individuals are listed by I.D., genus, species, and subbasin. See *Results and Discussion of Phase I Objectives* for a description of each locus. Individuals designated with NA are not present in the archived collection. Individuals designated with (-) need to be reanalyzed.

NUMBER	Cri1	Cri2	Csn1	Dlu1	GENUS	SPECIES	SUBBASIN
1	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
2	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
3	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
4	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
5	11	12	11	11	Chasmistes	brevirostris	UPPER KLAMATH
6	11	11	11	11	CHA/CAT	BREV/SNY	UPPER KLAMATH
7	11	11	11	2*	Deltistes	luxatus	LOST RIVER
8	11	11	11	2*	Chasmistes	brevirostris	LOST RIVER
9	11	11	11	2*	Deltistes	luxatus	LOST RIVER
10	11	11	11	2*	Deltistes	luxatus	LOST RIVER
11	NA	NA	NA	NA	Deltistes	luxatus	LOST RIVER
12	11	11	11	2*	Deltistes	luxatus	LOST RIVER
13	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
14	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
15	11	12	11	11	Chasmistes	brevirostris	UPPER KLAMATH
16	11	12	11	11	Chasmistes	brevirostris	SPRAGUE
17	11	11	11	2*	Deltistes	luxatus	SPRAGUE
18	11	12	11	11	Chasmistes	brevirostris	SPRAGUE
19	11	11	11	11	Chasmistes	brevirostris	SPRAGUE
20	11	11	11	2*	Deltistes	luxatus	SPRAGUE
21	11	11	11	11	Deltistes	luxatus	SPRAGUE
22	11	11	11	2*	Deltistes	luxatus	SPRAGUE
23	11	22	11	2*	Chasmistes	brevirostris	SPRAGUE
24	NA	NA	NA	NA	Deltistes	luxatus	SPRAGUE
25	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
26	NA	NA	NA	NA	Catostomus	snyderi	UPPER WILLIAMSON
27	NA	NA	NA	NA	Chasmistes	brevirostris	SPRAGUE
28	11	11	11	2*	Deltistes	luxatus	SPRAGUE
29	11	11	11	2*	Deltistes	luxatus	SPRAGUE
30	11	12	11	11	Chasmistes	brevirostris	SPRAGUE
31	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
32	11	12	11	11	Chasmistes	brevirostris	UPPER KLAMATH
33	11	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
34	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
35	11	12	11	11	Chasmistes	brevirostris	SPRAGUE
36	11	11	11	11	Chasmistes	brevirostris	SPRAGUE
37	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
38	11	11	11	11	Chasmistes	brevirostris	SPRAGUE
39	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
40	11	11	11	2*	Chasmistes	brevirostris	UPPER KLAMATH
41	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
42	11	22	11	11	Chasmistes	brevirostris	UPPER KLAMATH
43	11	11	11	2*	Chasmistes	brevirostris	UPPER KLAMATH
44	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
45	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
46	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
47	11	11	11	2*	Chasmistes	brevirostris	UPPER KLAMATH
48	11	11	11	11	Deltistes	luxatus	UPPER KLAMATH

NUMBER	Cri1	Cri2	Csn1	Dlu1	GENUS	SPECIES	SUBBASIN
Cri1	11	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
Cri2							
Csn1							
Dlu1							
GENUS							
SPECIES							
SUBBASIN							
49							
50	11	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
51	11	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
52	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
53	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
54	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
55	11	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
56	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
57	11	12	12	11	Catostomus	snyderi	UPPER WILLIAMSON
58	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
59	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
60	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
61	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
62	11	11	22	11	Catostomus	snyderi	UPPER WILLIAMSON
63	11	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
64	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
65	-	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
66	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
67	-	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
68	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
69	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
70	11	11	-	-	Catostomus	snyderi	UPPER WILLIAMSON
71	11	11	11	11	Catostomus	snyderi	UPPER WILLIAMSON
72	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
73	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
74	-	-	11	-	Catostomus	snyderi	UPPER WILLIAMSON
75	11	11	12	11	Catostomus	snyderi	UPPER WILLIAMSON
76	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
77	11	12	11	11	Catostomus	snyderi	SPRAGUE
78	12	12	11	11	Catostomus	snyderi	SPRAGUE
79	11	11	11	11	Catostomus	snyderi	LOST RIVER
80	11	11	11	11	Catostomus	snyderi	LOST RIVER
81	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
82	11	11	11	11	Catostomus	snyderi	UPPER KLAMATH
83	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE
84	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE
85	NA	NA	NA	NA	Catostomus	snyderi	LOST RIVER
86	11	22	11	11	Chasmistes	brevirostris	LOST RIVER
87	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
88	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
89	11	-	11	11	Chasmistes	brevirostris	LOST RIVER
90	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
91	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
92	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
93	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
94	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
95	11	22	11	11	Chasmistes	brevirostris	LOST RIVER
96	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
97	11	11	11	11	Chasmistes	brevirostris	LOST RIVER

98	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
99	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
100	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
101	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
102	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
103	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
NUMBER	Cri1	Cri2	Csn1	Dlu1	GENUS	SPECIES	SUBBASIN
Cri1	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
Cri2							
Csn1							
Dlu1							
GENUS							
SPECIES							
SUBBASIN							
104							
105	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
106	12	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
107	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
108	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
109	11	11	11	11	Chasmistes	Brevirostris	LOST RIVER
110	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
111	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
112	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
113	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
114	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
115	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
116	11	-	11	11	Chasmistes	brevirostris	LOST RIVER
117	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
118	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
119	11	22	11	11	Chasmistes	brevirostris	LOST RIVER
120	11	12	11	11	Catostomus	snyderi	SPRAGUE
121	12	12	11	11	Catostomus	snyderi	SPRAGUE
122	11	11	11	11	Catostomus	snyderi	SPRAGUE
123	12	22	11	11	Catostomus	snyderi	SPRAGUE
124	11	12	11	11	Catostomus	snyderi	SPRAGUE
125	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
126	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
127	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
128	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
129	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
130	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
131	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
132	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
133	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
134	11	11	11	11	Deltistes	luxatus	LOST RIVER
135	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
136	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
137	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
138	11	11	11	2*	Deltistes	luxatus	LOST RIVER
139	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
140	11	11	11	2*	Deltistes	luxatus	LOST RIVER
141	11	11	11	2*	Deltistes	luxatus	LOST RIVER
142	NA	NA	11	NA	Deltistes	luxatus	LOST RIVER
143	22	22	11	11	Catostomus	rimiculus	ROGUE
144	22	22	11	11	Catostomus	rimiculus	ROGUE
145	22	22	11	11	Catostomus	rimiculus	ROGUE
146	22	22	11	11	Catostomus	rimiculus	ROGUE

