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David Wolfe Wagman for the degree of Doctor of Philosophy in Fisheries Science presented on November 12, 2003.

Title: Species Identification Of Klamath Basin Suckers (Pisces: Catostomidae) And An Assessment Of Hybridization Using Anonymous Nuclear Loci

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Douglas F. Markle

Low copy number anonymous nuclear loci were used to search for species markers in four species of Klamath Basin suckers. We sequenced 28 randomly chosen loci representing 10,421 bp; 21 loci were similar to sequences in GenBank. Eight fixed sequence differences were found among Klamath species. Locus 120 contained rare but diagnostic markers for *Deltistes luxatus* and for *Catostomus rimiculus*. Locus 4 also contained three rare but unique sites in *Catostomus rimiculus*. No sequence differences were found between *Chasmistes brevirostris* and *Catostomus snyderi*. Loci 4 and 120 exhibited allele frequency differences between Rogue River *C. rimiculus* and all Klamath Basin suckers. Genotype BB of locus 4 was a fixed diagnostic marker and genotype BB of locus 120 was a frequency dependent marker for Rogue *C. rimiculus*.

Although Klamath suckers represent three genera, very limited variation was found among 10,431 base pairs. We examined phylogenetic patterns of five loci in eleven catostomid genera and 25 species to determine if the homogeneity in the Upper Klamath Basin was due to massive hybridization and introgression or to retention of ancestral sequences. Two loci with no similarity to GenBank sequences (non-coding loci) and three loci with substantial similarity to GenBank sequences (coding loci) gave similar results, providing support for various subfamilies and tribes, more support for eastern genera and little support for western genera. Each

locus was a mosaic of species or population markers, sometimes providing discriminatory power for allopatric populations of a species, such as *C. macrocheilus*, while not discriminating other species. Upper Klamath Basin species were noteworthy in their lack of autapomorphies, but had similar numbers of derived informative sites as other catostomins. Upper Klamath Basin species consistently shared ancestral or equivocal informative sites either with moxostomatins or a variable group of western species and shared derived sites with other western species, especially *C. occidentalis*. The data suggest that Upper Klamath Basin species have retained a largely ancestral genome at these loci. Thus, the failure of this technique to uncover significant variation in Upper Klamath Basin species may be a reflection of their plesiomorphic genome at these loci and not necessarily hybridization.

Species Identification of Klamath Basin Suckers (Pisces: Catostomidae) and an Assessment of Hybridization Using Anonymous Nuclear Loci

By

David Wolfe Wagman

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Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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David Wolfe Wagman, Author

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CONTRIBUTION OF AUTHORS

Dr. Douglas F. Markle was involved in the design, analysis, funding and writing of each chapter.

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DEDICATION

I would like to dedicate this dissertation to my mentors these many years and my mother and father, Drs. Althea and William Wagman, all of whom said I could do it.

Species Identification Of Klamath Basin Suckers (Pisces: Catostomidae) And An Assessment Of Hybridization Using Anonymous Nuclear Loci

Chapter 1

Introduction

Klamath Basin is located within a semi arid high desert between southeastern Oregon and northern California (Figure 1.1). The Basin can be divided into three distinct sub-basins: 1) Upper Klamath Lake (UKL) and its tributary rivers, the Sycan, Sprague and Williamson drain the northeastern reaches of the Klamath Basin; 2) the Lost River sub-basin drains the southeastern corner of the Klamath Basin and includes Clear Lake and Gerber Reservoirs, Tule and Lower Klamath Lakes; 3) the Klamath River drains UKL, south through northern California to the Pacific Ocean.

This isolated Basin has a high degree of biological endemism (Moyle, 1976). Endemic catostomids include Klamath Largescale sucker (*Catostomus snyderi*), Lost River sucker (*Deltistes luxatus*), and Shortnose sucker (*Chasmistes brevirostris*). *Deltistes luxatus* and *Ch. brevirostris* are lacustrine (Scoppettone and Vinyard, 1991) and located in Upper Klamath Lake and Lost River sub-basins with some expatriated individuals showing up in downstream reservoirs of the Klamath River (Dejardanin and Markle, 2000). These fish were once numerous throughout the sub-basins and their decline has led to endangered species listing for both (U.S. Fish and Wildlife Service, 1988). Although not endemic, Klamath Smallscale sucker (*Catostomus rimiculus*) are also present within the Basin. The Klamath *Catostomus* species are not federally protected and are riverine fish, distributed generally at the extremes of the Basin; *C. snyderi* in UKL and Lost River and *C. rimiculus* disjunct in the Klamath River below Keno, Oregon and in the adjacent Rogue River, although a single specimen has been captured in Upper Klamath Lake (Markle et. al., in preparation).

The endangered listings of the lake suckers have fueled attempts to restore these species. Although classified in three different genera, biologists have consistently had

difficulty identifying adult specimens from among the different Klamath sucker species due to the ontogenetic nature of the morphological characters used. Furthermore spawning behaviors have similarities that make identification even more arduous. *Deltistes luxatus* and *Ch. brevirostris* spawn at similar times in early to late spring, in Upper Klamath Lake springs and tributary rivers and in the Lost River sub-basin (Willow Cr.) (Buettner and Scoppettone, 1990). *Catostomus snyderi* spawn earlier and move higher up the tributary rivers of Upper Klamath Lake (Buettner and Scoppettone, 1990) and Barnes Valley Cr., tributary to Gerber Reservoir within the Lost River.

Markle (et al., unpublished data) have completed the most exhaustive morphological analysis on Klamath Basin suckers and based upon morphology, identify four species but find many statistical similarities. Lip features such as the cleft between the lower lip lobes and the lobe extensions past the maxilla are generally reliable field markers for most specimens. Some adult specimen have a combination of features which makes identification difficult. Sub-adult life stages are more difficult to discriminate due to their small size and the ontogenetic changes they experience; physical characters used to distinguish adults may not be fully formed and change with age. Juvenile suckers can be identified using lethal vertebral and gillraker counts (Simon and Markle, 1991). Sucker larvae are very similar morphologically across species and drift downstream and congregate in slower currents and pools. Morphologically, no reliable characteristic can distinguish larvae of the different species.

A further complication among Klamath Basin catostomids is the possibility of hybridization. Hubbs (1955) reported that catostomids hybridize in nature. Miller and Smith (1981) contend that hybridization has occurred between *Ch. brevirostris* and *C. rimiculus* and *C. snyderi*, making identification more challenging. These authors contend that no pure *Ch. brevirostris* populations remain presently (Miller and Smith, 1981). Markle (et al, unpublished data) suggest that specimens used for taxonomic purposes in the past by Gilbert (1898) may no longer be represented in present day populations, particularly in regards to *Ch. brevirostris,* although this may not be due to hybridization but rather the extinction of a unique population (Harriman Springs). Based on morphological characteristics of nose and lip papillae, different investigators (Andreasen,

1976; Bienz and Ziller, 1987; Koch and Contreras, 1973; Miller and Smith, 1981) suggested that species have become hybridized. Introgression is thought to be greatest within Upper Klamath Lake between *Ch. brevirostris* and C. *snyderi* and within extant *Ch. brevirostris* populations hybridizing with *C. rimiculus* in the Klamath River (Miller and Smith, 1981), possibly because of damming modifications.

Markle and Simon (1991) investigated meristic and morphological systematics of Upper Klamath Lake juvenile suckers, and found that of the 950 fish examined, 3% could be the result of inter-species breeding. Although this study found a low level of hybridization, the problem of identification is still confounded by this phenomenon. Miller and Smith (1981) claim that hybridization is most frequent within the Klamath River (J.C. Boyle reservoir) and G. Smith contends that *Chasmistes brevirostris* may have ceased to be a recognizable species (personal communication, ASIH, La Paz, Mex. 2000).

Consequently, the federal recovery plan (U. S. Fish and Wildlife Service, 1993) for the two lacustrine species includes the objective of characterizing and conserving genetic diversity of populations of all four Klamath Basin species. This objective has led to a large study of the morphology and molecular genetics of Klamath Basin catostomids. These research efforts included the Klamath Tribes Hatchery, the Bureau of Reclamation, Klamath Falls, Oregon; Dr. Thomas Dowling of Arizona University, Drs. Greg Tranah and Bernie May of University of California at Davis and Drs. Marty Cavaluzzi, Douglas Markle and myself. Each participant used the same adult samples with a different technical approach. Dr. Dowling investigated mitochondrial (mt) DNA genetics, Drs. Tranah and May used micro-satellites and allozymes, and Drs. Cavaluzzi and Markle used morphology. My approach involved the development of nuclear loci as species markers.

Although the morphological evidence suggests some hybridization, genetic characters provide a means to test for distinction or hybridization amongst Catostomidae. The primary goal of this research was to develop genetic markers for each Klamath Basin sucker species using anonymous nuclear loci. This technique was chosen because we felt it would provide the greatest amount of independent polymorphic loci which would be

definitive species markers, have the ability to detect hybridization and could be used to answer broader phylogenetic questions across the family of suckers.

Anonymous nuclear loci have previously been used to distinguish populations of green turtles (*Chelonia mydas*) (Karl et al. 1992), American oysters (*Crassostrea virginica*) (Karl and Avise, 1993; Hare et al., 1996) and for phylogeny reconstruction of wrasses (Labroidei) (Streelman and Karl, 1997). This technique randomly samples a genomic library for unique low copy sequences, amplifies these sequences for each species using polymerase chain reaction (PCR), and detects polymorphisms by single stranded conformational polymorphism (SSCP) (Orita, et al., 1989; Marklund, et al. 1995) or denaturing electrophoresis (Sambrooke, 1989). In this study, polymorphic sequences or loci were sequenced and aligned to determine variation within species. Three types of data emerged from these analyses; 1) gel electrophoresis patterns of alleles for each locus; 2) allele sizes based on number of nucleotides and 3) sequence analysis of individual sites within loci to determine autapomorphic and synapomorphic relationships. Autapomorphies define each species and the sharing of these unique sequences would identify first generation (F1) hybrids.

Genetically, suckers (family, Catostomidae) are tetraploids (genome 2n = 100 chromosomes) (Uyeno and Smith, 1972) and presumed to have arisen 50 mya (Ferris and Whitt, 1978). Over half (53%) of the genome, however, demonstrates a loss of duplicate gene expression (Ferris and Whitt, 1978; Buth, 1979); thus nuclear loci may be either 2n or 4n. Molecular studies on Klamath Basin suckers have mostly used functional proteins that are subject to potential ontogenetic variation (Koehn, 1969; Andreasen, 1976; Buth, 1979). These authors found no reliable protein patterns to establish species discrimination. Minimal DNA research had been previously performed on Klamath Basin suckers. Harris and Markle (1993) found some unique restriction polymorphisms using mtDNA among the four species. Although these studies indicate that mtDNA may be used to establish a species marker, Hillis and coworkers (1996) advocate using mtDNA only in allopatric situations. Sympatric species, with similar life histories and behaviors have the potential of hybridizing; mitochondrial DNA can rarely resolve hybrids because of the maternal mode of mtDNA inheritance. Given the morphological and behavioral

similarities of these suckers, definitive identification of hybrids would require at least a diploid locus such as those produced by the anonymous nuclear loci technique. Potentially, anonymous nuclear loci could help to establish wide ranging species identification markers that could be used to distinguish all species of Catostomidae.

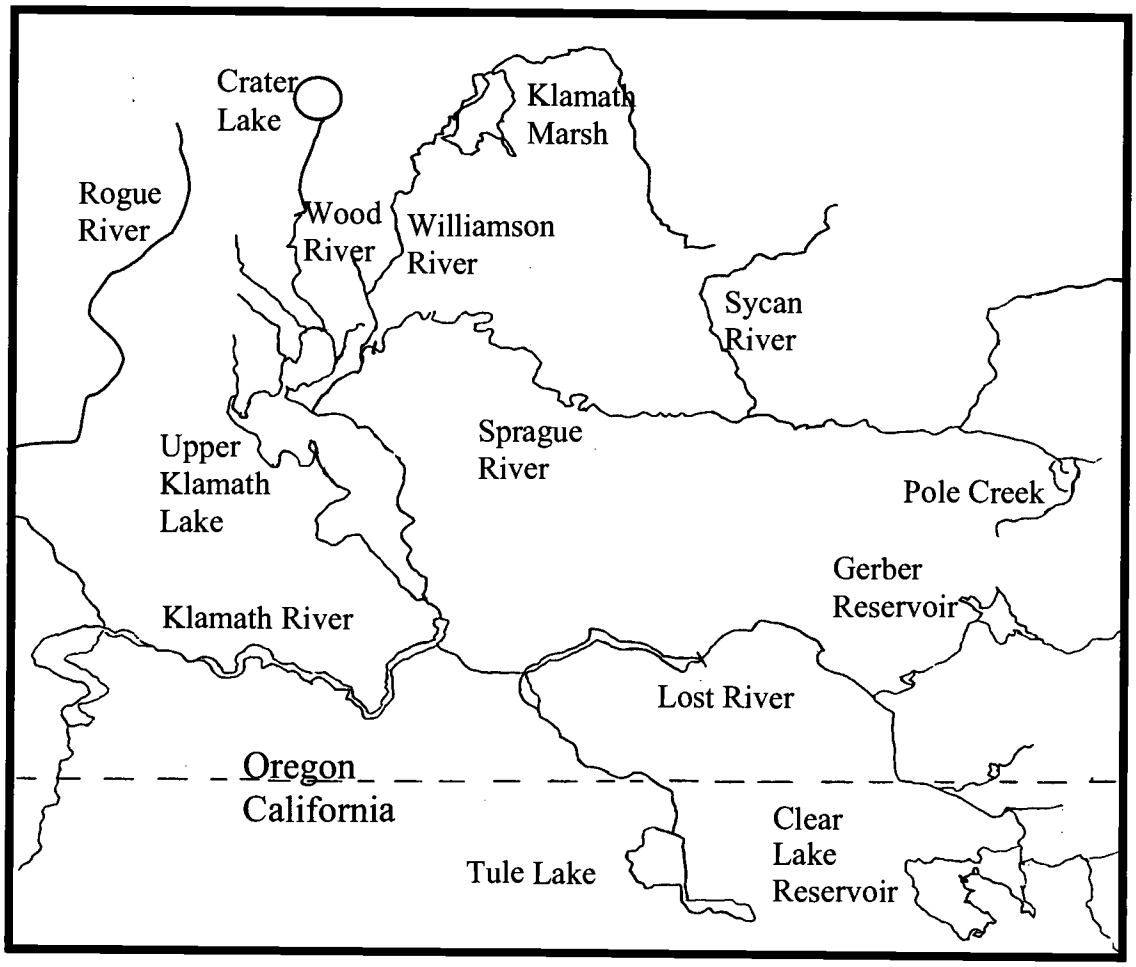

Figure 1.1. Klamath and Rogue River Basins

The goals of this study were: 1) to isolate and characterize variable genetic loci as species markers for Klamath Basin suckers; and 2) to investigate the potential for hybridization among these species.

The following five chapters summarize research using anonymous nuclear loci concerning Klamath Basin and other North American catostomids. Chapter Two describes the anonymous nuclear loci technique and examines their use to search for Klamath Basin sucker species markers. Among 28 loci, representing 10,421 bases of sequence only two frequency differences, and upon sequencing alleles among sucker species, revealed sequence autapomorphies for *C. rimiculus* and *D. luxatus* respectively but no unique genetic difference among *Ch. brevirostris* or *C. snyderi.*

Chapter Three examined the extent of variation using five loci, within eleven catostomid genera and 25 species. Two non-coding monomorphic (39 and 81) and three coding polymorphic (4, 120, 184) loci were used. The level of variation was similar between coding and non-coding loci and monomorphic loci proved to be variable outside of Klamath Basin. Phylogenetic signals among loci were weak resulting in poor resolution of known clades. The best signal was found in locus 81, which resolved Moxostomatini as a monophyletic clade. Western Catostomids had no definition but exhibited autapomorphic variation. Examination of informative bases showed no site saturation and the patterns of sharing usually showed two character states. But allele sharing was not consistent between members of tribes and genera within the family, especially with regards to Upper Klamath Basin species. Using the outgroup criterion of Watrous and Wheeler (1981), these patterns suggest that Upper Klamath Basin species are mostly plesiomorphic and that these loci provide a mosaic of phylogenetic evidence. Although hybridization may be problematic within the Klamath Basin, these loci suggest that the Upper Klamath Basin species' genomes are conservative and these loci could not detect hybridization. Our sampling of the genome suggests that even if the Klamath species originated as allopatric species, when they joined in sympatry, there would be little genetic difference between species to establish reproductive isolation. Ferris and Whitt (1978) hypothesized that the family Catostomidae resulted from a hybridization event between Cyprinidae species (based on chromosomal number, n=50). If the family was formed by hybridization and a modern species retains a largely plesiomorphic genome, it may mean that the ancestral propensity to hybridize has been retained in the modern species.