147	22	22	11	11	Catostomus	rimiculus	ROGUE
148	22	22	11	11	Catostomus	rimiculus	ROGUE
149	22	22	11	11	Catostomus	rimiculus	ROGUE
150	22	22	-	11	Catostomus	rimiculus	ROGUE
151	22	22	-	11	Catostomus	rimiculus	ROGUE
152	22	22	-	11	Catostomus	rimiculus	ROGUE
153	22	22	-	11	Catostomus	rimiculus	ROGUE
154	22	22	11	11	Catostomus	rimiculus	ROGUE
155	22	22	11	11	Catostomus	rimiculus	ROGUE
156	22	22	11	11	Catostomus	rimiculus	ROGUE
157	22	22	11	11	Catostomus	rimiculus	ROGUE
158	22	22	11	11	Catostomus	rimiculus	ROGUE
NUMBER	Cri1	Cri2	Csn1	Dlu1	GENUS	SPECIES	SUBBASIN
Cri1	22	22	11	11	Catostomus	rimiculus	ROGUE
Cri2							
Csn1							
Dlu1							
GENUS							
SPECIES							
SUBBASIN							
159							
160	22	22	11	11	Catostomus	rimiculus	ROGUE
161	22	22	11	11	Catostomus	rimiculus	ROGUE
162	22	22	11	11	Catostomus	rimiculus	ROGUE
163	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
164	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
165	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
166	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
167	11	22	11	11	Chasmistes	brevirostris	LOST RIVER
168	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
169	-	22	11	11	Chasmistes	brevirostris	LOST RIVER
170	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
171	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
172	-	12	11	11	Chasmistes	brevirostris	LOST RIVER
173	22	22	11	11	Catostomus	rimiculus	ROGUE
174	11	11	11	2*	Deltistes	luxatus	LOST RIVER
175	11	11	11	2*	Deltistes	luxatus	LOST RIVER
176	11	11	11	2*	Deltistes	luxatus	LOST RIVER
177	11	11	11	2*	Deltistes	luxatus	LOST RIVER
178	22	22	11	11	Catostomus	rimiculus	ROGUE
179	22	22	11	11	Catostomus	rimiculus	ROGUE
180	22	22	11	11	Catostomus	rimiculus	ROGUE
181	22	22	11	11	Catostomus	rimiculus	ROGUE
182	22	22	11	11	Catostomus	rimiculus	ROGUE
183	22	22	11	11	Catostomus	rimiculus	ROGUE
184	22	22	11	11	Catostomus	rimiculus	ROGUE
185	22	22	11	11	Catostomus	rimiculus	ROGUE
186	22	22	11	11	Catostomus	rimiculus	ROGUE
187	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
188	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
189	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
190	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
191	11	-	12	11	Chasmistes	brevirostris	LOST RIVER
192	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
193	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
194	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
195	11	12	11	11	Chasmistes	brevirostris	LOST RIVER

196	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
197	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
198	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
199	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
200	12	12	11	11	Catostomus	snyderi	SPRAGUE
201	12	12	11	11	Catostomus	snyderi	SPRAGUE
202	11	11	11	11	Catostomus	snyderi	SPRAGUE
203	11	11	11	11	Catostomus	snyderi	SPRAGUE
204	11	22	11	11	Catostomus	snyderi	SPRAGUE
205	11	22	11	11	Catostomus	snyderi	SPRAGUE
206	11	11	11	11	Catostomus	snyderi	SPRAGUE
207	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
208	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
209	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
210	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
211	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
212	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
213	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
NUMBER	Cri1	Cri2	Csn1	Dlu1	GENUS	SPECIES	SUBBASIN
Cri1	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
Cri2							
Csn1							
Dlu1							
GENUS							
SPECIES							
SUBBASIN							
214							
215	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
216	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
217	11	11	11	-	Chasmistes	brevirostris	LOST RIVER
218	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
219	11	12	11	11	Chasmistes	brevirostris	LOWER KLAMATH
220	11	11	11	11	Chasmistes	brevirostris	LOWER KLAMATH
221	11	22	11	11	Chasmistes	brevirostris	LOWER KLAMATH
222	11	12	11	11	Chasmistes	brevirostris	LOWER KLAMATH
223	11	12	11	11	Chasmistes	brevirostris	LOWER KLAMATH
224	11	11	11	11	Chasmistes	brevirostris	LOWER KLAMATH
225	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
226	12	-	11	11	Chasmistes	brevirostris	LOWER KLAMATH
227	11	22	11	11	Chasmistes	brevirostris	LOWER KLAMATH
228	11	12	11	11	Chasmistes	brevirostris	LOWER KLAMATH
229	11	11	11	11	Chasmistes	brevirostris	LOWER KLAMATH
230	11	11	11	11	Chasmistes	brevirostris	LOWER KLAMATH
231	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
232	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
233	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
234	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
235	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
236	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
237	12	12	11	11	DELTIKISTES	LUXATUS	LOWER KLAMATH
238	11	11	11	11	Chasmistes	brevirostris	LOWER KLAMATH
239	11	11	11	11	Chasmistes	brevirostris	LOWER KLAMATH
240	11	11	11	11	Chasmistes	brevirostris	LOWER KLAMATH
241	11	11	11	11	Chasmistes	brevirostris	LOWER KLAMATH
242	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
243	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
244	11	11	11	11	Chasmistes	brevirostris	LOST RIVER

245	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
246	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
247	11	11	11	2*	Deltistes	luxatus	LOST RIVER
248	11	11	11	23	Deltistes	luxatus	LOST RIVER
249	11	11	11	2*	Deltistes	luxatus	LOST RIVER
250	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
251	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
252	11	22	11	11	Chasmistes	brevirostris	LOST RIVER
253	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
254	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
255	11	11	11	2*	Deltistes	luxatus	LOST RIVER
256	11	11	11	23	Deltistes	luxatus	LOST RIVER
257	11	11	11	2*	Deltistes	luxatus	LOST RIVER
258	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
259	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
260	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
261	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
262	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
263	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
264	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
265	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
266	22	12	11	11	Catostomus	rimiculus	LOWER KLAMATH
267	12	12	11	11	DELTA/CAT	LUX/SNY	LOWER KLAMATH
268	11	11	11	11	Chasmistes	brevirostris	LOST RIVER
NUMBER	Cri1	Cri2	Csn1	Dlu1	GENUS	SPECIES	SUBBASIN
Cri1	11	12	11	11	Chasmistes	brevirostris	LOST RIVER
Cri2							
Csn1							
Dlu1							
GENUS							
SPECIES							
SUBBASIN							
269							
270	11	11	11	11	Deltistes	luxatus	LOST RIVER
271	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
272	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
273	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
274	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
275	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
276	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
277	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
278	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
279	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
280	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
281	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
282	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
283	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
284	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
285	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
286	22	22	11	11	Catostomus	rimiculus	LOWER KLAMATH
287	11	12	11	2*	Chasmistes	brevirostris	UPPER KLAMATH
288	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
289	22	22	11	11	Catostomus	rimiculus	UPPER KLAMATH
290	11	12	11	11	Chasmistes	brevirostris	UPPER KLAMATH
291	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
292	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
293	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH

294	11	11	11	11	Deltistes	luxatus	UPPER KLAMATH
295	11	12	11	11	Chasmistes	brevirostris	UPPER KLAMATH
296	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
297	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
298	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
299	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
300	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
301	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
302	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
303	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
304	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
305	11	11	11	2*	Deltistes	luxatus	UPPER KLAMATH
306	11	11	11	11	Deltistes	luxatus	UPPER KLAMATH
307	11	11	11	11	Chasmistes	brevirostris	UPPER KLAMATH
308	11	22	11	11	Catostomus	snyderi	UPPER KLAMATH
309	11	11	11	11	Catostomus	snyderi	SPRAGUE
310	11	12	11	11	Catostomus	snyderi	SPRAGUE
311	11	11	11	11	Catostomus	snyderi	SPRAGUE
312	11	11	11	2*	Catostomus	snyderi	SPRAGUE
313	NA	-	11	-	Catostomus	snyderi	SPRAGUE
314	11	12	11	11	Catostomus	snyderi	SPRAGUE
315	11	11	11	11	Catostomus	snyderi	SPRAGUE
316	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE
317	-	-	-	-	Catostomus	snyderi	SPRAGUE
318	11	11	11	11	Catostomus	snyderi	SPRAGUE
319	11	11	11	11	Catostomus	snyderi	SPRAGUE
320	11	11	12	2*	Catostomus	snyderi	SPRAGUE
321	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE
322	11	22	11	11	Catostomus	snyderi	SPRAGUE
323	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE
NUMBER	Cri1	Cri2	Csn1	Dlu1	GENUS	SPECIES	SUBBASIN
Cri1	11	11	11	2*	Catostomus	snyderi	SPRAGUE
Cri2							
Csn1							
Dlu1							
GENUS							
SPECIES							
SUBBASIN							
324							
325	11	11	11	11	Catostomus	snyderi	SPRAGUE
326	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE
327	11	11	11	11	Catostomus	snyderi	SPRAGUE
328	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE
329	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE
330	11	12	11	11	Catostomus	snyderi	SPRAGUE
331	11	11	11	11	Catostomus	snyderi	SPRAGUE
332	11	11	11	11	Catostomus	snyderi	SPRAGUE
333	NA	NA	NA	NA	Catostomus	snyderi	SPRAGUE

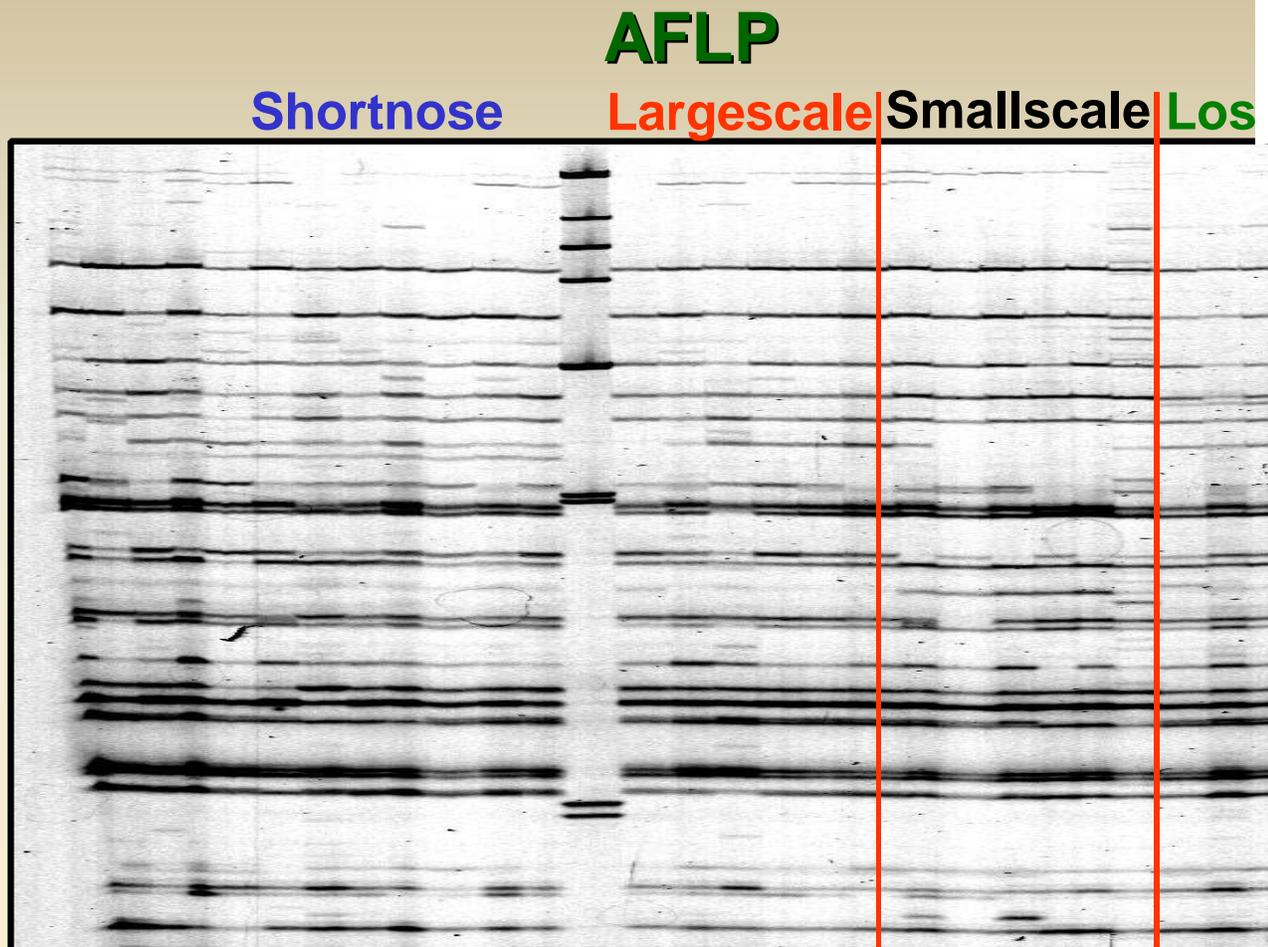


Figure 1. AFLP gel demonstrating a large number of bands shared by the Klamath basin sucker species.