Chapter Four discusses the potential for bias within the anonymous nuclear loci technique, the conservation of the Catostomidae genome and the genetic relationships of western catostomids.

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Chapter 2:

Anonymous Nuclear Loci as Species Markers in Klamath Basin Suckers (Pisces: Catostomidae).

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Abstract

Low copy number anonymous nuclear loci were used to search for species markers in four species of Klamath Basin suckers. We identified four classes of markers: fixed sequence species differences, strictly diagnostic markers, operationally diagnostic markers, and allele frequency dependent markers. Only the first two could be used independently to identify individuals to species.

We sequenced and examined 28 randomly chosen loci representing 10,421 base pairs; 21 had homologies to gene sequences identified in GenBank. Only eight fixed sequence differences were found among the Klamath species. Locus 120 (aldehyde dehydrogenase) contained four unique sites in *Deltistes luxatus* and a single insertion in *Catostomus rimiculus*. Locus 4 (collagen I) contained three unique sites in *Catostomus rimiculus*. No fixed sequence differences were found between *Chasmistes brevirostris* and *Catostomus snyderi*. Locus 4 and locus 120 exhibited allele frequency differences between Rogue River *C. rimiculus* and all Klamath Basin suckers. The homozygous BB genotype of locus 4 was an operationally diagnostic marker for Rogue *C. rimiculus* but corroboration is currently lacking. The homozygous BB genotype of locus 120 was a frequency dependent marker for Rogue *C. rimiculus* but, again, corroboration is lacking.

Some loci, such as loci 4, 81 and 184, were much better markers for suckers outside Klamath Basin (Wagman and Markle, 2003b). This suggests that the technique is effective in detecting differences and that the genetic similarity detected in Klamath Basin suckers is not an artifact. Genetic similarity may result from different evolutionary processes such as ancestral polymorphism in conservative loci, recent divergence (sympatric speciation), or hybridization. Although these evolutionary processes will have opposite speciation effects, at some time, they will appear identical causing similar genomic and phenotypic changes. Because of the low genetic variability detected by this study we could not identify the evolutionary process involved.

Introduction

Klamath Basin is located in southeastern Oregon and northern California and is composed of three sub-basins. Upper Klamath Lake (UKL) and its tributary rivers, the Sycan, Sprague and Williamson drain the northeastern reaches of the Klamath Basin and flows to the Klamath River. The Lost River sub-basin is a closed drainage without passage to the ocean, includes Clear Lake and Gerber Reservoirs, Tule and Lower Klamath Lakes, and drains the southeastern corner of the Basin. Historically, water from these sub-basins remained separate (Figure 1.1) while only during flooding does water from Klamath River (below UKL) flowed into the lower Klamath Lake of the Lost River sub-basin (Rykbost and Todd, 2002).

Upper Klamath Basin contains three endemic species of catostomids: Lost River sucker (*Deltistes luxatus*), shortnose sucker (*Chasmistes brevirostris*), and Klamath largescale sucker (*Catostomus snyderi*). These species are generally sympatric throughout UKL and Lost River sub-basins. A fourth catostomid, Klamath smallscale sucker (*Catostomus rimiculus*) resides in the Rogue River Basin and the Klamath River sub-basin where it becomes sympatric with expatriated *Ch. brevirostris*, *C. snyderi* and *D. luxatus* from the upper sub-basins (Desjardins and Markle, 2000). *Deltistes luxatus* and *Ch. brevirostris* are lacustrine, using both lake springs and tributary rivers for spawning, and were once numerous throughout the UKL and Lost River sub-basins (Buettner and Scoppetone, 1990). Their population decline led to endangered species listings (U.S. Fish and Wildlife Service, 1988; 1989). *Catostomus snyderi* and *C. rimiculus* are riverine fish, distributed more or less at the extremes of the basin, with *C. snyderi* in the UKL and Lost River sub-basins' rivers (Buettner and Scoppetoni, 1990) and *C. rimiculus* in the lower Klamath and Rogue rivers. However, a single specimen has been captured in Upper Klamath Lake (Markle et al., in preparation).

As a result of the endangered species listings, efforts are underway to restore the lake suckers. These efforts are hampered by the lack of life stage independent characteristics that identify each species. Adult forms of the four species can be recognized by morphology, each with recognizable geographic forms (Markle et al. in

preparation). Overlapping ranges and the capture of suspected adult hybrids can make adult identification difficult. The adult morphological characters used to distinguish these species can not fully differentiate juveniles or larval life forms. *Deltistes luxatus* juveniles can be identified with the lethal technique of counting gillraker and vertebrae (Markle and Simon, 1993). Larvae are extremely delicate and cannot be identified by these ontogenetic characteristics because they are not fully developed. Characters not subject to ontogenetic variation would improve the identification of suckers at all developmental stages. Genetic characters may contribute to the resolution of questions regarding taxonomic identification at all ontogenetic stages, population structure, and hybridization.

Sucker genetics are complex. Catostomids are tetraploids (genome 2n = 100 chromosomes) and as a "group evolved from an ancestor similar to the minnow" (Uyeno and Smith 1972). In addition "catostomids have twice the number of chromosomes and cellular DNA content of related families of Cypriniformes, e.g. the diploid species of cyprinids" (Ferris and Whitt, 1978). Over half (53%) of the genome, however, demonstrates a loss of duplicate gene expression (Tsoi et al., 1989; Ferris and Whitt, 1978; Buth, 1979). Thus nuclear loci may be either 2n or 4n.

Previous studies used molecular techniques to distinguish catostomids. Allozymes were used by Buth (1978; Buth et al. 1987, Crabtree and Buth 1987) to distinguish sucker species and by Ferris and Whitt (1978) to investigate catostomid phylogeny. This method requires fresh frozen tissues from several organ systems and multiple developmental stages. The small size of larvae makes this technique difficult. Tranah and May (pers. comm, 1999) used allozymes to distinguish Klamath Basin adults but found very low variation between species. However because of poor tissue quality only a few loci were examined. Past molecular studies on Klamath Basin suckers have mostly used functional proteins that are subject to potential ontogenetic variation (Koehn, 1969; Andreasen, 1976; Buth, 1979). These studies found no reliable protein patterns to establish species identifications.

Mitochondrial DNA (mt DNA) analysis has been used with some success for Klamath Basin suckers. A preliminary restriction fragment length polymorphism (RFLP) study of mtDNA in Klamath Basin suckers by Harris and Markle (1993) found that

Upper Klamath suckers share a majority of restriction patterns, indicating a highly conserved mitochondrial genome across genera and species. Dowling used two mitochondrial genes and found 62 composite haplotypes that segregated into four clades, but some clades contained more than one species and some species were in more than one clade (Markle et al. in preparation). Although these studies indicate that mtDNA may be a useful species marker, Hillis et al. (1996) advocate use of mtDNA only in allopatric situations. Sympatric species, with similar life histories and behaviors, have the potential of hybridizing, and mitochondrial DNA can only establish maternal inheritance.

A cooperative effort to establish genetic and morphological species markers in Klamath Basin catostomids was begun in 1992 and involved the Klamath Tribes, the Bureau of Reclamation, Arizona State University, University of California at Davis, and Oregon State University. Each researcher used the same samples with a different methodological approach, including mtDNA, allozymes, genomic micro-satellites, morphology, and anonymous nuclear loci.

The goal of this study was to isolate and characterize anonymous nuclear loci for each Klamath Basin sucker species. The anonymous nuclear loci technique has been used to distinguish populations of green turtles (*Chelonia mydas*) (Karl et al. 1992), American oysters (*Crassostrea virginica*) (Karl and Avise, 1993; Hare et al., 1996) and for phylogeny reconstruction of wrasses (Labroidei) (Streelman and Karl, 1997). This technique randomly samples a genomic library for unique low copy clones, amplifies the clones for each species using polymerase chain reaction (PCR), and detects polymorphisms by single stranded conformational polymorphism (SSCP) or denaturing electrophoresis (Orita et al., 1989; Marklund et al. 1995; Sambrooke, 1989). Polymorphic clones or loci are sequenced and aligned to determine variation in the species.

Materials And Methods

Fish Samples

Adult Klamath Basin (*Ch. brevirostris, D. luxatus, C. snyderi. C. rimiculus*) and Rogue River suckers used for this study were collected by U. S. Bureau of Reclamation (BOR) and are listed in Appendix 1. Eight specimens were sampled non-lethally for tissues and of the original 333 specimens, 296 were available for this study. Tissue samples were numbered consecutively and herein referenced with the acronym AR. Carcasses of 325 specimens were deposited in the Oregon State University Fish Collection (catalog numbers are referenced with the prefix OS). Sampling attempted to target specimens from suspected spawning groups in spring and early summer, 1993-1994, but over half (183, 55%) were collected outside the spawning seasons from August to November, 1993 (Upper Klamath Lake samples) and could constitute mixed spawning groups.

DNA Preparation for Cloning

A genomic library was constructed from a *Ch. brevirostris,* AR-041 (OS 015963-b), captured in the lower Williamson River. DNA was isolated from muscle tissue following Taggard et al. (1992) with two slight modifications; DNA was precipitated overnight and pelleted by centrifugation. Total DNA (10 ug) was restriction digested with *Sau* 3AI (Promega, Madison, WI) following the manufacturer's directions. Restricted DNA was size fractionated on a 0.8% agarose gel containing ethidium bromide next to a size ladder made from *Hin* DIII-restricted Lambda Phi X 174 (Sigma, City, ST). The gel was visualized using ultraviolet (UV) light and the region between 300-600 bp was excised. DNA was eluted and 2.1 ug of restricted DNA was recovered.

Vector DNA Preparation

Plasmid pUC18 (Promega) was restriction digested with *Bam* HI (Promega). *Bam*H1 and *Sau*3A1 have similar restriction sequences and are compliments. Restricted DNA was extracted with Tris saturated phenol (pH 7.6) and chloroform (24:1 isopropanol) and the aqueous phase collected and precipitated. DNA was centrifuged and re-suspended in 17 ul water. The vector was de-phosphorylated using calf intestinal

phosphatase (Promega, following manufacturer's directions). This reaction was precipitated and stored at –73 ^{0}C.

Ligation Reaction

Ligation of sucker restricted DNA to dephosphorylated pUC18 vector followed Sambrooke et al. (1989). Two ligation protocols were used, 1:1 and 1:3 ratios of vector arms to insert DNA arms. Each 20-ul reaction contained either 25 ng or 75 ng of insert DNA, 50 ng of dephosphorylated, *Bam*H1 restriction digested pUC18 DNA, ligation buffer (Promega), 1 unit of T4 ligase (Promega), and water. Ligation was completed after incubating for 3.5 hours at room temperature (RT). The ligation reaction was mixed with competent DH5α *E. coli* and transformation was accomplished by incubating the reaction on ice, followed by a heat shock. Luria Broth (LB) was added and incubated for 1 hr at 37 °C with shaking at 200 rpm. Ampicillin inoculated LB plates were prepared with IPTG and X-Gal for blue-white color determination. The two ligation libraries were then plated in densities of 10, 25 and 100 ul per plate. Plates were inverted and incubated overnight. Glycerol stocks of each library were made and frozen. The 1:1 and 1:3 libraries were approximately 50 % recombinant and contained an estimated 412 and 2012 recombinant clones, respectively. Clones were randomly chosen and grown for DNA isolation.

Clone DNA Isolation

Over 200 recombinant clones were harvested and grown overnight in LB inoculated with ampicillin. Sterile glycerol stocks of each culture were made and frozen. The remaining culture was used for a mini-prep DNA isolation using the STET protocol of Sambrooke et al. (1989). DNA was precipitated with 100% isopropanol and pelleted by centrifugation. The DNA pellet was air dried and resuspended in sterile water. All clone DNA preparations were quantified by spectrophotometry.

Clone DNA Amplification

To insure that the insert DNA was in the correct size range, insert DNA was amplified by PCR using pUC18 primers and visualized on ethidium bromide containing 1.1% agarose (BRL) gels. Amplified clones were sized based on comparison to *Hin*D III-restricted Lambda Phi X 174 size markers. Only clones in a size range of 300-700 bp were used for further analysis because this size corresponds to a single sequencing run on the ABI automated sequencer at Oregon State University. PCR reactions of 25 ul were optimized using master mix solutions set for four concentrations of $MgCl_2$ (1, 2, 3, 4 mM). Master mix solutions contained 10 X polymerase buffer (Promega), 5 mM dNTP (BRL), the appropriate amount of 25 mM $MgCl_2$ and water. The appropriate volume of master mix solution was aliquoted into an iced sterile tube, forward and reverse primers and *Taq* polymerase (Promega) were added. Template DNA was added and the solution mixed. A three-step PCR method was used: an initial 3 minute de-naturation at 94 °C for one cycle; and a three-step program of 45 second de-naturation at 93 °C, 45 second annealing, and 60 second extension at 72 °C (annealing temperatures varied with each clone and ranged between 45 and 58 °C; Appendix 2). The PCR reaction was optimized for each clone by varying the $MgCl_2$ concentration and annealing temperatures experimentally.

Southern Blot Preparation

It is important to use low copy DNA in order to minimize confusion resulting from closely related genes. We used a Southern slot blot method described by Katatos, et al. (1979) to survey the copy number of the insert DNA within the sucker genome. The volume that would contain 1 ug of insert DNA was calculated, taking into account insert size and measured concentration of the preparation. This was used to qualitatively examine copy number of each insert from the *C. brevirostris* genome. MSI nitroPure (City, ST) nitrocellulose blots were made using a vacuum slot blotting system, according Sambrooke et al. (1989). The DNA was linked to the membrane by an Ultra Lum (City, ST) ultraviolet Crosslinker (UVC 515) at 1200 X 100 microjoules. Blots were air dried

and stored at – 20^0C . Each blot contained a positive control of total sucker DNA and a negative control of pUC18 DNA.

Probe Preparation

*Sau*3A1 digested DNA (315 ng) was labeled using α–[^{32} P] –dCTP (NEN, Massachusetts) and the Multiprime system^{TM} from Amersham (Amsterdam). Unincorporated label and dNTPs were eluted from the labeled probe DNA using Elutip-d (Schleicher and Schuell, New York) following the manufactures procedures. The resulting probe had a radiation level of 162,970 cpm/ul and a specific activity of 92.7 uCi.

Southern Blot Hybridization

Individual blots were layered between fine meshed sheets, placed into a hybridization bottle and pre-hybridized 1 hour at 65 °C in 200 ml 5X SSC, 1% sarcosyl in a Biometra hybridization oven. Probe DNA was denatured for 5 minutes at 95°C and placed on ice. Pre-hybridization solution was replaced with fresh 5X SSC and 1% sarcoyl and contained 3 X 10^6 cpm labeled probe. Hybridization occurred overnight at 65 °C. Southern blots were washed twice for 30 minutes each in 200 ml of 3 X SSC, 0.5% sarcosyl at 65 °C and additionally washed twice in 200 ml of 3 X SSC for 30 minutes. Blots were air dried and exposed on Kodak X-Omat AR film at –73°C overnight. Films were developed using an automated X-Omat developer.

Low copy number clones were 95% of all clones, identified by their weak signal on the film. Low copy number clones were randomly chosen for sequencing and primer selection.

DNA Preparation for Sequencing

Clones were grown in ampicillin-inoculated LB medium overnight. DNA was isolated with Fisher's Wizard Midiprep DNA isolation kit. DNA was quantified by

spectrophotometry and diluted appropriately for the automated sequencer depending on insert size. Standard forward and reverse pUC18 sequencing primers were used for sequencing. Raw sequences were compared using the program SeqEd (version 1.0.1. Applied Biosystems, Inc.) and aligned by eye. Clean clone sequences were searched for open reading frames (ORF) and stable primers located using the Macintosh-based program Oligo (Version 4.0, National Biosciences Inc.) with the basic default parameters. Coding regions were avoided, primer sites were located distant from any ORF to maximize length, efficiency and amplification success of the product, and approximately 50 bp from the start and end of the insert DNA. Primer synthesis occurred at the Center for Gene Research (Oregon State University) yielding 25-40 nmoles of each forward and reverse specific primer. Primer stocks were diluted to 50 pM in water and frozen. Amplification stocks of 20 pM were prepared and used routinely for PCR. Primers are listed for each clone in Appendix 2.

Surveying Genetic Variation

One individual from the three Upper Klamath sub-basin sucker species was used to find sequence differences between the species: *D. luxatus* (OS 015922) from Upper Klamath Lake, *Ch. brevirostris* (OS 015963-b) from the lower Williamson River, *C. snyderi* (OS 015900-f) from the upper Williamson River. Due to reclassification of the samples, *C. rimiculus* (OS 015908-a,b,d and OS 0159009i) from Topsy Reservoir were sequenced for loci 4 and 120 only. None of these individuals showed any morphological or genetic evidence of hybridization in subsequent studies. Each fish was amplified with a locus's primers. Total PCR product was visualized on 2% agarose gels containing ethidium bromide (2.5 ul/100 ml gel 10 mg/ml). Clean PCR products were cut from the gel and eluted using 0.45 um Ultrafree-MC filter units (Millipore) following the manufacturer's protocol. Eluted DNA was precipitated with 1/10th of a volume of 3M sodium acetate and 2.2 volumes of 95% ethanol (Sambrooke et al., 1989) and re-suspended in 25 ul water. Quality of re-suspended DNA (5 ul) was determined by electrophoretic separation on a mini-2% agarose gel and quantified by comparison to a

Hin DIII-restricted lambda PhiX 174 standard ladder. DNA was sequenced in both directions using the PCR primers and the sequences were aligned using SeqEd.

Once variation was detected either denaturing polyacrylamide gel electrophoresis (PAGE) or single stranded conformational polymorphism (SSCP; Glenn, 1996; Marklund et al, 1995) analysis was used for Klamath species survey. Denaturing PAGE was used for detection of size variation. These gels were made of Long Ranger™ acrylamide (FMC) following the manufactures' directions using a square tooth 55-sample comb (Appendix 3). Amplified DNA from individuals was mixed with 100% formamide in a 1.4:1 ratio. Samples were denatured at 83°C and placed on ice. Wells were loaded with 5 ul sample into pre-warmed (40-50 °C) gels and run for 2-3 hours at constant power of 50 watts at room temperature (RT). Gels were disassembled, overlaid with a gel-staining gasket (Appendix 3) and secured by binder clamps. The gel was placed in the dark and stained with Sybr Gold (Molecular Probes, Eugene, OR) for 30 minutes. Gels were inverted and illuminated on an UV light source and photographed with a Kodak MP-3 system using Polaroid 667 film.

Gels for SSCP were made from MDE acrylamide (FMC) following the manufactures' protocol. Gels polymerized overnight with a square tooth 55-sample comb and were loaded at RT. Samples were prepared as for denaturing PAGE, 5 ul were loaded into each lane and the gel was run for 5 minutes at 50 watts to allow the samples to fully penetrate the gel matrix. The gel was disconnected from its power source and moved into a 4 °C cabinet. Gels ran for 20-24 hours at 4 watts per gel. Gels were stained and photographed as in denaturing PAGE.

Alleles were scored from photographs for eight polymorphic loci. When the adult gel patterns were compared to early life stages it became apparent that contamination from the A allele of locus 184 had occurred in the PCR amplification. Juveniles and larvae exhibited the homozygous BB genotype (50 and 32% respectively, data not shown) but this genotype was not found in the adults initially (Figure 2.1). Typically genotypic patterns for locus 184 have either two bands of equal intensity (AA), three bands of equal intensity (AB) or a doublet which appears as a wide single band (BB) (Figure 2.1). Contaminated PCR samples would include bands that stain in a less intense

manner (Lane 22, Figure 2.1). As a result, only bands of equal intensity were scored for the final analysis.

Basic population statistics were generated using web based Genepop program (Raymond and Rousset, 1995). Statgraphics Plus (version 3.0, 1997) was also used for statistical comparisons.

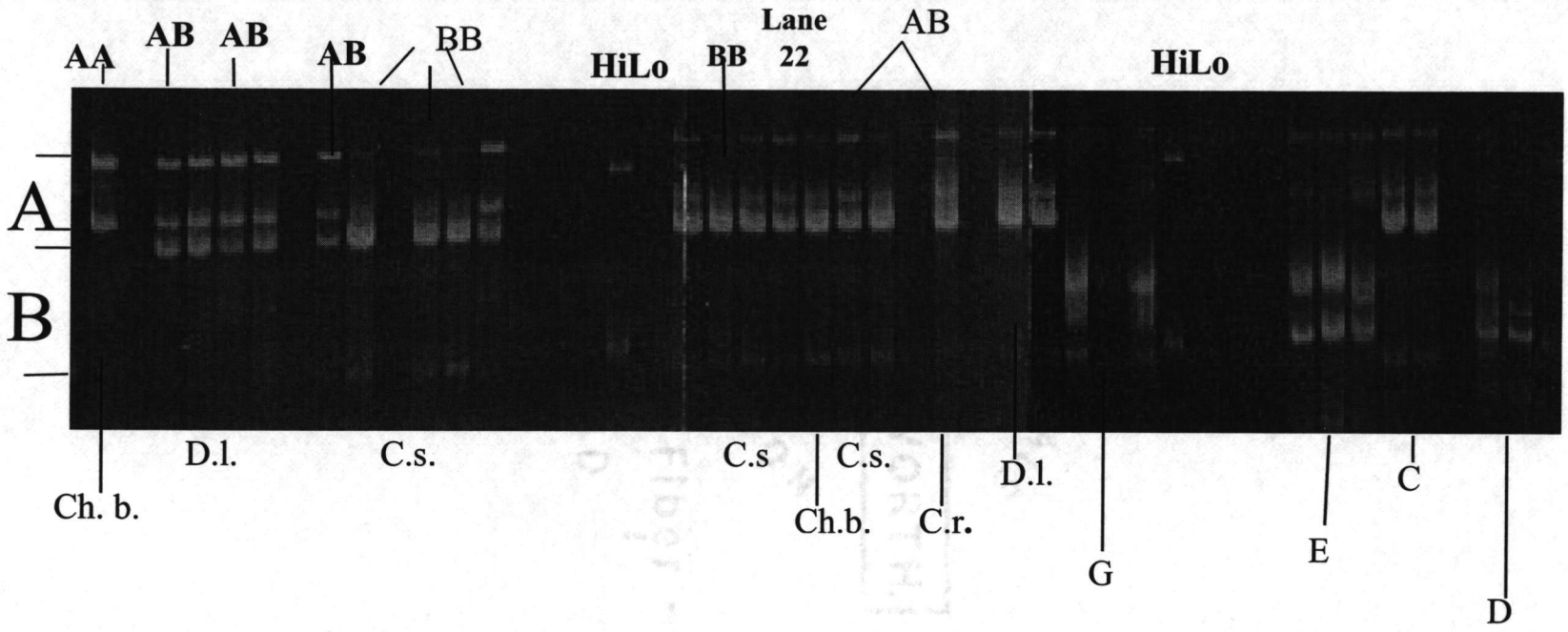


Figure 2.1: SSCP genotypes of Klamath Basin and outgroup catostomids for Locus 184. Allele A is found in all suckers due to PCR cross contamination; only bands of equivalent staining intensity were scored. AB genotypes had bands of equal staining intensity between the alleles, AA genotypes always had an absence of the B allele and BB genotypes were scored when the B allele intensity was greater than that of the A alleles. Klamath Basin suckers; *Chasmistes brevirostris* (Ch.b.), *D. luxatus* (D.l.), *C. snyderi* (C.s.), *C. rimiculus* (C.r.). Alleles C, D, E, and G are found in catostomids from outside of Klamath Basin. Allele C is found in *C. occidentalis;* Allele D is found in *C. macrocheilus* (Hood R.); Allele E is found in *X. texanus;* Allele G is found in *C. macrocheilus* (Siuslaw R.).

Results

Genomic copy number was qualitatively assessed for 202 clones. Ninety five percent of the clones were determined to have a low copy number (data not shown). The high number of low copy number clones was expected. Karl et al. (1992), Karl and Avise (1993), and Streelman and Karl (1997) report low copy clone percentages of 91, 79, and 65% respectively. These reports distinguished single copy clones from low, moderate and high copy number loci. Our best estimate is qualitative and included only low, moderate and high copy number designations. Only low copy number clones were used in our analysis.

Each clone was referred to as a genetic locus, identified by an arbitrary numbering system and/or by the name of the coded protein (Table 2.1). The inserts of forty-four clones were sequenced for primer selection. Appendix 2 contains the GenBank accession number, clone and PCR sizes, PCR conditions and primers, clone sequence and the protein and/or nucleic acid identities as determined from Genbank for each clone. Sixteen clones were either difficult to optimize for PCR in all species, were duplicates of another clone, or were not used for primer selection. Twenty-eight clones were successfully amplified and sequenced in both directions for one to three individuals from the three Upper Klamath sub-basin species. Four individuals were sequenced for *C. rimiculus* for two loci.

Table 2.1: Twenty-eight anonymous nuclear loci used in this study. Search type refers to either a GenBank protein (amino acids, aa) search using the Blastx program or a nucleotide (nt) search using the Blastn program.

Locus	PCR Size (bp)	residues	Sequence identities and similarities
2	501	20/22 aa	unknown protein, *Mycoplasma genitalium*
4	454	16/27 aa	collagen F1 - freshwater sponge, *Ephydatia muelleri*
8	543	14/36 aa	NADH dehydrogenase subunit 8 *Crithidia oncopelti*
9	348	22/74 aa	13KD protein, *Saccharomyces cerevisiae*
9		39/41 nt	diphtheria toxin repressor gene, *Corynebacterium diphtheriae*
13	320	17/32 aa	probable membrane protein, *Saccharomyces cerevisiae*
19	421	40/125 aa	serine/threonine kinase, *Homo sapiens*
39	338	32/36 nt	ependymin precursor gene, *Notropis chrysoleucas*
54	286	13/39 aa	hypothetical protein HI1195, *Haemophilus influenzae*
61	215	28/30 aa	myelin basic protein kinase-like protein, *Xenopus laevis*
61		75/88 nt	ERK2, exon 1, *Mus musculus*
63	278	23/24 nt	cosmid T10B9, *Caenorhabditis elegans*
67	305	17/49 aa	potassium channel, *Rattus norvegicus*
76	190	21/34 aa	KIAA0335, *Homo sapiens*
79	249	16/40 aa	pyruvate, orthophosphate dikinase, *Thermotoga maritima*
81	261	20/20 nt	cadherin-7 mRNA , *Homo sapiens*
81		20/20 nt	mRNA for guanylyl cyclase C, *Xenopus laevis*
82	262	34/34 nt	Rat transferrin receptor mRNA, 3' end.
88	482	19/28 aa	Hypothetical protein RV2035, *Mycobacterium tuberculosis*
94	372	23/64 aa	AbcA , *Dictyostelium discoideum*
		147/156nt	transposon Tsn1-3 transposase pseudogene, *Salvelinus namaycush*
107	543	23/85 aa	Hypothetical protein, len: 1676 aa, *Plasmodium falciparum*
117	498	21/21nt	Human DNA sequence PAC 272J12, chromosome 22q12-qter
119	460	19/19 nt	12p13 BAC RPCI11-436I9, *Homo sapiens*

Table 2.1: continued.

120	430	17/34 aa	tumor-associated aldehyde dehydrogenase *Rattus norvegicus*
126	306	16/26 aa	orf2, *Battrachocottus baikalensis*
142	361	21/21 nt	chromosome 5, PAC clone 162O17, LBNL H147, *Homo sapiens*
146	381	12/33 aa	No definition line found, *Caenorhabditis elegans*
146		26/26 nt	no arches mRNA , *Danio rerio*
176	401	11/23 aa	hypothetical protein Hl1418, *Haemophilus influenzae* Rd
182	373	63/109 aa	brain-specific angiogenesis inhibitor 1 precursor, *Homo sapiens*
184	434	16/18 aa	Ankyrin$_G$, *Homo sapiens*
187	382	35/122 aa	envelope glycoprotein, Human immunodeficiency virus type 1
	10421	Total bps	

The 28 loci represented 10,421 base pairs of sequence. Twenty-one loci contained open reading frames (ORF) (overall 70.5% (31/44)) and had sequence similarities to sequences found in GenBank (Table 2.1). Only two loci, 4 and 120, contained fixed sequence species differences or autapomorphies for *C. rimiculus* and *D. luxatus* respectively. Four polymorphic sites were found in the three *C. rimiculus* (AY352299) sequenced from Klamath River: 3 transversions (position 323 C→A, 347 G→T, 356 C→A) in locus 4 and a single insertion (position 291) in locus 120 (AY366138) (Appendix 2). Locus 120 also had a unique *D. luxatus* allele (AY366135) found in two individuals from Upper Klamath Lake. This allele contained a transition at position 308 (T→C) and three transversions at positions 345 (T→A), 365 (T→A) 426 (G→T), (Appendix 2). Detection of this allele required sequence analysis and was limited to three *D. luxatus* samples. Initially we thought these sequence differences were shared among all Klamath Basin species. These differences were not detected by our electrophoretic techniques but only upon sequencing were they discovered. They did not occur in the other samples sequenced. We did not determine if these eight unique sites were present for all Klamath samples.

Eight loci exhibit polymorphic electrophoretic alleles within Klamath Basin suckers. Four loci (117, 119, 126, and 146) have rare variant alleles occurring in less than 5% of the samples and four have common variants (loci 4, 120, 142, and 184).

Rare variants

Locus 117 (AY351359) had no open reading frame (ORF) and weak identity (21 nucleotides (nt)) to a human sequence on chromosome 22q12 (Table 3.1, Appendix 2). The two alleles were detected by SSCP and were the result of a position 123 transition (C→T). This rare allele was found in two of 150 individuals (both *C. rimiculus*). Locus 119 (AY351360) also had no ORF and weak identity (19 nt) to a human sequence on chromosome 12p13. The two alleles were detected by SSCP and were the result of a position 361 transversion (G→ T) (Table 2.1, Appendix 2). The rare allele was found in two of 150 individuals (both *C. rimiculus*). Locus 126 (AY351361) contained an ORF that had 61% identity and 72% overall identity (19/26 amino acids) to a highly specific repeat in ORF 1 found in a sequence from *Battrachocottus baikalensis* (Kholodilov, N.G., unpublished). The two alleles were the result of a position 143 transition (G→A) and only found in 2 of 48 individuals (*Ch. brevirostris* and *D. luxatus*) during SSCP analysis. Locus 146 (AY351365) had an ORF that was moderately homologous to a protein from *Caenorhabditis elegans* (23/33 aa) and had strong identity to a short 26 nt long *Danio rerio* mRNA sequence. Locus 146 had two SSCP alleles caused by a transition (G→A) at position 249. The rare allele was detected in 2 of 48 individuals (both *C. rimiculus*.).

Common variants

The common allele variant group contained two types of polymorphisms: length or size polymorphic alleles (loci 4 and 120) detected with denaturing PAGE and sequence variant alleles (loci 142 and 184) detected with SSCP. The longest of any

length polymorphic alleles were designated as the "A" allele. Alleles that migrated the least were designated as the "A" allele in SSCP analysis.

Locus 4

Locus 4 (AF362135) had 59% identity (73% overall identity, 20/27 amino acids (aa)) to a collagen 1 gene from a fresh water sponge, *Ephydatia muelleri* (Table 2.1, Appendix 2). This ORF spanned nucleotide positions 140 to 60 and corresponded to amino acid positions 31-58 of the protein. The two alleles were caused by two deletions (total length 11-bp) between positions 244-247 and 255-261 (AF362136) (Appendix 2) and exhibited allelic frequency differences between the four Klamath Basin species (Table 2.2). *Ch. brevirostris* and *D. luxatus* had very high frequencies of allele A (95 and 96% respectively), while *C. snyderi* demonstrated an intermediate frequency (86%), and *C. rimiculus* exhibited a low frequency of allele A (16%). An analysis of variance (ANOVA) of the frequency of the "A" allele found significantly different frequencies among the Klamath Basin suckers (ANOVA, $p = 0.00001$). A Fisher's least significant difference (LSD) multiple range test identified three groups, *Ch. brevirostris* plus *D. luxatus*, *C. snyderi*, and *C. rimiculus.*

Rogue River *C. rimiculus* were fixed BB homozygotes, but Klamath River *C. rimiculus* had a genotypic frequency of 56% and an allele frequency of 66%. Upper Klamath Lake species were fixed homozygotes, AA (Table 2.2) but the B allele was found in the upper reaches of the tributaries (15-64% allele frequency). The Lost River sub-basin had the B allele in low frequencies ($\leq$8%) found in reservoirs, and not found in the river proper (Table 2.2).