AFLP - SSCP

Largescale | Smallscale | Lost River

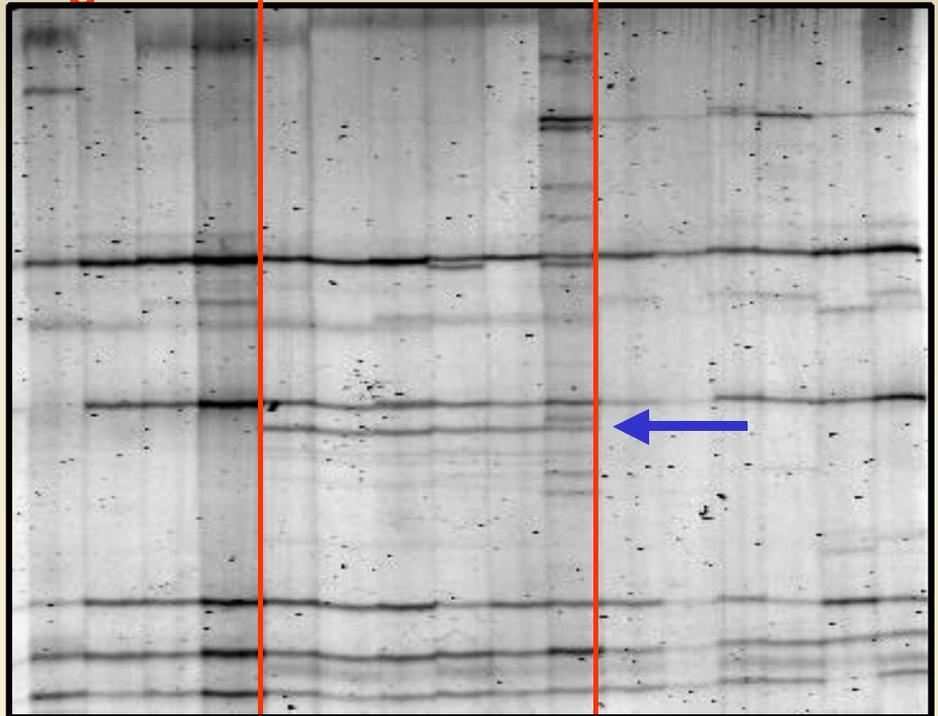


Figure 2. SSCP gel of the AFLP shown in Fig1. The blue arrow indicates a band unique to Klamath smallscale suckers. SSCP separate bands based on nucleotide sequences.

Klamath smallscale marker (Cri1) - S

Shortnose

Lost River

Largescale

Smallscale

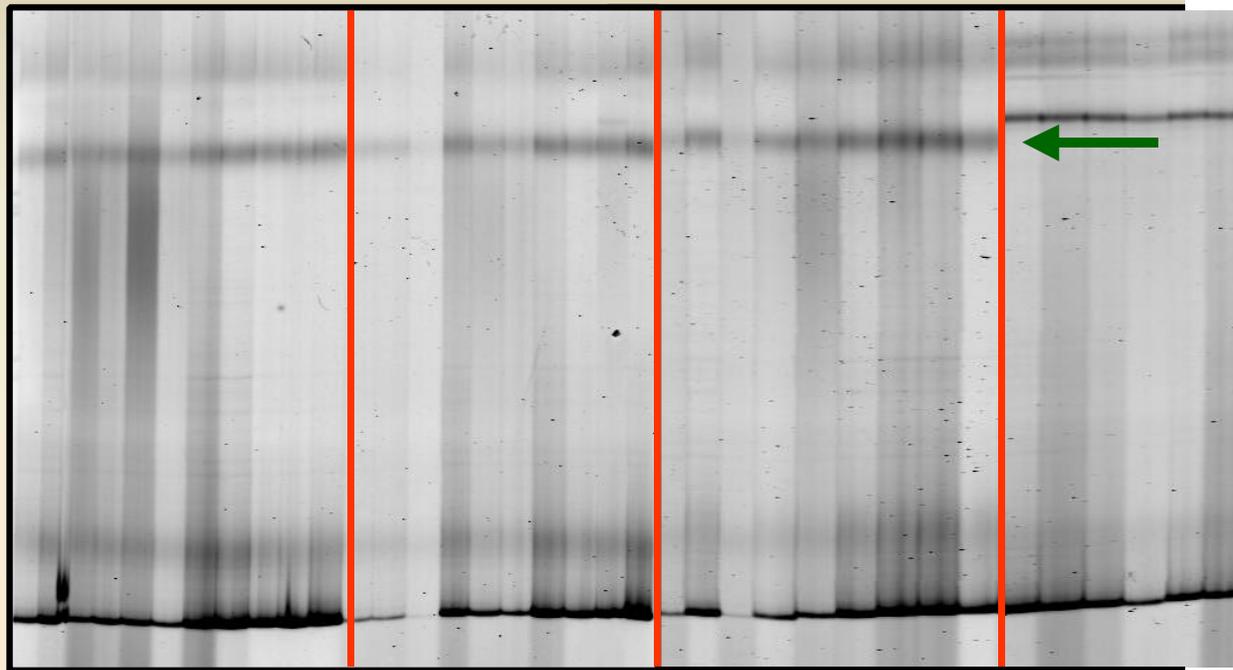


Figure 3. SSCP gel of Klamath smallscale locus (Cri1) showing the unique smallscale allele (indicated by the blue vertical line in Fig 2). The green arrow indicates the alternate allele, present in Shortnose, Lost River, and largescale suckers.

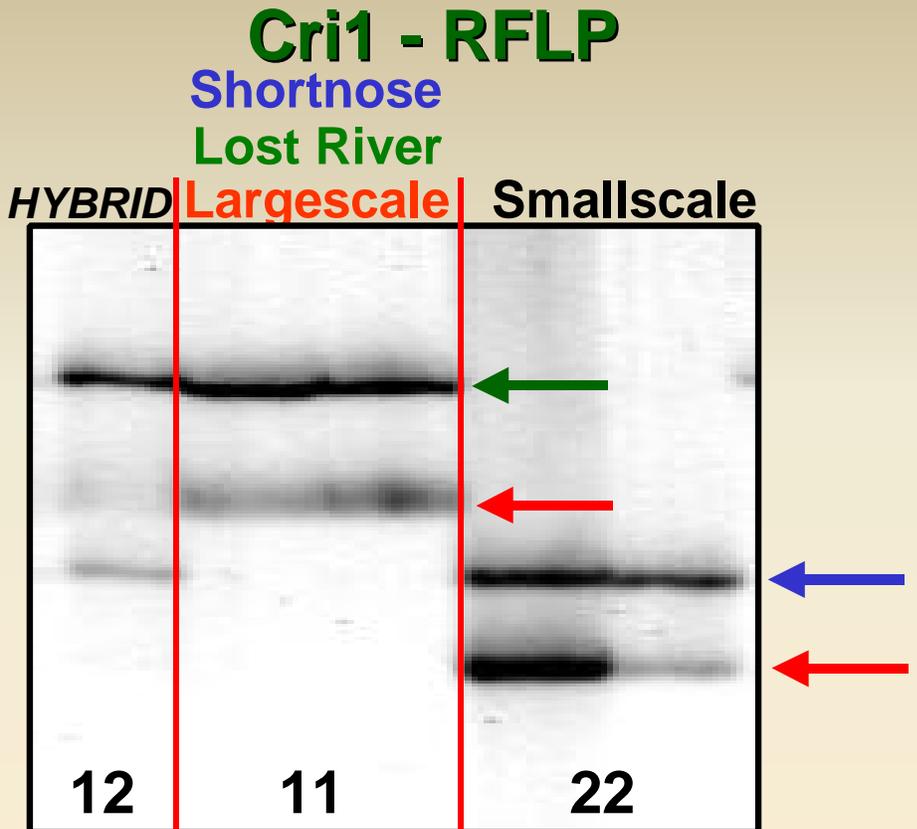


Figure 4. RFLP of Klamath smallscale locus (Cri1). The green arrow indicates the uncut band present in Shortnose, Lost River, and largescale suckers. The blue arrow indicates the cut band present in smallscale suckers. Both bands are present in individuals designated as *hybrids*. Red arrows indicate the homozygous bands present as an artifact of the tetraploid genome.

Cri2

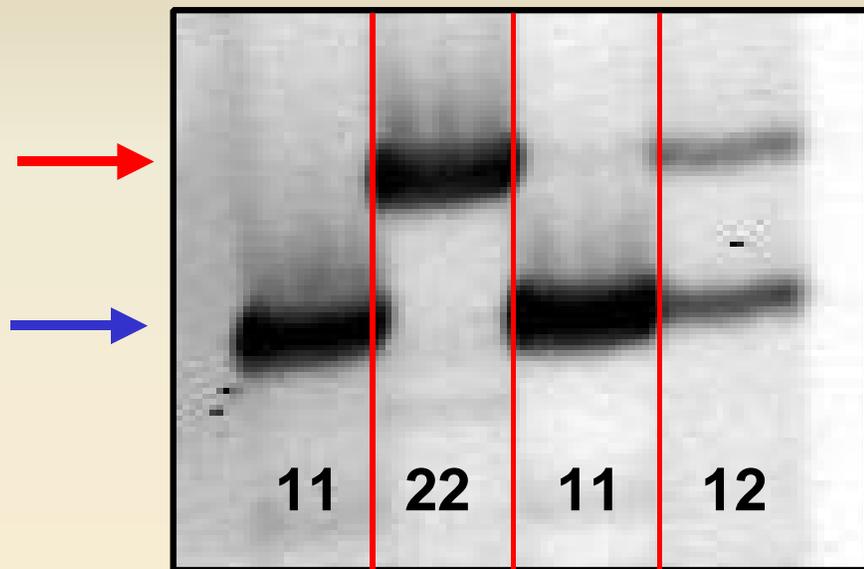


Figure 5. Klamath smallscale locus (Cri2). The blue arrow indicates the 1 allele that is fixed in Lost River suckers and the red arrow indicates the 2 allele fixed in smallscale suckers. Both alleles are present in Shortnose and largescale suckers.

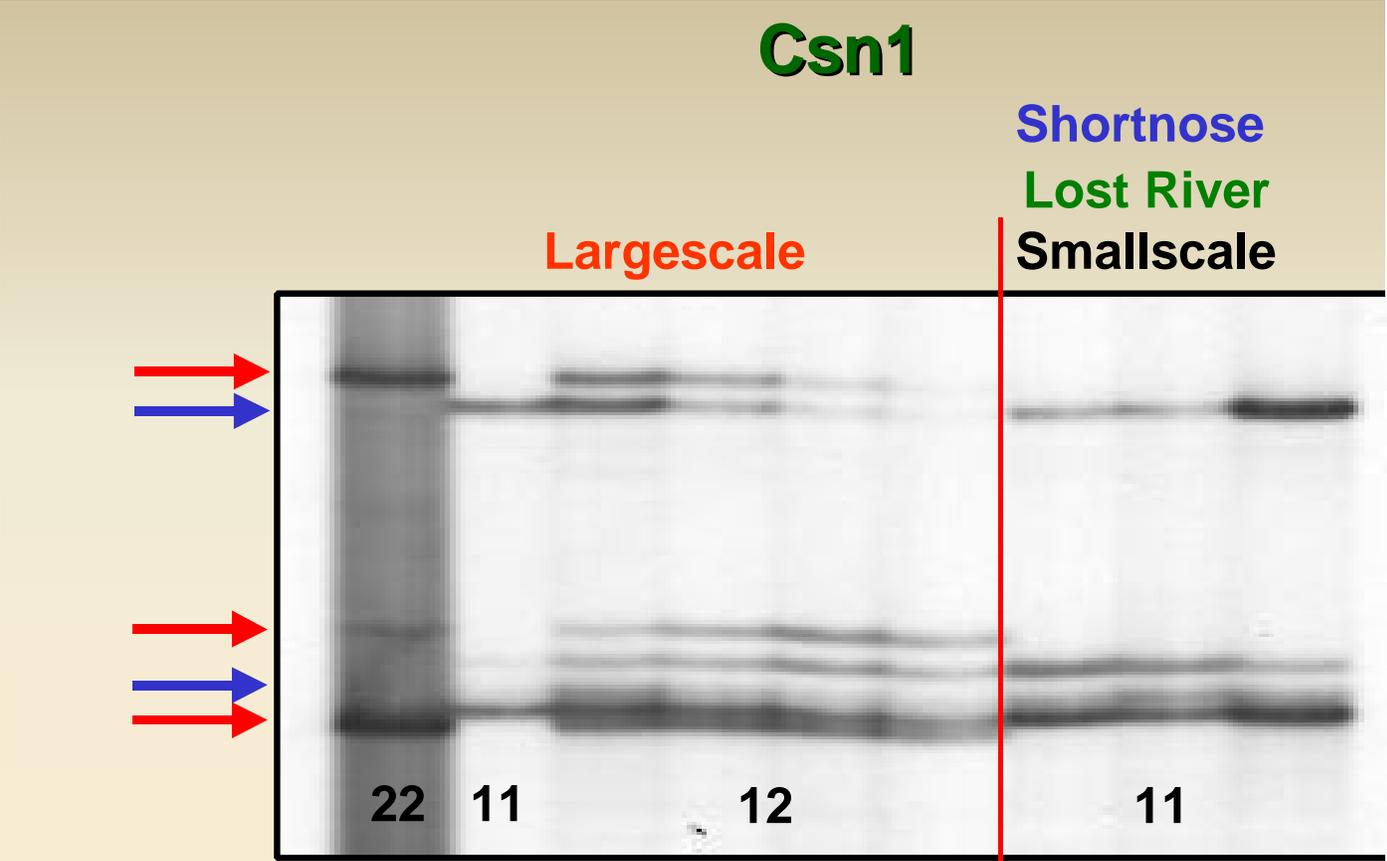


Figure 6. SSCP of Klamath largescale locus (Csn1). The red arrows indicate the 2 alleles present in largescale suckers. The blue arrows indicate the 1 alleles present in Shortnose, Lost River, and smallscale suckers.