Table 2.2: Genotypic and allelic frequencies (Freq.) for Locus 4 for all species and sites. n = sample size. Ch.b. = *Chasmistes brevirostris*, C.s. = *Catostomus snyderi*, C.r. = *Catostomus rimiculus*, D.l. = *Deltistes luxatus*.

Species	Site	n	AA	AB	BB	Freq. A	Freq. B
Ch.b.	all	97	0.91	0.08	0.01	0.95	0.05
C.s.		101	0.78	0.15	0.07	0.86	0.14
C.r.		52	0.13	0.06	0.81	0.16	0.84
D.l.		42	0.95	0.025	0.025	0.96	0.04
		292					
All	Sprague River	34	0.79	0.09	0.12	0.84	0.16
Ch.b.		5	1	0	0	1	0
C.s.		24	0.75	0.08	0.17	0.79	0.21
D.l.		5	0.8	0.2	0	0.9	0.1
all/C.s.	Sycan River	7	0.43	0.43	0.14	0.36	0.64
all/C.s.	Upper Williamson River	27	0.74	0.22	0.04	0.85	0.15
all	Lower Williamson River	17	1	0	0	1	0
Ch.b.		13	1	0	0	1	0
C.s.		2	1	0	0	1	0
D.l.		2	1	0	0	1	0
all	Upper Klamath Lake	30	1	0	0	1	0
Ch.b.		13	1	0	0	1	0
C.r.		1	1	0	0	1	0
D.l.		16	1	0	0	1	0
all	Gerber Reservoir	25	0.8	0.2	0	0.9	0.1
Ch.b.		14	0.86	0.14	0	0.93	0.07
C.s.		11	0.73	0.27	0	0.86	0.14
all	Lost River	5	1	0	0	1	0
C.s.		1	0	0	0	0	0
D.l.		4	1	0	0	1	0
all	Clear Lake Reservoir	85	0.92	0.06	0.02	0.95	0.05
Ch.b.		42	0.86	0.12	0.02	0.92	0.08

Table 2.2: continued.

C.s.		28	1	0	0	1	0
D.l.		15	0.93	0	0.07	0.93	0.07
all	Topsy Reservoir	27	0.37	0.15	0.48	0.44	0.56
Ch.b.		4	1	0	0	1	0
C.s.		1	0	1	0	0.5	0.5
C.r.		22	0.27	0.14	0.59	0.34	0.66
all/Ch.b.	Copco Reservoir	5	0.8	0.2	0	0.9	0.1
all/C.r.	Rogue River	29	0	0	1	0	1
Ch.b.	Klamath Tribes Hatchery	1	1	0	0	1	0

Locus 120

Locus 120 (AF362137) had 45% identity (69% overall identity, 26/37 aa) to a tumor associated aldehyde dehydrogenase from *Rattus norvegicus* (Table 2.1, Appendix 2). The ORF spanned from nucleotide position 150 – 1 and corresponded to amino acids 1-50 of the protein sequence. The two alleles differed by a 4 bp deletion spanning positions 300-304 (AF362138) (Appendix 2) that exhibited frequency differences between the four species (Table 2.3). *Chasmistes brevirostris* and *C. snyderi* had high frequencies of allele A (85-87% respectively), *D. luxatus* exhibited an intermediate frequency (76%) and *C. rimiculus* had a low frequency (26%). The frequencies were significantly different (ANOVA, $p = 0.00001$). Fisher's LSD multiple range test identified three groups, *Ch. brevirostris* plus *C. snyderi*, *D. luxatus* plus *Ch. brevirostris* and *C. rimiculus*. The two *Catostomus* species were significantly different but *Ch. brevirostris* overlapped with only the upper sub-basin species (*C. snyderi*, *D. luxatus*).

In the Rogue River, 72% of *C. rimiculus* were fixed homozygotes, BB, while in the Klamath River the proportion was 55%. In Upper Klamath sub-basin 73% of all species were fixed homozygotes, AA (Table 2.3). Therefore no case can be made that any of the four species were fixed for either allele.

Table 2.3: Genotypic and allelic frequencies (Freq.) for Locus 120 for all species and sites. Abbreviations are the same as Table 2.2.

Species	Site	n	AA	AB	BB	Freq. A	Freq. B
Ch.b.	all	92	0.73	0.25	0.02	0.85	0.15
C.s.		89	0.74	0.25	0.01	0.87	0.13
C.r.		51	0.02	0.49	0.49	0.26	0.74
D.l.		38	0.63	0.26	0.11	0.76	0.24
		270					
All	Sprague River	29	0.59	0.34	0.07	0.76	0.24
Ch.b.		5	0.6	0.2	0.2	0.7	0.3
C.s.		19	0.63	0.37	0	0.82	0.18
D.l.		5	0.4	0.4	0.2	0.6	0.4
all/C.s.	Sycan River	7	0.43	0.57	0	0.71	0.29
all/C.s.	Upper Williamson River	24	0.87	0.13	0	0.94	0.06
all	Lower Williamson River	17	0.88	0.12	0	0.94	0.06
Ch.b.		13	0.85	0.15	0	0.92	0.08
C.s.		2	1	0	0	1	0
D.l.		2	1	0	0	1	0
all	Upper Klamath Lake	26	0.73	0.19	0.08	0.83	0.17
Ch.b.		11	0.73	0.27	0	0.86	0.14
C.r.		1	0	0	1	0	1
D.l.		14	0.79	0.14	0.07	0.86	0.14
all	Gerber Reservoir	21	0.71	0.29	0	0.86	0.14
Ch.b.		12	0.67	0.33	0	0.83	0.17
C.s.		9	0.78	0.22	0	0.89	0.11
all	Lost River	4	0.25	0.75	0	0.63	0.38
C.s.		1	0	1	0	0.50	0.50
D.l.		3	0.33	0.67	0	0.67	0.33
all	Clear Lake	81	0.74	0.21	0.05	0.85	0.15
Ch.b.		41	0.78	0.20	0.02	0.88	0.12
C.s.		26	0.77	0.19	0.04	0.87	0.13

Table 2.3:continued.

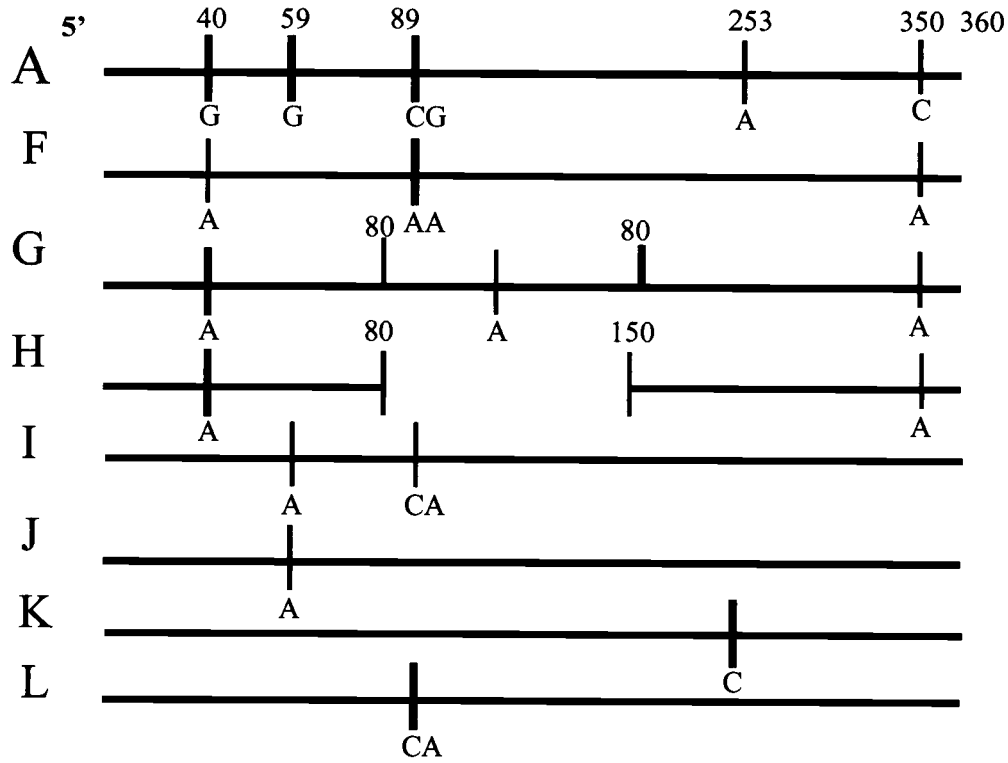
D.l.		14	0.57	0.29	0.14	0.71	0.29
all	Topsy Reservoir	26	0.15	0.73	0.12	0.52	0.48
Ch.b.		4	0.50	0.50	0	0.75	0.25
C.s.		1	1	0	0	1	0
C.r.		21	0.05	0.81	0.14	0.45	0.55
all/Ch.b.	Copco Reservoir	5	0.40	0.60	0	0.70	0.30
all/C.r.	Rogue River	29	0	0.28	0.72	0.14	0.86
Ch.b.	Klamath Tribes Hatchery	1	1	0	0	1	0

Locus 142

Locus 142 (AY351364) was the only polyploid locus identified by this study. This locus did not contain an ORF and had weak sequence identity (21 nt) to a human chromosome 5 sequence (Table 2.1, Appendix 2). Locus 142 had at least 12 alleles in seven genotypes based on SSCP analysis (Figure 2.2). Individuals ampified for locus 142 had from one to three different alleles within a genotype. The alleles were highly related with sequences differing by 1 to 5 single nucleotide changes. Because catostomids are allotetraploids (Ferris and Whitt 1978, Buth 1979) we assumed this was a tetraploid locus and that we had a gene cluster or family of similar sequences derived from a common ancestral sequence. Twelve alleles have been found, but only eight have been sequenced. Alleles B, C, D, E, migrated very closely to the A allele and isolation has been difficult. The A allele was identical to the clone sequence (Figure 2.2, appendix 2).

Locus 142 suggested allelic segregation because all alleles were not equally available to all genotypes (Figure 2.2). Assuming that B, C, D, and E alleles had similar sequences to A, genotype groups appeared to have closely related alleles with minor single base changes (Figure 2.2A). Genotypes 1-4 only had A-E alleles, genotype 5 had F, G, and H alleles, genotype 6 had I and J alleles and genotype 7 had K and L alleles (Figure 2.2B). Genotype 5 alleles, F, G, and H, shared a transition at position 40 (G→A)

A-



B- Locus 142

GENOTYPE #	ALLELES
1	A_4
2	A_2B_2
3	A_2C_2
4	$A_2 D_1 E_1$
5	$F_2 G_1 H_1$
6	$I_2 J_2$
7	$K_2 L_2$

Figure 2.2: **A-** Line diagrams representing 8 of the 12 alleles for Locus 142. "A" allele was identical to the clone sequence. Positions that deviated from the A allele are represented by vertical bars with the corresponding bases under them. The repeated position 80 in allele G represents an 80bp repeat. The gap in allele H represents a 70bp deletion. **B-** Genotype number and alleles found in each genotype.

and a transversion at 350 (C→A). Allele G contained an 80 bp repeat of bases 1-80 inserted after base 80. Allele H was identical to G except for a 70 bp deletion of positions 81-149. Genotype 6 alleles, I and J, shared a unique transition at position 59 (G→A) and allele I contained a unique transition at position 90 (G→A) (Figure 2.2).

The K and L alleles of genotype 7 did not share any unique changes, allele L shared a transition (G→A) at position 90 with allele I and allele K had a unique transversion at position 253 (A→C) (Figure 2.2). Although the allele usage was not exclusive, frequency differences were found between the four species. *Chasmistes brevirostris* used seven genotypes, *C. snyderi* six, *D. luxatus* five and *C. rimiculus* four of the seven locus 142 genotypes (Table 2.4). The most common allele (A) was found in four genotype groups in all four Klamath Basin suckers, but with different frequencies (Table 2.4). Genotype 1 was significantly less common in *C. rimiculus* (4%) while genotype 3 was significantly more common (70%). Genotype 5 occurred in 21 specimens, 14 *Ch. brevirostris* from Upper Klamath Lake, Sprague River and Clear Lake Reservoir and 7 *C. snyderi* from Clear Lake and Gerber Reservoirs. Genotype 6 was found in all species and accounted for 10% of *Ch. brevirostris*, 13% of *C. snyderi,* 20% of *C. rimiculus* and 36% of *D. luxatus.* Major genotypes per species were *Ch. brevirostris*, genotypes 1, 2, 5, and 6 (89 % of specimens); *C. snyderi,* genotypes 1, 2, 3, and 6 (90%); *C. rimiculus*, genotypes 3 and 6 (90%); and *D. luxatus*, genotypes 1 and 6 (83%).

Table 2.4: Genotypic (GT) frequencies for Locus 142 for all species and sites. Abbreviations are the same as Table 2.2.

	Genotype number:		1	2	3	4	5	6	7
Species	site	n	AAAA	AABB	AACC	AADE	FFGH	IIJJ	KKLL
Ch. b.	all	92	0.48	0.16	0.05	0.01	0.15	0.10	0.04
C.s.	all	96	0.43	0.15	0.19	0	0.07	0.13	0.04
C.r.	all	46	0.04	0.07	0.70	0	0	0.20	0
D. l.	all	36	0.47	0.06	0.08	0.03	0	0.36	0
		270							

Table 2.4: continued.

all	Sprague River	33	0.27	0.24	0.09	0	0.06	0.24	0.09
Ch. b.		7	0.14	0.29	0.29	0	0.29	0	0
C.s.		22	0.27	0.23	0.05	0	0	0.32	0.14
D. l.		4	0.50	0.25	0	0	0	0.25	0
all / C.s.	Sycan River	7	0.29	0.29	0.43	0	0	0	0
all / C.s.	Upper Williamson River	27	0.41	0.11	0.44	0	0	0.04	0
all	Lower Williamson River	15	0.47	0.20	0.07	0	0.07	0.07	0.13
Ch. b.		11	0.45	0.27	0.09	0	0.09	0	0.09
C.s.		2	0	0	0	0	0	0.50	0.50
D. l.		2	1.00	0	0	0	0	0	0
all	Upper Klamath Lake	28	0.43	0	0.11	0.04	0.07	0.32	0.04
Ch. b.		12	0.58	0	0.08	0.08	0.17	0	0.08
C.r.		1	1.00	0	0	0	0	0	0
D. l.		15	0.27	0	0.13	0	0	0.60	0
all	Gerber Reservoir	25	0.48	0.12	0	0	0.12	0.24	0.04
Ch. b.		14	0.57	0.07	0	0	0.07	0.21	0.07
C.s.		11	0.36	0.18	0	0	0.18	0.27	0
all	Lost River	5	0.40	0	0.20	0.20	0	0.20	0
C.s.		1	0	0	1.00	0	0	0	0
D. l.		4	0.50	0	0	0.25	0	0.25	0
all	Clear Lake Reservoir	75	0.57	0.09	0.04	0	0.17	0.11	0.01
Ch. b.		39	0.49	0.10	0.03	0	0.21	0.15	0.03
C.s.		25	0.68	0.08	0.04	0	0.20	0	0
D. l.		11	0.64	0.09	0.09	0	0	0.18	0
all	Topsy Reservoir	25	0.16	0.12	0.64	0	0	0.08	0
Ch. b.		4	0.50	0.50	0	0	0	0	0

Table 2.4: continued.

C.s.		1	1.00	0	0	0	0	0	0
C.r.		20	0.05	0.05	0.80	0	0	0.10	0
all / Ch.b.	Copco Reservoir	5	0.40	0.60	0	0	0	0	0
all / C.r.	Rogue River	25	0	0.08	0.64	0	0	0.28	0

Locus 184

Locus 184 (AY351372) had an 88% identity (99% overall identity, 18/18 aa) to human Ankyrin$_G$ (Kordeli, et al 1995). The ORF within this locus spanned nucleotide positions 480 – 533 and corresponded to amino acid positions 4081 - 4098 of the protein sequence. Locus 184 had two alleles caused by a position 353 transversion (C→A) (AY366162) and exhibited a gradual decrease in allelic frequency between the four Klamath Basin species (Table 2.5). *Deltistes luxatus* had a high frequency (60%) for allele A, *Ch. brevirostris* and *C. snyderi* were intermediate (44% and 38%, respectively) and *C. rimiculus* was low (23%). Rogue River *C. rimiculus* had a significantly lower frequency of this allele (14%). Frequencies of allele A were significantly different between the four species (ANOVA, $p = 0.0013$). Fisher's (LSD) multiple range test identified three groups, *D. luxatus, Ch. brevirostris* plus *C. snyderi*, and *C. rimiculus*

Table 2.5: Locus 184 genotypic and allelic frequencies by species and site. Abbreviations are the same as Table 2.2.