Dlu1

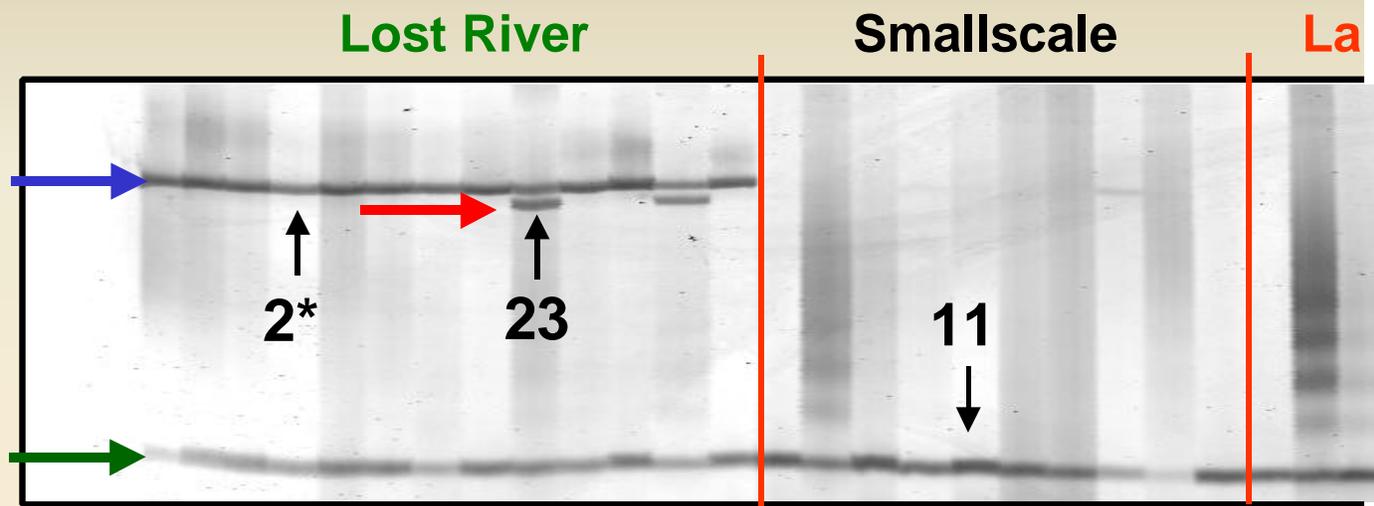


Figure 7. Lost River sucker locus (Dlu1). The green arrow in the control locus amplified in all species (scored as the 1 allele), the blue arrow indicates the 2 allele present in Lost River suckers, and the red arrow indicates the rare 3 allele present in Lost River suckers. Genotype scores are indicated with black arrows.

Dlu 404

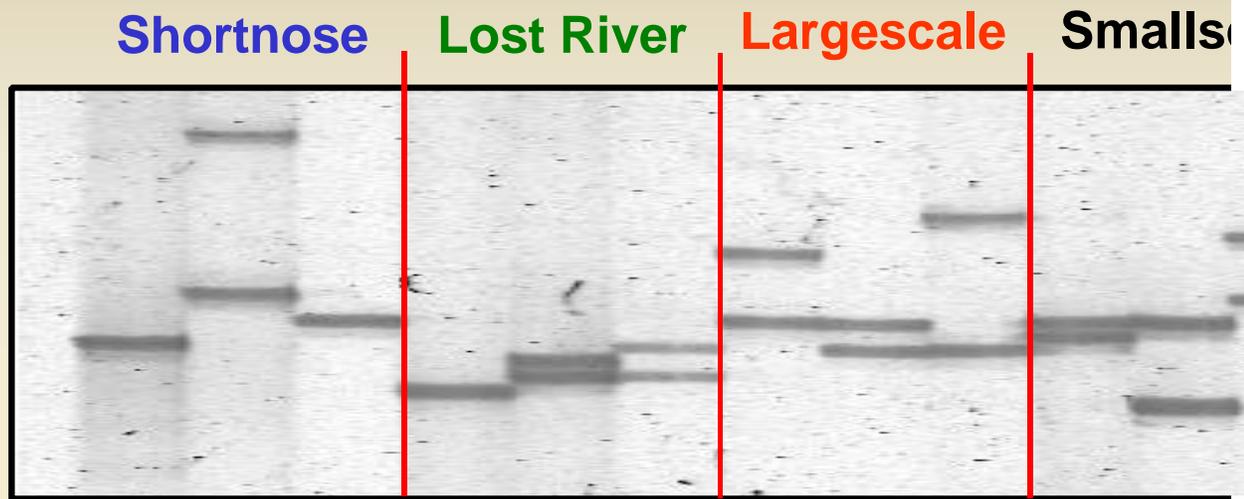


Figure 8. Polymorphic microsatellite with tetrameric (GATA)_n r

Dlu 201

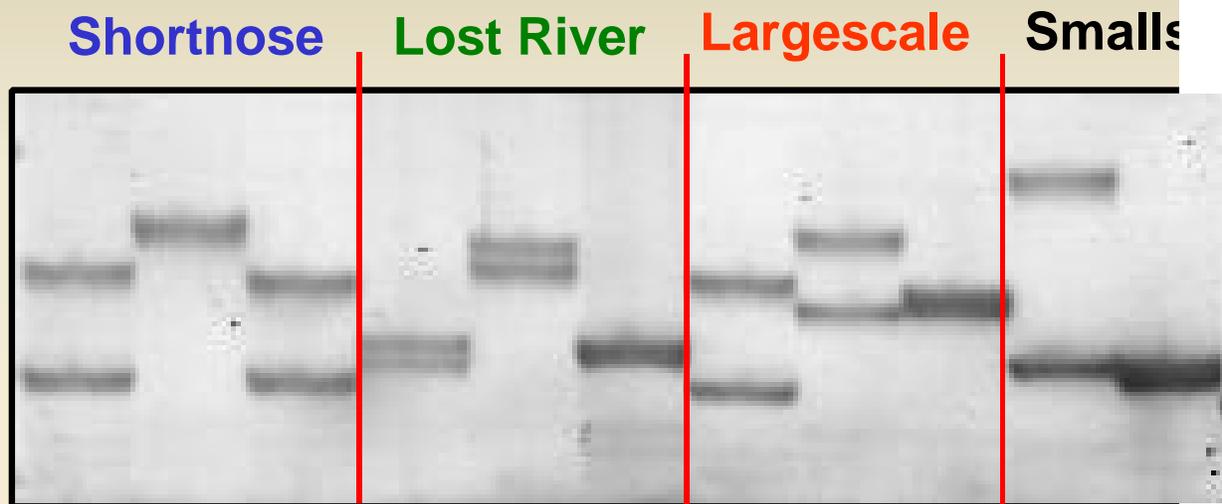


Figure 9. Polymorphic microsatellite with dimeric $(CA)_n$ rep

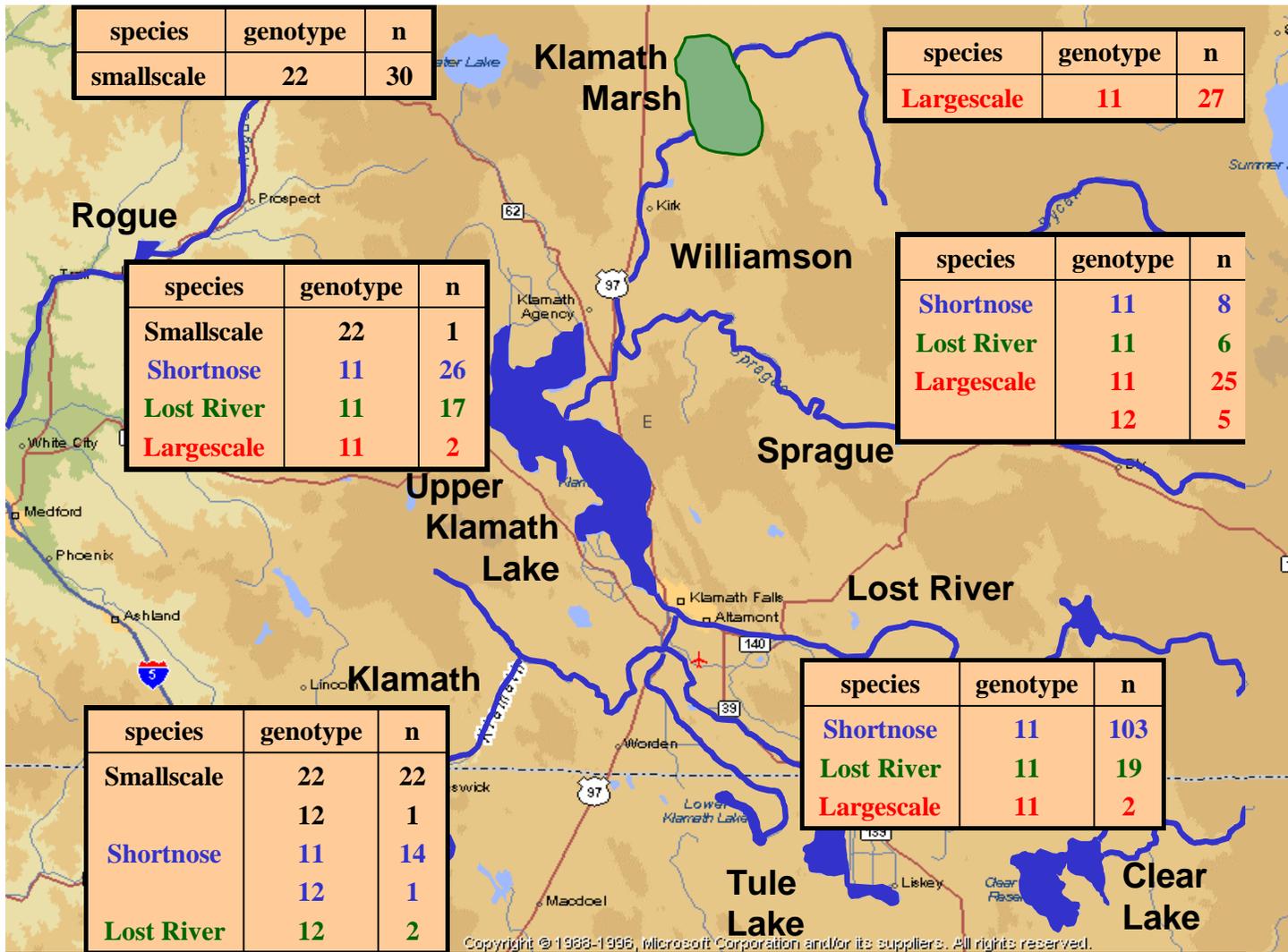


Figure 10. Distributions and numbers (n) of Cril genotypes for all species