Species	Site	n	AA	AB	BB	Freq. A	Freq. B
Ch.b.	all	89	0.29	0.30	0.40	0.44	0.56
C.s.		82	0.17	0.43	0.40	0.38	0.62
C.r.		48	0.10	0.25	0.65	0.23	0.77
D.l.		34	0.26	0.68	0.06	0.60	0.40
		253					

Table 2.5: continued.

all	Sprague River	59	0.14	0.34	0.52	0.15	0.85
Ch.b.		8	0.13	0.38	0.50	0.31	0.69
C.s.		16	0.13	0.19	0.69	0.22	0.78
D.l.		5	0.20	0.80	0	0.60	0.40
all / C.s.	Sycan River	7	0.29	0.14	0.57	0.36	0.64
all / C.s.	Upper Williamson	21	0.05	0.67	0.29	0.38	0.62
all	Lower Williamson	15	0.20	0.27	0.53	0.33	0.67
Ch.b.		11	0.27	0.18	0.55	0.36	0.64
C.s.		2	0	0.50	0.50	0.25	0.75
D.l.		2	0	0.50	0.50	0.25	0.75
all	Upper Klamath Lake	27	0.19	0.56	0.26	0.46	0.54
Ch.b.		11	0	0.36	0.64	0.18	0.82
C.r.		1	0	1.00	0	0.50	0.50
D.l.		15	0.33	0.67	0	0.67	0.33
all	Gerber Reservoir	22	0.27	0.41	0.32	0.48	0.52
Ch.b.		13	0.38	0.23	0.38	0.50	0.50
C.s.		9	0.11	0.67	0.22	0.44	0.56
all	Lost River	4	0.25	0.75	0	0.63	0.38
C.s.		1	0	1.00	0	0.50	0.50
D.l.		3	0.33	0.67	0	0.67	0.33
all	Clear Lake Reservoir	71	0.37	0.37	0.27	0.55	0.45
Ch.b.		37	0.43	0.30	0.27	0.58	0.42
C.s.		25	0.32	0.36	0.32	0.50	0.50
D.l.		9	0.22	0.67	0.11	0.56	0.44
all	Topsy Reservoir	23	0.26	0.17	0.57	0.35	0.65
Ch.b.		3	0.33	0.33	0.33	0.50	0.50
C.s.		1	0	0	1.00	0	1.00
C.r.		19	0.26	0.16	0.58	0.34	0.66
all/ Ch.b.	Copco Reservoir	5	0	0.60	0.40	0.30	0.70

Table 2.5: continued.

all /C.r.	Rogue River	28	0	0.29	0.71	0.14	0.86
Ch.b.	Klamath Tribe Hatchery	1	0	0	1.00	0	1.00

Discussion

Surprisingly, among the four species from three genera of Klamath Basin suckers, there were only eight fixed single nucleotide polymorphisms (SNP) in 10,421 bp. These were found in only two species, *D. luxatus* and *C. rimiculus*. Locus 120 contained a single insertion unique to three Topsy Reservoir *C. rimiculus* and four diagnostic single nucleotide polymorphisms (SNPs) (3 transversion and 1 transition) that identify 2 *D. luxatus* from Upper Klamath Lake. Locus 4 contained three SNPs (3 transversions) found in four Topsy Reservoir *C. rimiculus*. These may represent fixed sequence species differences, but these alleles had small sample sizes and therefore have limited power to segregate Klamath Basin catostomids.

Eight loci were polymorphic and only four of these had common polymorphic genotypes or variant loci shared among the four species. We found three types of species markers within the common variant loci: strictly diagnostic, operationally diagnostic and frequency dependent. We define a strictly diagnostic species marker as an allele or genotype that is only found in one species and indicates that an individual can be identified to. This can be a rare SNP such as those found loci 4 and 120 or a more traditional fixed species difference (such as an indel or an allele size variant). An operationally diagnostic species marker is an allele or genotype fixed for one population (drainage or sub basin) of a species and may, with corroborative information, indicate that an individual can be identified to species. A frequency dependent species marker is an allele or genotype showing a statistically significant difference in frequency between species and may, with corroborative information, indicate that a population can be identified to species.

The homozygous BB genotype of Locus 4 was an operationally diagnostic species marker for *C. rimiculus* in the Rogue River (Table 2.2). If allele B is a autapomorphy for *C. rimiculus* then the hybrid gene flow argument is that all *C. rimiculus* are BB, and all *Ch. brevirostris, C. snyderi*, and *D. luxatus* are AA. All heterozygotes (AB) would be F1 or F2 hybrids and/or backcrosses, although some F2 and F2 backcrosses could show a parental genotype. Both alleles must either pre-date differentiation of the species or indicate ancient hybridization prior to Klamath *C. rimiculus* dispersal into the Rogue River, or its separation from the Klamath Basin. However, the B allele was widespread in the Lost River sub-basin and upper Williamson River of the UKL sub-basin and a single *C. rimiculus*, genotype AA, has been documented in UKL (Table 2.2). Therefore, if locus 4 is a species marker, hybrids must be viable to account for the frequencies in the Klamath River (56% B allele) and the higher reaches of the UKL (15%) and Lost River sub-basins (7-14%). Hybridization is either on-going, especially between *C. rimiculus* and *C. snyderi* (Klamath River), or the B allele was an ancestral polymorphism which had been lost in Upper Klamath Lake by genetic drift and isolated from the higher reaches of the sub-basin and the reservoirs of Lost River sub-basin where it is maintained by random mating. The Rogue River *C. rimiculus* samples were limited to one site on a single day and may represent a single spawning group or sampling artifact.

The B allele of locus 120 was a frequency-dependent marker for *C. rimiculus* (Table 2.3). Again, 72% of the Rogue River fish were homozygous, BB, for this locus and therefore the possibility that it may be a sampling artifact can not be dismissed. This allele suggested either hybridization throughout Klamath Basin or an ancestral polymorphism. The former requires gene movement up river from the Lower Klamath sub-basin to the Upper Klamath and Lost River sub-basins. Infrequent recent exchange between Lost River and Klamath River water (Rykbost and Todd, 2002) suggests that the movement is ancient. The single *C. rimiculus* identified in the upper sub-basins suggests that the movement occurred in individuals backcrossed to the upper basin parent species. Ancestral polymorphism seems simpler with genetic drift in both the Rogue River and the Klamath Basin, but in opposite directions.

Allele frequency dependent markers were seen in loci 4, 120 and 184. Species exhibited different allele frequencies for these loci. The significant allele usage between species is clearest in *C. rimiculus* from all sites. This species had significantly lower frequencies for the A allele for these loci. Individual loci exhibited different allele frequencies between species; these differences found only three distinct groups, *D. luxatus. C. rimiculus, and Ch. brevirostris/ C. snyderi.*

The loci used in this study ranged in size from 190-543 bp. Our selection of 28 low copy number anonymous nuclear loci resulted in a large number of protein coding genes. Over 70% of these loci had open reading frames (ORF) despite our selection of primers to avoid ORFs and maximize more variable introns. We would expect ORF containing loci to be conservative because of protein functionality, but third position changes and small intron regions connected to coding exons might have been expected to be more variable. They were not and could be used to distinguish only two of the four Klamath suckers.

Loci 81, 82, 117, 119, 142, 146 (Table 2.1) contained no ORFs, and a similar lack of polymorphisms as those containing an ORF. The most variable locus was 142, genotypic gel patterns indicated this locus was tetraploid, the only one found. Because of the allotetraploid genome in catostomids, we expected to find twice as many alleles for these loci as in a diploid organism (Dawson, 1962). Amplification of this locus found up to three alleles within an individual suggesting a tetraploid gene family. Overall, twelve alleles were found suggesting a highly variable set of genes. Although these alleles were variable they showed no species specific usage. The significance of these alleles was seen in the patterns of alleles within genotypes. The alleles seemed to be grouped into specific genotypes, and did not exhibit random assortment. Random assortment would be present if alleles of all types were found together, such as A with F, G, H, I, J, K, or L. The absence of random assortment (Table 2.4) suggested either assortative mating through mate recognition or selection against particular allele combinations.

These loci indicate that the Klamath/Rogue Basins catostomid genomes are conserved and highly related. The sequence similarity of random loci and low levels of polymorphism across the four species does not support their current classification in three

genera unless the samples are of hybrid origin, (G. Smith, personal communication, June 17, 2000). Further, most differences followed geographic, rather than taxonomic, patterns with *C. rimiculus* from the lower Klamath Basin and Rogue River and *D. luxatus* more often the "distinct" entities while species from two genera in Upper Klamath Basin (*Ch. brevirostris*, and *C. snyderi*) were more similar (Tables 2.2, 2.3, 2.5).

There are a number of explanations for these results. For example, the 28 loci used in this study may be so highly conserved that they only contain deep evolutionary information, such as an east west division of the family (Chapter 3). Alternatively, the catostomid genome maybe so highly conserved that these loci are insufficient to represent the recent evolutionary history of these lineages (Chapter 3). A phylogenetic analysis addresses these issues in chapter 4 and discusses the comparison of Klamath Basin samples with congeneric species samples from the northwest and different genera and species from around the country.

Widespread hybridization between species could also explain the lack of genetic difference found in this study. Smith's (1992) phylogeny of Catostomidae hypothesized that the Klamath sucker species colonized the Basin through three distinct routes. *C. snyderi* is basal to a group including *C. rimiculus, C. tahoensis* and *C. microps* suggestive of a coastal dispersal pattern. Smith includes *D. luxatus* with *Xyrauchen* as a large bodied form and basal to *Chasmistes* suggesting Colorado-Great Basin origin. Ancestral *Chasmistes* fossils suggest a Great Basin connection (Miller and Smith, 1981). Each of these colonizations has left little clear route from their source and, few if any, other representative species from these connections. Although we do find morphological similarities between species, there has not been a convergence of phenotypes in all populations (Markle et. al., in preparation). The genetic similarities suggested that if hybridization was the cause, it would have to be the result of a Basin-wide event that involved all populations throughout the entire Klamath Basin either as a singular event or one that is continuing because all alleles for each locus were found in all sites (except within the Rogue Basin). The hybrid genotypes would have completely supplanted the genomes of the distinct Klamath species because no unique alleles were found for a given species. This event would have had to occur before the separation of the Rogue River

from the Klamath Basin because the genomes are so similar. However the fact that distinct phenotypes occur throughout the Basin, suggested either few genetic differences result in phenotypic differences or the highly related genome is plastic and can produce different phenotypic expressions. We suggest in Markle et. al. (in preparation) that the similarities seen in these species is due to interbreeding species representing a hybridizing species group or syngameon. Within these groups, hybridization was ongoing while morphological characters remain diagnostic for each species. In these cases hybridization is a source of genetic variation.

Another explanation for the genetic similarities found in Klamath Basin is the process of sympatric speciation. Although this is a rare and unpopular hypothesis, Sku'lason and Smith (1995) have compiled 24 biological examples supporting this speciation method. Among these examples 62% (15 of 24) are fish taxa. The driving force behind sympatric speciation is food resource partitioning leading to unique phenotypes. Among the phenotypic changes, Sku'lason and Smith have listed "jaw size and jaw shape, and (body) size, shape and number of gill rakers as their first five phenotypic differences among sympatric morphs. These are some of the same morphological characteristics (lip morphology and gillraker number) used to identify Klamath Basin suckers (Markle et. al, in preparation). And because these characters are important for the acquisition of food resources, the morphological variability may suggest that these species use different food resources and that the ancestral genome has evolved phenotypic variations based on resource partitioning. If Klamath Basin was colonized by a single sucker species instead of Smith's hypothesis of three separate colonizations then the genetic similarities we found could be ancestral, suggestive of an ancestral form that colonized both the Klamath and Rogue Basins. This explanation was suggested by data (Chapter 3) that showed that the genomes of Klamath suckers are conservative sharing many ancestral characters with distant sister taxa such as *Cycleptus*, *Moxostoma* and *Carpiodes*. The genetic similarity between groups evolving from a common ancestor will be very great and unless reproductive isolation can be completely accomplished, mating between phenotypes will continue to produce populations of genetically similar individuals.

Our data suggested that the four species of suckers in the Klamath and Rogue Basins are closely related and do not warrant separate generic separation. At this point, it is not clear which evolutionary process (sympatric speciation, syngameon or hybridization) was the cause of low genetic variability. Although hybridization may appear to be a destructive speciation event or a waste of each species' reproductive potential, it may also be a natural and necessary source of genetic variation, as would be expected from a syngameon (Markle et al. in preparation) or early during resource partitioning divergence evolution (sympatric speciation). Consequently, it is not currently advisable to make management decisions detrimental to existing species categorization.

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Chapter 3:

Genetic identity and the case for hybridization in Upper Klamath Basin suckers

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Abstract

Although Klamath Basin suckers represent three genera, only two frequency dependent allele differences were found in 28 random, low copy-number, anonymous nuclear loci representing 10,431 base pairs. We examined phylogenetic patterns in five of these loci in eleven catostomid genera and 25 species to determine if the homogeneity in the Upper Klamath Basin was due to massive hybridization and introgression or to retention of ancestral sequences. Two non-coding loci and three coding loci gave generally similar results, providing support for various subfamilies and tribes, some support for eastern genera but no support for western genera. Each locus was a mosaic for species or population markers, sometimes providing discriminatory power for allopatric populations of a species, such as *C. macrocheilus*, while not discriminating other species. Upper Klamath Basin species were noteworthy in their lack of autapomorphies, but had similar numbers of derived informative sites as other catostomins. Upper Klamath Basin species consistently shared ancestral or equivocal informative sites either with moxostomatins or a variable group of western species and shared derived sites with other western species, especially *C. occidentalis*. These loci suggest that Upper Klamath Basin species have retained a largely ancestral genome at these loci. Thus, the failure of this technique to uncover significant variation in Upper Klamath Basin species may be a reflection of their plesiomorphic genome at these loci and not necessarily hybridization.

Introduction

Low copy number anonymous nuclear loci have been used to distinguish male-mediated gene flow between populations of green turtles (*Chelonia mydas*) (Karl et al. 1992), population structure in American oysters (*Crassostrea virginica*) (Hare and Karl, 1998; Karl and Avise, 1993) and phylogeny of labroid fishes (Streelman and Karl, 1997). However, the technique was not successful in distinguishing four sympatric species of Klamath Basin suckers. Although the Klamath suckers represent three genera, only eight fixed nucleotide differences were found in 28 random loci representing 10,431 base pairs (bp) (Wagman and Markle, 2003a).

The failure of this technique to uncover variation in Klamath suckers species could be due to widespread hybridization or to retention of ancestral sequences. Suckers are known to hybridize (Buth et al., 1987; Miller and Smith, 1981; Smith, 1992), but the near identity in our results would suggest massive hybridization in Klamath suckers. These results could also be produced if Klamath species retained ancestral character states for these loci. The ancestral genome argument requires phylogenetic comparisons to demonstrate that Klamath species retain ancestral states. In the following, we examine phylogenetic patterns in five low copy number anonymous nuclear loci in catostomids to better understand the low sequence variation in Klamath suckers.

Materials And Methods

We surveyed 25 species representing 11 of the 13 North American catostomid genera (Nelson, 1994). Several species were represented by samples from multiple drainages: *C. macrocheilus* from seven drainages, and *Erimyzon oblongus* and *C. columbianus* from two drainages. Specimens examined are listed in Table 3.1. Sequence data for Klamath Basin samples were reported in Wagman (Chapter 2).

Muscle or fin tissues were extracted as described in Wagman (Chapter 2) following the procedure of Taggard et al. (1992) with slight modifications. DNA was

precipitated overnight at –20 ^{0}C followed by a 4 ^{o}C centrifugation for 20 minutes at 13,000 X g and centrifuged again after washing with 70% ethanol. DNA was quantified by spectrophotometry.

DNA Amplification

We chose three coding loci that were polymorphic (loci 4, 120 and 184) and two non-coding loci that were monomorphic (loci 39 and 81) in the three Upper Klamath Basin species. The polymorphic loci were described in Wagman (2003). Locus 39 had no open reading frame (ORF), no protein identity in GenBank, and weak nucleotide identity to the ependymin gene (Appendix 2). Locus 81 had no ORF, and weak nucleotide homologies to cadherin and guanylyl cyclase C (Appendix 2). All five loci were not successfully amplified for all species (Table 3.1).

DNA was amplified by polymerase chain reaction (PCR) and visualized on ethidium bromide (2 ul/ 100 ml (10 mg/ml) 2% agarose (BRL) gels. Reactions for PCR were optimized using master mix solutions set for a reaction volume of 25 ul and a DNA volume of 6 ul (up to 300 ng total DNA). Four master mix concentrations of $MgCl_2$ were used (1, 2, 3, 4 mM). Master mix solutions contained 10 X polymerase buffer (Promega), 5 mM dNTP (BRL), the appropriate amount of 25 mM $MgCl_2$ and water for 50 reactions. The appropriate volume of master mix solution (16.75 ul / 25 ul reaction) was aliquoted into an iced sterile tube. Specific forward and reverse primers constructed for each locus from a *Ch. brevirostris* genomic library (Appendix 2) were added (1 ul 20pM-primer solution / 25 ul PCR reaction). TAQ polymerase (1-1.25 units per reaction) was added to the master mix and aliquoted (19 ul/ reaction) into the tube. Template DNA was added last.

Reactions were run in a Perkin Elmer 9600 thermocycler using a three-step PCR method: an initial 3 minute de-naturation at 94 ^{o}C for one cycle; and a three-step program of 45 second de-naturation at 93 ^{o}C, 45 second annealing, and 60 second extension at 72 ^{o}C. Annealing temperatures for non-Klamath taxa were lower than those used for Klamath Basin species to facilitate primer annealing (successful annealing temperatures varied with each species and ranged between 45° and 58°C). The PCR reaction was

optimized for each species by varying the $MgCl_2$ concentration and annealing temperatures experimentally.

Table 3.1: Specimens sequenced for loci 4, 39, 81, 120, 184. Catalog number is the museum catalog for carcasses or other identifying number. Upper Klamath species in boldface. Accession numbers (AF#, AY#) are listed for sequences submitted to GenBank. "X" refers to a sequence used for comparison and is identical to the GenBank sequence for a particular taxon and locus. UKL. = Upper Klamath Lake. OS = Oregon State Fish Museum, NCSM = North Carolina State Museum, UAIC = University of Alabama Ichthyological collection. Arizona = Dr. T. Dowling , Arizona State University, Reid = S. Reid US Fish and Wildlife

Taxon	Drainage	Catalog Number	Locus 4	Locus 39	Locus 81	Locus 120	Locus 184
CATOSTOMINI							
Chasmistes brevirostris	UKL. OR	OS015954B	X				
	Williamson R. OR.	OS015963b	AF362135	AY351345	AY351352	AF362137	AY351372
	Lost R. OR	OS015947f		X	X		
	Klamath R. OR	OS017487a		X	X		
		OS017487b		X	X		
Ch. cujui	Pyramid L. NV	Arizona 7		AY366088	AY366108	AY366134	AY366162
		Arizona 8	AY352292	X		X	X
Deltistes luxatus	UKL. OR	OS015922	AY352293			X	AY366163
		OS015931b		AY366089	AY366109		
		OS015926				AY366135	
		OS015927				X	
Xyrauchen texanus	Dexter Lab (refuge)	OS 015279,97	AY352294	AY366090	AY366110	AY366136	AY366164
		OS 015279,98	X	X	X	X	X
		OS015279,99			X	X	
Catostomus	Williamson R. OR	OS015900f	AY352298	AY366091	AY366111	AY366137	AY366165
(Catostomus) snyderi							
C. (C.) rimiculus	Klamath R. OR	OS0159008a	AY352299			AY366138	
		OS0159008b	X			X	
		OS0159008d	X			X	
		OS0159009i	X				
C. (C.) occidentalis	Larabee Cr. CA	OS 015623-1	AY352295	AY366092	AY366112	AY366140	AY366167

Table 3.1: continued.

humboltianus		OS 015623-2	X	X	X	X	X
C. (*C.*) *microps*	Coffee Mill Cr. CA	Reid1-10		X	AY366113	AY366141	AY366166
C. (*C.*) *sp.*	Wall Canyon, NV	OS 014101-14	AY352296	AY366093	AY366114	AY366142	AY366168
		OS 014101-15		X	X	X	
C. (*C.*) *warnerensis*	Summer L. OR	OS 014271 d		X	AY366115		AY366179
		OS 014271 e		AY366094		AY366143	X
		OS 014271 f		X		X	X
C. (*C.*) *macrocheilus*	Willamette R. OR	OSX 43				X	X
		OSX 44		AY366097	X	X	AY366169
	Hood R. OR	OS 015886, 231	X	AY366096	X	X	AY366173
		OS 015885, 230	X	AY366095	X	X	
	Siuslaw R. OR	OS 015461-6	X	X	X	X	AY366171
		OS 015461-7		X	X	X	X
	Woahink R. OR	OS 013656a		X	X	AY366145	AY366170
		OS 013656c	X	X	X	X	X
	Umpqua R. OR	OS 015427-8			X	X	X
		OS 015427-9		X	X	X	X
		OS 015427-10		X	X		
	Millicoma R. OR	OS 015442-2	X	X	X		
		OS 015442-3	X	X	AY366116	AY366146	X
		OS 015442-5			X	X	X
		OS 015442-7		X	X		
	Coquille R. OR	OS 015433	AY352301	AY366098	AY366117	AY366144	AY366172
		OS 015434	X	X	X	X	X
C. (*C.*) *commersoni,*	Seneca, MD	UAIC 12255.03	AY352303		AY366121	AY366150	AY366174
C. (*Pantosteus*)	Booners Cr. OR	OS 017548a		X		X	AY366177

Table 3.1: continued.

columbianus		OS 017548b	AY352297	AY366[illegible]	AY366118	AY366147	
	Kiger Cr. OR	OS 017553a	AY352300	AY366100	AY366119	AY366148	AY366175
C. (P.) platyrhynchus,	Willamette R. OR	OS 017518a	AY352302	AY366101	AY366120	AY366149	AY366176
		OS 017518b	X	X	X	X	X
MOXOSTOMATINI							
Hypentelium etowanum	Cahaba R. AL	UAIC 11310.08		AY366102	AY366123	AY366151	
H. nigricans	Valley Cr. NC	NCSM 29084	AY352305	AY366103	AY366122		
Erimyzon oblongus	Mill Branch Cr., AL	UAIC 11109.09			AY366125	AY366152	
	Fall Cr. NC	NCSM 29398			AY366124	AY366153	AY366178
Minytrema melanops	Little Bear Cr. AL	UAIC 11141.01		AY366105	AY366126	AY366154	AY366180
Thoburnia rhothoeca	Catawba Cr. VA	UAIC 11009.05	AY352304	AY366104	AY366127	AY366155	AY366184
Moxostoma duquesnei	Turkey Cr. AL	UAIC 11711.01		AY366106	AY366129		AY366181
M. macrolepidotum	Deep R. NC	NCSM 29384			AY366128	AY366156	AY366182
M. pappillosum	Deep R. NC	NCSM 29385		AY366107	AY366130	AY366157	AY366183
ICTIOBINAE							
Carpiodes carpio	Honey Cr. NB	UAIC 11219.08			AY366131	AY366158	
Ca. cyprinus	High Rock Res. NC	NCSM 29738-5			AY366132	AY366159	
CYCLEPTINAE							
Cycleptus elongatus	Duck R. TN	NCSM 29618			AY366133	AY366160	

DNA Sequencing

Successful PCR amplifications were size fractionated on 2 % agarose gels containing ethidium bromide (2.5ul/100ml gel 10mg/ml) and the band excised. DNA was isolated using a 0.45 um Ultrafree-MC filter units (Millipore) following the manufacturer's directions. Isolated DNA was re-suspended in 15 ul of ultra-pure water and sequencing performed by OSU Center for Gene Research and Davis Sequencing (Davis, California) using primers prepared at OSU.

Analyses

The Klamath Basin species were heterozygous for three loci 4, 120, 184. In all cases we used the most common allele for the species. For locus 184 only, the reported sequence for *C. rimiculus* was based on identity of numerous SSCP gels to *Ch. brevirostris.* No other sequence data were based on SSCP identity. For species represented by one or a few individuals, we attempted to choose the most derived allele based on a combination of sequence data, uniqueness of SSCP gels, signal strength on the chromatographs, and ease of isolation.

Sequence ambiguities were corrected by comparing chromatographs from bi-directional sequences. Sequences were aligned using BioEdit (Hall, T. A. 1999). Three loci (4, 81,184) contained deletions that followed the forward primer sequence, suggesting sequencing artifacts. These ambiguous regions were removed from the final alignment: locus 4 (seven bases), locus 81 (19 bases) and locus 184 (40 bases).

Species with multiple populations were collapsed into the smallest grouping when sequences were identical. Pairwise comparisons of sequence alignments were used to construct a similarity matrix. Autapomorphies for all populations or species were identified and they plus invariant sites removed to create a matrix of informative sites. Informative sites were examined for consistency with current taxonomy and to determine if Klamath Basin species retained potentially ancestral bases. Each site was coded for the ancestral state at the Catostomini level, such that ancestral or equivocal states were distinguished from derived informative sites. The ancestral criterion was outgroup

analysis, that state found in some but not all members of the in-group and in the out-group (Watrous and Wheeler 1981). Polarity was determined by mapping sites on the phylogeny of Harris and Mayden (2001) (Figure 3.1). All of the Upper Klamath Basin species are Catostomini so their sister taxon, and most appropriate outgroup, is the Moxostomatini. In addition, for some loci, we have additional outgroups in two other subfamilies, the Ictiobinae and Cycleptinae.

We were primarily interested in evidence of ancestral sequences in Klamath Basin species. We examined phylogenies for each locus using parsimony analysis with the heuristic search option of PAUP*4.0 (version 4.0b4a; Swofford, 1998). Bootstrap replicas of 10,000 were performed. The largely polytomous trees were used as a check on our interpretation of locus sequences (data not shown except for locus 81). Locus 81 was represented as 50% majority rule consensus trees (Figure 3.2).

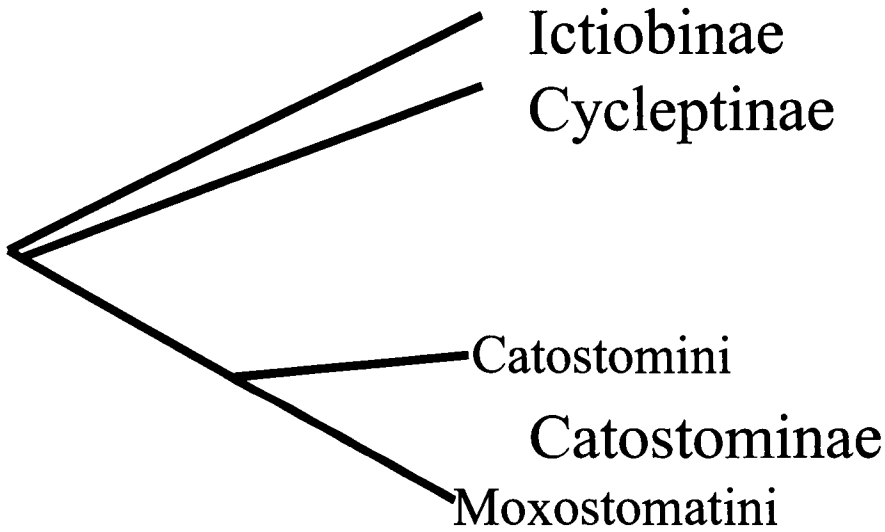

Figure 3.1: Phylogeny of Catostomidae, based on Smith, 1992 and Mayden and Harris, 2001.

Results

Locus 4

Locus 4 had nine different allele sizes ranging from 309-403 bp (Table 3.2). Five size variants appeared unique for individual species: *C. platyrhynchus* (369 bp), *C. commersoni* (381 bp), *H. nigricans* (398 bp), *T. rhothoeca* (403 bp) and *C. macrocheilus* (382 bp – excluding one Hood R. *C. macrocheilus,* Hood 230, OS 015885, that differed from all other *C. macrocheilus* because of a SNP and a 77bp deletion). Klamath Basin species shared a 397 bp allele with *C. occidentalis*. Autapomorphies were found for both species and populations within species (Table 3.2). For example, the two samples of *C. columbianus* shared a SNP and both Booners and Kiger Cr. populations of *C. columbianus* also contained a unique SNP each. The largest number of autapomorphies was found in *H. nigricans* (Table 3.2).

Excluding *C. macrocheilus* OS 015885, sequence similarity within the two tribes was 90.1-100% (Catostomini) and 92.3% (Moxostomatini) (Table 3.3). For genera or subgenera represented by two or more species, identity was 95.9% (*Chasmistes*), 90.1-100% (*Catostomus*), and 93.7-99.4% (*C. Pantosteus*). The three Upper Klamath Basin species were identical and more similar to *C. occidentalis* (99.7%) than to lower Klamath *C. rimiculus* (95.2%). This locus is fixed in Rogue R. *C. rimiculus,* but only found in 72.7% of Klamath R. *C. rimiculus* (16 of 22 individuals, frequency of B allele is 66%). The three Upper Klamath Basin species differed by 11-14.9% from moxostomatines.

There is no indication of site saturation at informative sites since each has only two states except the insertion site at 245-247 which has three (Table 3.4). The informative sites provided unique sequence support for both tribes, especially the indels at 216-218 and 386-394 and several SNPs (Table 3.4; Moxostomatini 83, 208, 213, 239, 242, 245-247, 307, 374; Catostomini 208, 213, 239, 242, 307, 374). For genera or subgenera represented by two or more species there were no synapomorphies. Polarity of twelve of the 22 informative sites was equivocal at the level of Catostomini. The three Upper Klamath species and the geographically adjacent, *C. occidentalis*, have derived

states at the other ten sites which suggests that the Klamath sequence does not represent an ancestral subfamily sequences. The parapatric lower Klamath *C. rimiculus*, resembled other western catostomins rather than the Upper Klamath species at its most abundant allele, which was fixed in Rogue River. It possessed the Upper Klamath allele at lower frequency (34%) in the Klamath River population (Wagman and Markle, 2003).

Table 3.2: Locus 4 allele sizes and autapomorphies listed as single nucleotide polymorphisms (SNP) and insertion/deletions (InDels). Upper Klamath species are in boldface. The *C. macrocheilus* group is Hood 231, Woahink, Siuslaw, Millicoma, and Coquille.

Taxon	Allele size (bps)	Autapomorphies SNP	Autapomorphies InDel (bps)	Site numbers
CATOSTOMINI				
Ch. brevirostris	397			
Ch. cujui	386			
D. luxatus	397			
X. texanus	385	3	1	11, 30, 162, 398
C. snyderi	397			
C. rimiculus	386	3		323, 347, 356
C. occidentalis	397			
C.sp. Wall Canyon	385			
C. macrocheilus 230 Hood	309	1	77	23-100, 274
C. macrocheilus group	382		4	137-140
C. commersoni	381	5	5	99, 170, 171, 172,178-182, 396
C. columbianus	386	1		72
C. columbianus Booners	386	1		354
C. columbianus Kiger	386	1		360
C. platyrhynchus	369	3	7, 10	62-69, 73-82, 84, 178, 403
MOXOSTOMATINI				
H. nigricans	398	18	3 , 2	70, 85, 98-100, 127, 129, 131, 132,169,181,207,214-215,220, 223, 232, 263, 304, 342, 346, 405, 410
T. rhothoeca	403	7		26, 55, 75, 107, 214, 228, 301
Total		43	8	

Table 3.3: Identity matrix of 16 taxa using locus 4. The *C. macrocheilus* group is Hood 231, Woahink, Siuslaw, Millicoma, and Coquille.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *Ch. brevirostris*	100															
2 *Ch. cujui*	95.9	100														
3 *D. luxatus*	100	95.9	100													
4 *X. texanus*	94.9	98.9	94.9	100												
5 *C. snyderi*	100	95.9	100	94.9	100											
6 *C. rimiculus*	95.2	98.7	95.2	97.6	95.2	100										
7 *C. occidentalis*	99.7	95.7	99.7	94.7	99.7	94.9	100									
8 *C.sp.* Wall Canyon	95.9	100	95.9	98.9	95.9	98.7	95.7	100								
9 *C. macrocheilus* Hood 230	76.5	79.7	76.5	79.0	76.5	78.7	76.5	79.7	100							
10 *C. macrocheilus* group	94.9	98.9	94.9	97.9	94.9	97.6	94.7	98.9	78.7	100						
11 *C. commersoni*	93.9	97.1	93.9	96.1	93.9	96.3	93.7	97.1	77.4	96.1	100					
12 *C. columbianus* Booners	95.4	99.4	95.4	98.4	95.4	98.1	95.2	99.4	79.5	98.4	96.6	100				
13 *C. columbianus* Kiger	95.4	99.4	95.4	98.4	95.4	98.1	95.2	99.4	79.5	98.4	96.6	99.4	100			
14 *C. platyrhynchus*	90.4	94.3	90.4	93.2	90.4	93.0	90.1	94.3	82.6	93.2	91.7	93.7	93.7	100		
15 *H. nigricans*	85.1	87.5	85.1	86.6	85.1	86.8	85.3	87.5	70.3	86.6	85.3	87.0	87.0	83.1	100	
16 *T. rhothoeca*	89.0	91.5	89.0	91.0	89.0	90.8	88.7	91.5	73.4	90.5	88.8	91.0	91.0	87.3	92.3	100

Table 3.4: Locus 4 informative sites. Shaded cells are ancestral or equivocal states at the Catostomini level (12 are equivocal). Upper Klamath Basin species in boldface; del represents gap associated with insertion/deletion polymorphisms. The *C. macrocheilus* included Hood, Siuslaw, Woahink, Millicoma, and Coquille.