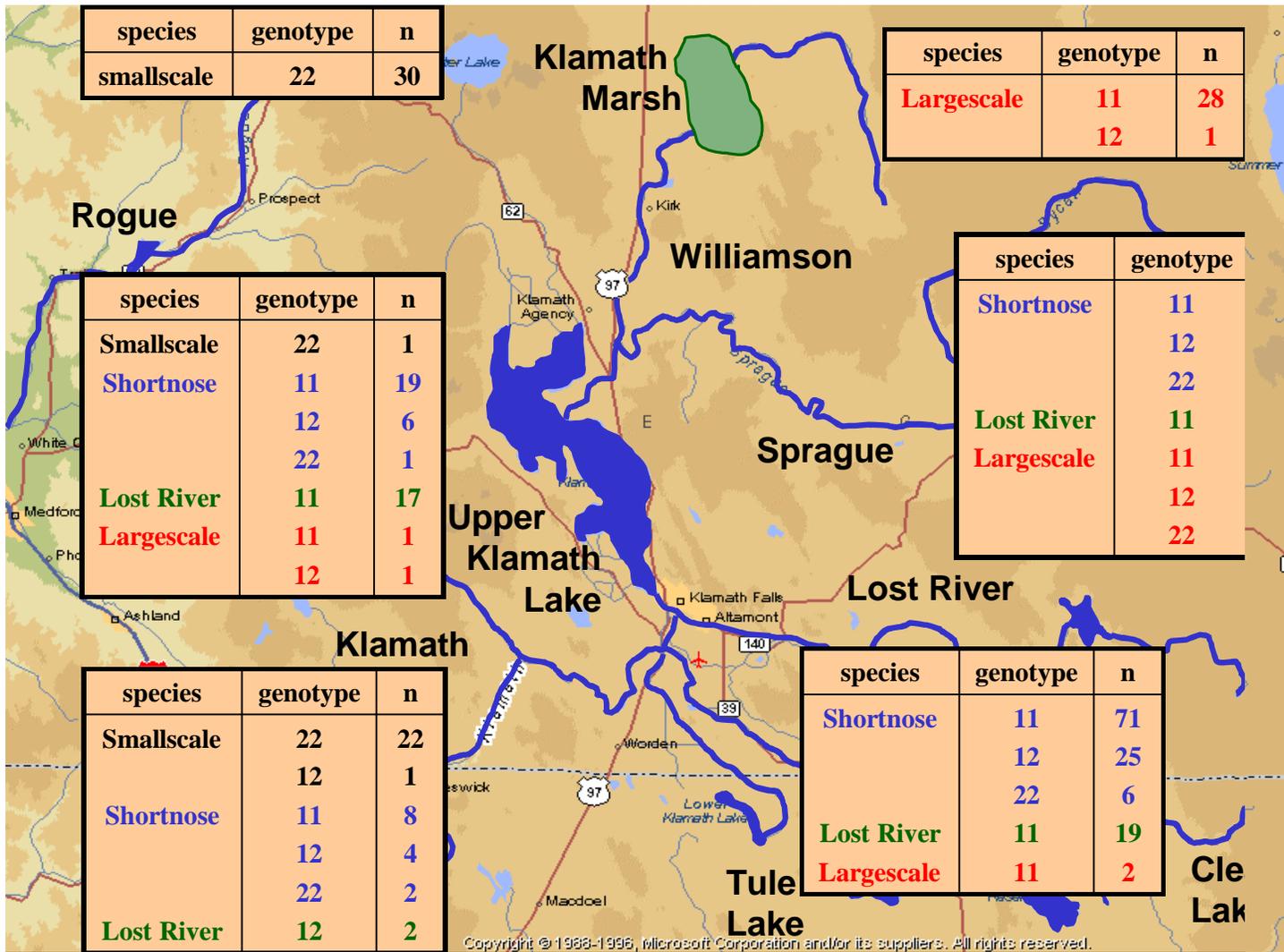


Figure 11. Distributions and numbers (n) of Cri2 genotypes for all species

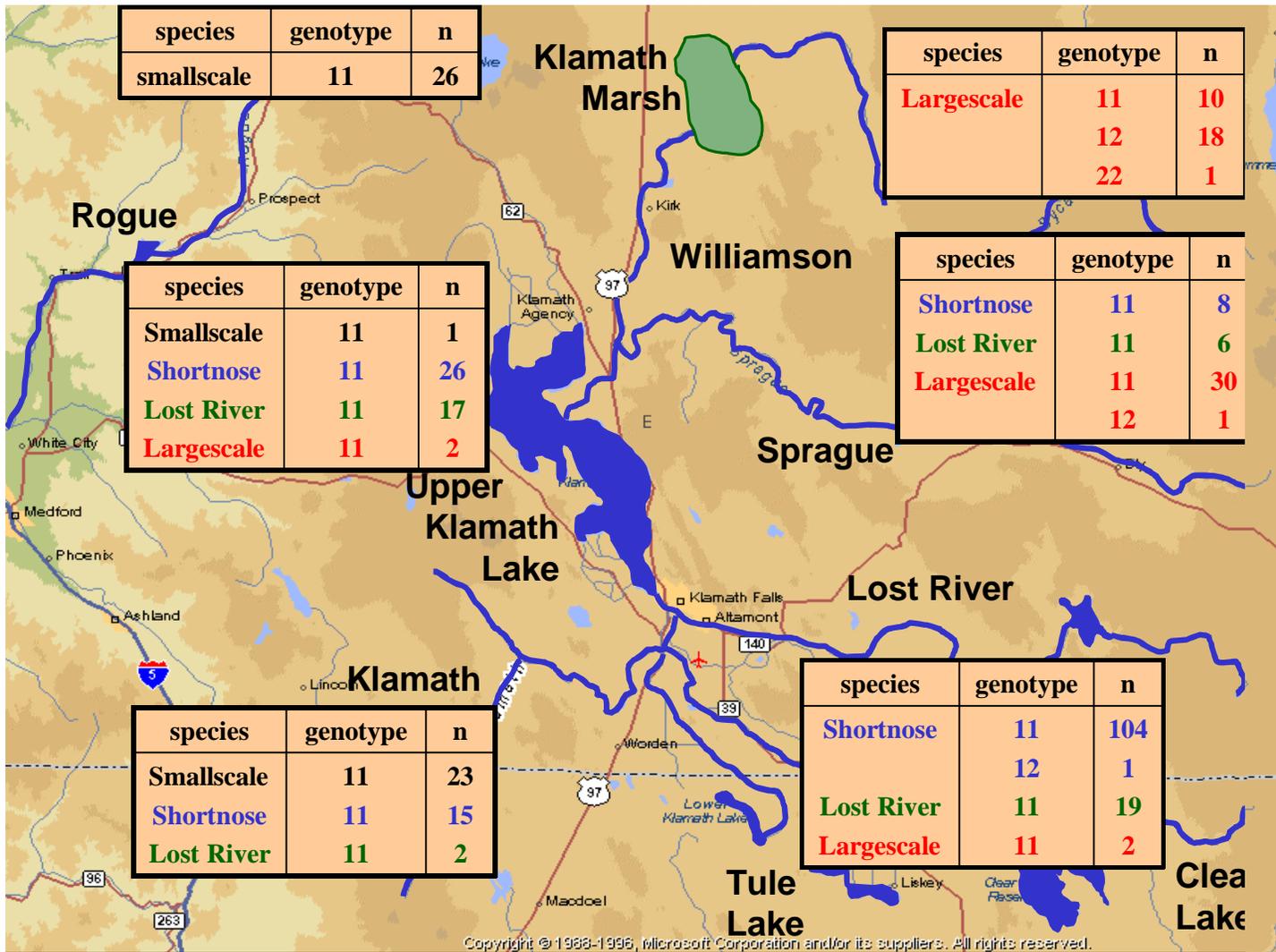


Figure 12. Distributions and numbers (n) of Csn1 genotypes for all species

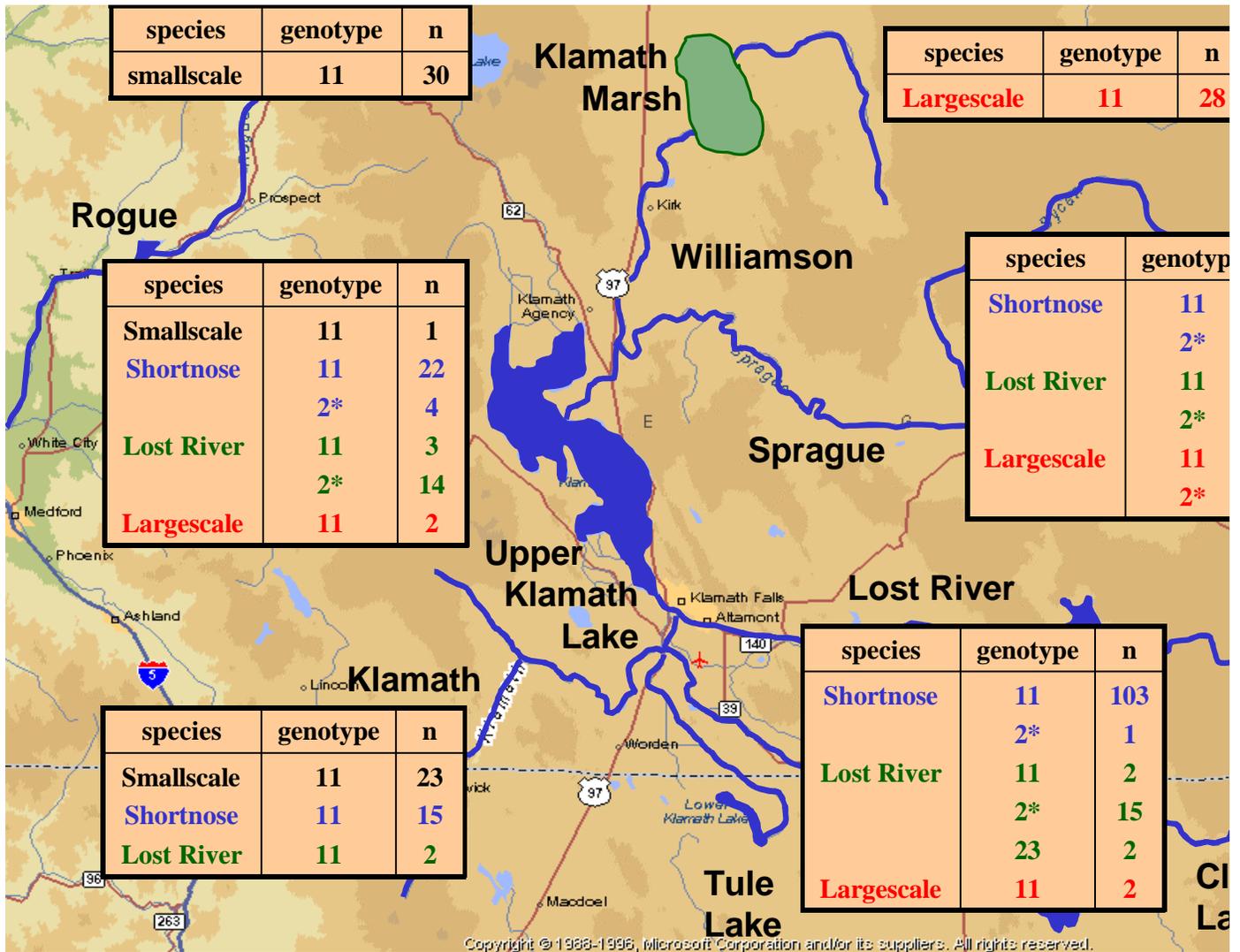


Figure 13. Distributions and numbers (n) of Dlu1 genotypes for all species