Site(s) number	11	34	53	83	94	180	193	208	213	216-218	239	242	244	245-247	251	252	254	255-261	307	328	374	386-394
Taxon																						
CATOSTOMINI																						
Ch. brevirostris	G	A	T	A	C	A	T	C	C	del	C	T	T	CAG	G	A	T	TTTCTTT	del	A	G	del
Ch. cujui	G	A	T	A	T	G	T	C	C	del	C	T	del	del	T	C	G	del	del	A	G	del
D. luxatus	G	A	T	A	C	A	T	C	C	del	C	T	T	CAG	G	A	T	TTTCTTT	del	A	G	del
X. texanus	A	A	T	A	T	G	T	C	C	del	C	T	del	del	T	C	G	del	del	A	G	del
C. snyderi	G	A	T	A	C	A	T	C	C	del	C	T	T	CAG	G	A	T	TTTCTTT	del	A	G	del
C. rimiculus	G	A	T	A	C	G	T	C	C	del	C	T	del	del	T	C	G	del	del	T	G	del
C. occidentalis	G	A	C	A	C	A	T	C	C	del	C	T	T	CAG	G	A	T	TTTCTTT	del	A	G	del
C.sp. 74 Wall Canyon	G	A	T	A	T	G	T	C	C	del	C	T	del	del	T	C	G	del	del	A	G	del
C. macrocheilus	G	A	T	A	T	G	T	C	C	del	C	T	del	del	T	C	G	del	del	A	G	del
C. commersoni	G	A	T	A	C	del	T	C	C	del	C	T	del	del	T	C	G	del		A	G	del
C. columbianus	G	A	T	A	T	G	T	C	C	del	C	T	del	del	T	C	G	del	del	A	G	del
C. platyrhynchus	G	T	T	A	T	G	C	C	C	del	C	T	del	del	T	C	G	del	del	A	G	del
MOXOSTOMATINI																						
H. nigricans	G	T	C	T	T	G	C	A	A	CGA	A	A	T	TTA	T	C	G	del	C	T	T	TGAATAATC
T. rhothoeca	A	T	T	T	T	G	C	A	A	CGA	A	A	T	TTA	T	C	G	del	C	T	T	TGAATAATC

Locus 39

Locus 39 had four different allele sizes ranging from 268 - 273 bp (Table 3.5). One species, *T. rhothoeca* (271 bp), and two populations, *C. macrocheilus* from the Willamette R. (268 bp) and *C. columbianus* from Booners Cr. (273 bp), had unique size variants. Klamath Basin species shared a 269 bp allele with all other taxa representing both tribes of catostomines (Table 3.5).

Sequence similarity within the two tribes was 94.1-100% (Catostomini) and 96.3-100% (Moxostomatini) (Table 3.6). For genera or subgenera represented by two or more species, identity was 99.2% (*Chasmistes*), 94.1-100% (*C. Catostomus*) and 94.1-95.2% (*C. Pantosteus*). The greatest dissimilarity was found within *Pantosteus*. The three Upper Klamath Basin species were identical and 99.6% identical to two western suckers (*C. warnerensis* and *C. macrocheilus* from Willamette R.) and 99.6-100% identical to the moxostomatins, *Hypentelium* and *Moxostoma* (Table 3.6).

There is no indication of site saturation at the three informative sites since each has only two states (Table 3.7). The three Upper Klamath Basin species shared a common base at site 58 which is otherwise only found in moxostomatines (Table 3.7) but which had equivocal polarity.

Table 3.5: Locus 39 allele sizes and autapomorphic listed as single nucleotide polymorphisms (SNP) and insertion/deletions (InDels). Upper Klamath species are boldface. The *C. macrocheilus* group is Siuslaw, Woahink, Umpqua, Millicoma, and Coquille.

Species	Allele size (bps)	Autapomorphies		Site Numbers
		SNP	InDels (bps)	
CATOSTOMINI				
Ch. brevirostris	269			
Ch. cujui	269	1		183
D. luxatus	269			
X. texanus	269	1		235
C. snyderi	269			
C. occidentalis	269	1		252
C. sp. Wall Canyon	269			
C. warnerensis	269			
C. macrocheilus Hood	269	1		20
Hood 230	269	3		258, 268, 270
Hood 231	269	2		5, 43
C. macrocheilus Willamette	268			
C. macrocheilus group	269			
C. columbianus	269-273	2	1, 1	6, 7, 88, 94
Booners Cr.	273	1	5	89-94, 175
Kiger Cr.	269	4	1	11, 172, 177, 193, 196
C. platyrhynchus	269	4		105,141,142,197
MOXOSTOMATINI				
H. etowanum	269			
H. nigricans	269			
M. melanops	269	3		15, 119, 191
T. rhothoeca	271	5		41, 179,189, 247, 268
Mox. duquesnei	269			
Mox. pappillosum	269			
Total		28	4	

Table 3.6: Identity matrix of 23 taxa using locus 39. The *C. macrocheilus* group is Woahink, Millicoma, and Coquille.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1 *Ch. brevirostris*	100																						
2 *Ch. cujui*	99.2	100																					
3 *D. luxatus*	100	99.2	100																				
4 *X. texanus*	99.2	99.2	99.2	100																			
5 *C. snyderi*	100	99.2	100	99.2	100																		
6 *C. occidentalis*	99.2	99.2	99.2	99.2	99.2	100																	
7 *C.sp.* Wall Canyon	99.2	99.2	99.2	99.2	99.2	99.2	100																
8 *C. warnerensis*	99.6	99.6	99.6	99.6	99.6	99.6	99.6	100															
9 *C. macrocheilus* Hood 230	97.7	97.7	97.7	97.7	97.7	97.7	97.7	98.1	100														
10 *C. macrocheilus* Hood 231	98.1	98.1	98.1	98.1	98.1	98.1	98.1	98.5	98.1	100													
11 *C. macrocheilus* Willamette	99.6	99.6	99.6	99.6	99.6	99.6	99.6	100	98.1	98.5	100												
12 *C. macrocheilus* Umpqua	98.8	98.8	98.8	98.8	98.8	98.8	99.6	99.2	97.3	97.7	99.2	100											
13 *C. macrocheilus* group	99.2	99.2	99.2	99.2	99.2	99.2	100	99.6	97.7	98.1	99.6	99.6	100										
14 *C. macrocheilus* Siuslaw	98.8	98.8	98.8	98.8	98.8	98.8	99.6	99.2	97.3	97.7	99.2	99.2	99.6	100									
15 *C. columbianus* Booners	95.2	95.2	95.2	95.2	95.2	95.2	95.2	95.6	94.5	94.9	95.6	95.6	95.2	94.9	100								
16 *C. columbianus* Kiger	96.2	96.2	96.2	96.2	96.2	96.2	96.2	96.6	94.8	95.1	96.6	95.9	96.2	95.9	95.2	100							
17 *C. platyrhynchus*	98.1	98.1	98.1	98.1	98.1	98.1	98.1	98.5	96.6	97.0	98.5	97.7	98.1	97.7	94.1	95.1	100						
18 *H. etowanum*	99.6	98.8	99.6	98.8	99.6	98.8	99.6	99.2	97.3	97.7	99.2	99.2	99.6	99.2	94.9	95.9	97.7	100					
19 *H. nigricans*	100	99.2	100	99.2	100	99.2	99.2	99.6	97.7	98.1	99.6	98.8	99.2	98.8	95.2	96.2	98.1	99.6	100				
20 *T. rhothoeca*	97.4	97.4	97.4	97.4	97.4	97.4	98.1	97.7	96.3	96.3	97.7	97.7	98.1	97.7	93.5	94.4	96.3	97.7	97.4	100			
21 *M. melanops*	98.8	98.1	98.8	98.1	98.8	98.1	98.1	98.5	96.6	97.0	98.5	97.7	98.1	97.7	94.1	95.1	97.0	98.5	98.8	96.3	100		
22 *Mox. duquesnei*	100	99.2	100	99.2	100	99.2	99.2	99.6	97.7	98.1	99.6	98.8	99.2	98.8	95.2	96.2	98.1	99.6	100	97.4	98.8	100	
23 *Mox. pappillosum*	99.6	99.6	99.6	99.6	99.6	99.6	99.6	100	98.1	98.5	100	99.2	99.6	99.2	95.6	96.6	98.5	99.2	99.6	97.7	98.5	99.6	100

Table 3. 7: Locus 39 informative sites. Shaded cells are ancestral or equivocal states at the Catostomini level (2 are equivocal). Upper Klamath Basin species in boldface. The *C. macrocheilus* group is Willamette, Umpqua, Siuslaw, Millicoma, Coquille.

Site(s) number Taxon	10	58	198
CATOSTOMINI			
Ch. brevirostris	G	G	A
Ch. cujui	G	A	A
D. luxatus	G	G	A
X. texanus	G	A	A
C. snyderi	G	G	A
C. occidentalis	G	A	A
C.sp. Wall canyon	G	A	T
C. warnerensis	G	A	A
C. macrocheilus group	G	A	T
C. macrocheilus Hood	T	A	A
C. macrocheilus Woahink	G	A	A
C. columbianus Booners	T	A	A
C. columbianus Kiger	G	A	A
C. platyrhynchus	G	A	A
MOXOSTOMATINI			
H. etowanum	G	G	T
H. nigricans	G	G	A
T. rhothoeca	G	A	T
M. melanops	G	G	A
Mox. duquesnei	G	G	A
Mox. pappillosum	G	A	A

Locus 81

Locus 81 had six allele sizes ranging from 197-241 bp (Table 3.8). Four size variants were unique for individual species: *E. oblongus* (197 bp), *T. rhothoeca* (229 bp), *Mox. duquesnei* (230 bp), and *Mox. macrolepidotum* (241 bp). The three Upper Klamath Basin species shared a 201 bp allele with some or all Catostominae, Ictobinae, and Cycleptinae. Only eight sites were variable for western Catostomini, producing a few autapomorphic SNPs for some species or populations.

Sequence similarity within tribes was highly variable: 98-100% in Catostomini and 66-100% in Moxostomatini. (Table 3.9). The three Upper Klamath Basin species appeared to have an ancestral sequence and were 100% identical to most western catostomines as well as *Hypentelium*, both ictiobines and the cycleptine. There was no indication of site saturation. Only 17 of 78 informative sites had three states and most involved large moxostomatine insertions at sites 67-90 and 131-147 (data not shown). With the exception of three SNPs involving *C. warnerensis, C. columbianus* and *E. oblongus,* 96.3% of the variation within locus 81 was within moxostomatins excluding *Hypentelium. Thoburnia rhothoeca*, *Mox. duquesnei* and *pappillosum* shared 40 unique sites. These plus *M. melanops* and *Mox. macrolepidotum* shared an additional 17 sites. *Minytrema melanops* and *Mox. macrolepidotum* shared 29 unique sites and *E. oblongus* contained 29 autapomorphic sites. Excluding *Hypentelium,* Moxostomatini were resolved as monophyletic, supported in 100% of bootstrap replicates (Figure 4.1).

Locus 120

Locus 120 had eight allele sizes ranging from 396- 413 bp (Table 3.10). Only *C. commersoni* (401 bp) had a unique size variant. This locus had many species and population autapomorphies. The three Upper Klamath Basin species shared a common size variant (402 bp) that differed by one base pair from a variant (403 bp) found in ten species including catostomines, moxostomatines, and cycleptines (Table 3.10).

Table 3.8: Locus 81 allele sizes and autapomorphies listed as single nucleotide polymorphisms (SNP) and insertion/deletions (InDels). Upper Klamath species are in boldface. The *C. macrocheilus* group is Willamette, Hood, Umpqua, Woahink, Siuslaw, and Coquille.

Taxon	Allele Size (bps)	Autapomorphies SNP	Autapomorphies In/Del (bps)	Site numbers
CATOSTOMINI				
Ch. brevirostris	201			
Ch. cujui	201			
D. luxatus	201			
X. texanus	201	2		118, 239
C. snyderi	201			
C. occidentalis	201			
C. microps	201			
C.sp. Wall Canyon	201			
C. warnerensis	201			
C. macrocheilus group	201			
C. macrocheilus Millicoma	201	1		238
C. commersoni	201			
C. columbianus	201			
C. platyrhynchus	201			
MOXOSTOMATINI				
H. etowanum	201			
H. nigricans	201			
E. oblongus	197	8	11, 1	17,19,43,59, 91-101, 104, 129, 165, 167, 239
E. oblongus NC	197	4		108, 109, 115, 222
E. oblongus Ala	197	5		67, 154, 177, 182, 188
M. melanops	231			
T. rhothoeca	229	6		37, 63, 127, 173, 207, 219
Mox. macrolepidotum	241			
Mox. duquesnei	230	3		19, 48, 191
Mox. pappillosum	231	4		6, 134, 146, 151
ICTIOBINAE				
Ca. carpio	201			
Ca. cyprinus	201			
CYCLEPTINAE				
Cycleptus elongatus	201			
Total		33	2	

Table 3.9: Identity matrix for 26 taxa for Locus 81. The *C. macrocheilus* group is Hood, Willamette, Umpqua, Woahink, Siuslaw, and Coquille.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *Ch. brevirostris*	100												
2 *Ch. cujui*	100	100											
3 *D. luxatus*	100	100	100										
4. *X. texanus*	99.0	99.0	99.0	100									
5 *C. snyderi*	100	100	100	99.0	100								
6 *C. occidentalis*	100	100	100	99.0	100	100							
7 *C. microps*	100	100	100	99.0	100	100	100						
8 *C. sp.* Wall Canyon	100	100	100	99.0	100	100	100	100					
9 *C. warnerensis*	99.0	99.0	99.0	98.0	98.0	98.0	98.0	99.0	100				
10 *C. macrocheilus* group	100	100	100	99.0	100	100	100	100	98.0	100			
11 *C. macrocheilus* Millicoma	99.5	99.5	99.5	98.5	99.5	99.5	99.5	99.5	98.0	99.5	100		
12 *C. commersoni*	99.5	99.5	99.5	98.5	99.5	99.5	99.5	99.5	98.0	99.5	99.0	100	
13 *C. columbianus*	98.5	98.5	98.5	97.5	98.5	98.5	98.5	98.5	100	98.5	98.0	98.0	100
14 *C. platyrhynchus*	100	100	100	99.0	100	100	100	100	98.0	100	99.5	99.5	98.5
15 *H. etowanum*	100	100	100	99.0	100	100	100	100	98.0	100	99.5	99.5	98.5
16 *H. nigricans*	100	100	100	99.0	100	100	100	100	98.0	100	99.5	99.5	98.5
17 *E. oblongus* NC	78.7	78.7	78.7	78.3	78.7	78.7	78.7	78.7	78.0	78.7	78.3	78.7	78.3
18 *E. oblongus* AL	78.3	78.3	78.3	77.8	78.3	78.3	78.3	78.3	78.0	78.3	77.8	78.3	77.8
19 *M. melanops*	76.8	76.8	76.8	76.0	76.8	76.8	76.8	76.8	76.0	76.8	76.4	77.2	76.0
20 *T. rhothoeca*	70.9	70.9	70.9	70.1	70.9	70.9	70.9	70.9	70.0	70.9	70.5	71.3	69.7
21 *Mox. macrolepidotum*	77.2	77.2	77.2	76.4	77.2	77.2	77.2	77.2	76.0	77.2	76.8	77.6	76.4
22 *Mox. duquesnei*	71.0	71.0	71.0	70.2	71.0	71.0	71.0	71.0	71.0	71.0	70.6	71.4	70.6
23 *Mox. pappillosum*	71.4	71.4	71.4	70.6	71.4	71.4	71.4	71.4	70.0	71.4	71.0	71.9	70.2
24 *Ca. carpio*	100	100	100	99.0	100	100	100	100	98.0	100	99.5	99.5	98.5
25 *Ca. cyprinus*	100	100	100	99.0	100	100	100	100	98.0	100	99.5	99.5	98.5
26 *Cy. elongatus*	100	100	100	99.0	100	100	100	100	98.0	100	99.5	99.5	98.5

Table 3.9: continued.

Taxon	14	15	16	17	18	19	20	21	22	23	24	25	26
1 *Ch. brevirostris*													
2 *Ch. cujui*													
3 *D. luxatus*													
4. *X. texanus*													
5 *C. snyderi*													
6 *C. occidentalis*													
7 *C. microps*													
8 *C. sp.* Wall Canyon													
9 *C. warnerensis*													
10 *C. macrocheilus* group													
11 *C. macrocheilus* Millicoma													
12 *C. commersoni*													
13 *C. columbianus*													
14 *C. platyrhynchus*	100												
15 *H. etowanum*	100	100											
16 *H. nigricans*	100	100	100										
17 *E. oblongus* NC	78.7	78.7	78.7	100									
18 *E. oblongus* AL	78.3	78.3	78.3	94.9	100								
19 *M. melanops*	76.8	76.8	76.8	73.3	72.0	100							
20 *T. rhothoeca*	70.9	70.9	70.9	67.0	67.0	82.6	100						
21 *Mox. macrolepidotum*	77.2	77.2	77.2	72.7	71.4	99.1	82.6	100					
22 *Mox. duquesnei*	71.0	71.0	71.0	66.5	66.5	85.0	93.0	85.8	100				
23 *Mox. pappillosum*	71.4	71.4	71.4	66.5	67.3	84.2	92.6	85.1	96.5	100			
24 *Ca. carpio*	100	100	100	78.7	78.3	76.8	70.9	77.2	71.0	71.4	100		
25 *Ca. cyprinus*	100	100	100	78.7	78.3	76.8	70.9	77.2	71.0	71.4	100	100	
26 *Cy. elongatus*	100	100	100	78.7	78.3	76.8	70.9	77.2	71.0	71.4	100	100	100

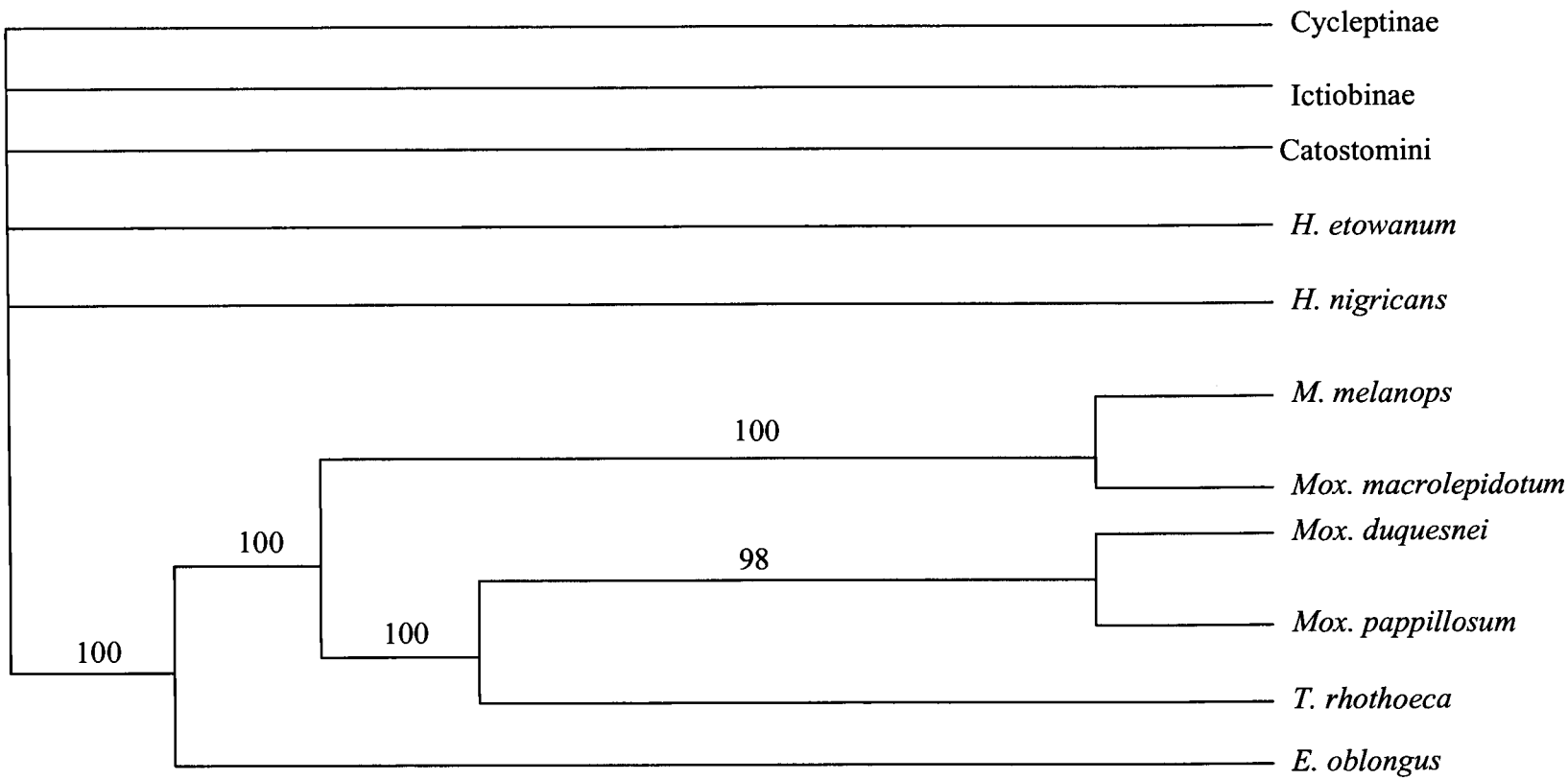

Figure 3.2: Locus 81 phylogenetic relationships of Catostomidae based on parsimony analysis. Topography depicts a strict consensus of 196 trees yielded by an un-weighted analysis (151 steps CI = 0.793, RC=0.679). Numbers above the branches are bootstrap percent replicates.

Table 3.10: Locus 120 allele sizes and autapomorphies listed as single nucleotide polymorphisms (SNP) and insertion/deletions (InDels). Upper Klamath Basin species in boldface. The *C. macrocheilus* group is Willamette, Hood, Umpqua, Siuslaw, and Coquille. Autapomorphies in parenthesis are not included in totals.

Taxon	Allele Size(bp)	Autapomorphies SNP	Autapomorphies InDel(bp)	Site numbers
CATOSTOMINI				
Ch. brevirostris	402			
Ch. cujui	405			
D. luxatus	402	4		308, 345, 365, 427
X. texanus	396	1		395
C. snyderi	402			
C. rimiculus	398		1	291
C. occidentalis	397	2		27, 349
C. microps	403	1		349
C.sp. Wall Canyon	397	2		140, 304
C. warnerensis	403			
C. macrocheilus group	397			
C. macrocheilus Woahink	396	1		426
C. macrocheilus Millicoma	397	1		107
C. commersoni	401	5	1, 1	27,318, 324, 325, 335, 336,422
C. columbianus Booners	398	1		144
C. columbianus Kiger	405			
C. platyrhynchus	403			
MOXOSTOMATINI				
H. etowanum	403			
E. oblongus NC	403	1		346
E. oblongus Ala	403			
M. melanops	403			
T. rhothoeca	403			
Mox. macrolepidotum	403			
Mox. pappillosum	403			
ICTIOBINAE				
Carpiodes		(15)	(21, 10)	41, 44, 94, 98, 101, 120, 168, 254, 263-283, 304, 305, 332, 363-372, 373, 418
Ca. carpio	413	1		185
Ca. cyprinus	413	1		360

Table 3. 10: continued.

CYCLEPTINAE			
Cycleptus elongatus	403		
Total		21	3

Sequence similarity was high within tribes; 93-100% in catostomines and 99-100% in moxostomatines (Table 3.11). Ictiobines were the most distinct, differing by 15-17% from all other catostomids. For genera and subgenera with two or more species, sequence similarity was: *Chasmistes* – 98.7%, *Catostomus* – 92.8-99.4%, *C. (Pantosteus)* – 96-98%, *Erimyzon* – 98.7%, *Carpiodes* – 99.5% and *Moxostoma* – 100%. The Upper Klamath *Ch. brevirostris* and *C. snyderi* were 99.7% identical to *C. platyrhynchus*; Cycleptus and all moxostomatines (Table 3.11) except the North Carolina sample of *E. oblongus*.

There was no indication of site saturation with only four informative sites having three states, one of which was the multi-base insertion/deletion at 256-262 (Table 3.12). Character state polarity at the level of catostomins was ambiguous at only two of the 14 sites. Derived informative sites, especially the sequence change at 256-262, and 17 other synapomorphies provided strong support for Ictiobinae (Tables 3.10, 3.12). There was no derived support for any other taxon. Several sites (238, 255, 256-262, 302) provided support for a western grouping of *X. texanus*, *C. rimiculus, C. occidentalis, C. sp., C. macrocheilus,* and Booners Cr. *C. columbianus.* Site 285 contained a deletion unique to the three Upper Klamath Basin species. With the exception of site 285, the three Upper Klamath Basin species were otherwise almost identical to moxostomatins and possessed the ancestral or equivocal state for all other informative sites.

Locus 184

Locus 184 had 13 size variants from 303 to 363 bp (Table 3.13). Six size variants were unique: *Ch. cujui* (361 bp), *C. warnerensis* (363 bp), *C. sp.* (353 bp), *C. commersoni* (303 bp), *C. platyrhynchus* (349 bp), and *T. rhothoeca* (330 bp). The three *Moxostoma* species shared a 327 bp allele. Autapomorphies were found for twelve taxa including several *C. macrocheilus* populations (Table 3.13).

Sequence similarity within tribes was 73.7-99.7% (Catostomini) and 80.9-99.6% (Moxostomatini) (Table 3.14). For genera or subgenera represented by two or more species, identity was 99.4% (*Chasmistes*), 77.6-99.7% (*C. Catostomus*), 87.5% (*C. Pantosteus*), 99.3-99.6% *(Moxostoma*). Most of the dissimilarity in catostomines is due to *C. commersoni* and its 49 bp autapomorphic deletion. Excluding *C. commersoni,* the western *C.* (*Catostomus*) had similarities of 92.5-99.7%. Among western *Catostomus, C. platyrhynchus* was most dissimilar and differed by an average of 11.6% from others and 12.5% from the other *Pantosteus*, *C. columbianus*. The three Upper Klamath Basin species were most similar (>97.7%) to other western species, *Ch. cujui, X. texanus, C. occidentalis* and *C. warnerensis*.

Three informative sites (236, 338 and 350) were approaching saturation with four states for each site (Table 3.15) while 14 others had three states and all other had two, again suggesting little evidence of site saturation. Character state polarity at the level of catostomins was equivocal at ten of 52 sites. There was some support for various taxonomic groupings; Catostomini and Moxostomatini have different states for four sites (86, 144, 303, 307). A fifth site (43) also exhibited tribal division with the exception of *C. commersoni*, although these five sites are equivocal in regards to the polarity of the phylogenetic signal. *Moxostoma was* supported by this locus by its unique deletion between 160-170 and derived states at six other sites (24, 73, 271, 273, 290, 341). Overall sharing of sites was sometimes high with, for example, *C. macrocheilus* sharing 43 sites with *C. microps*. The three Upper Klamath Basin species shared a unique base at site 78 (Table 3.15). Generally, Upper Klamath Basin species were most like the western species, *Ch. cujui, X. texanus*, and *C. occidentalis*, which differed by nine informative sites. However other western species, *C. macrocheilus*, *C. microps* and *C. columbianus*, differed from Upper Klamath Basin species at all or most of 18 informative sites. In contrast, at least one moxostomatin matched the Klamath sequence at all but ten sites (Table 3.14).

Overall, the patterns for the five loci, the number of autapomorphies per species was similar between the two tribes with catostomins having more at loci 39 and 120 and moxostomatins more at the other three loci (Table 3.16). The weighted average number of autapomorphies was slightly higher for moxostomatins. The three Upper Klamath Basin species were noteworthy in their extremely low number of autapomorphies.

For the three loci with some informative variation in Catostomini (loci 4, 120 and 184), moxostomatins tended to have the least number of derived sites while catostomins, including the Upper Klamath species, had about 5 per taxon (Table 3.17). Of course variation in locus 81 (not shown in Table 3.17) was almost entirely in moxostomatins.

Table 3.11: Identity matrix for 27 taxa for locus 120. The *C. macrocheilus* group is Hood, Willamette, Umpqua, Siuslaw, and Coquille.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *Ch. brevirostris*	100												
2 *Ch. cujui*	98.7	100											
3 *D. luxatus*	98.7	97.5	100										
4 *X. texanus*	95.5	96.2	94.8	100									
5 *C. snyderi*	100	98.7	98.7	95.5	100								
6 *C. rimiculus*	95.3	96.0	94.5	98.4	95.3	100							
7 *C. occidentalis*	95.0	96.2	94.3	98.2	95.0	98.7	100						
8 *C. microps*	96.7	98.0	96.0	96.7	96.7	96.7	97.2	100					
9 *C.sp.* Wall Canyon	94.8	96.0	94.0	98.2	94.8	97.7	97.9	96.5	100				
10 *C. warnerensis*	96.7	98.0	96.0	96.7	96.7	96.3	96.5	99.0	97.5	100			
11 *C. macrocheilus* group	95.5	96.7	94.8	99.4	95.5	98.4	98.7	97.2	98.7	97.2	100		
12 *C. macrocheilus* Woahink	95.5	96.2	95.0	99.4	95.5	98.4	98.2	96.7	98.2	96.7	99.4	100	
13 *C. macrocheilus* Millicoma	95.8	97.0	95.0	98.7	95.8	98.2	98.4	97.0	98.4	97.0	99.2	99.2	100
14 *C. commersoni*	96.7	96.0	95.5	93.5	96.7	93.8	93.5	94.8	92.8	94.5	93.5	93.5	93.5
15 *C. columbianus* Booners	95.5	96.7	94.8	98.9	95.5	98.2	98.4	96.7	98.2	96.7	99.4	99.4	99.4
16 *C. columbianus* Kiger	98.0	98.7	97.2	96.5	98.0	96.0	96.0	97.7	95.8	97.7	97.0	97.0	97.0
17 *C. platyrhynchus*	99.7	99.0	98.5	95.7	99.7	95.5	95.3	97.0	95.0	97.0	95.8	95.8	95.8
18 *H. etowanum*	99.7	99.0	98.5	95.7	99.7	95.5	95.3	97.0	95.0	97.0	95.8	95.8	95.8
19 *E. oblongus* NC	98.5	97.7	97.7	95.2	98.5	95.0	94.8	96.2	94.5	96.2	95.3	95.3	95.3
20 *E. oblongus* AL	99.7	99.0	98.5	95.7	99.7	95.5	95.3	97.0	95.0	97.0	95.8	95.8	95.8
21 *M. melanops*	99.7	99.0	98.5	95.7	99.7	95.5	95.3	97.0	95.0	97.0	95.8	95.8	95.8
22 *T. rhothoeca*	99.7	99.0	98.5	95.7	99.7	95.5	95.3	97.0	95.0	97.0	95.8	95.8	95.8
23 *Mox. macrolepidotum*	99.7	99.0	98.5	95.7	99.7	95.5	95.3	97.0	95.0	97.0	95.8	95.8	95.8
24 *Mox. pappillosum*	99.7	99.0	98.5	95.7	99.7	95.5	95.3	97.0	95.0	97.0	95.8	95.8	95.8
25 *Ca. carpio*	84.7	85.1	84.2	84.6	84.7	84.9	84.7	85.6	84.6	85.3	85.1	85.1	85.1
26 *Ca. cyprinus*	84.7	85.1	84.2	84.6	84.7	84.9	84.7	85.6	84.6	85.3	85.1	85.1	85.1
27 *Cy. elongatus*	99.7	99.0	98.5	95.7	99.7	95.5	95.3	97.0	95.0	97.0	95.8	95.8	95.8

Table 3.11: continued.

Taxon	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 *Ch. brevirostris*														
2 *Ch. cujui*														
3 *D. luxatus*														
4 *X. texanus*														
5 *C. snyderi*														
6 *C. rimiculus*														
7 *C. occidentalis*														
8 *C. microps*														
9 *C.sp.* Wall Canyon														
10 *C. warnerensis*														
11 *C. macrocheilus* group														
12 *C. macrocheilus* Woahink														
13 *C. macrocheilus* Millicoma														
14 *C. commersoni*	100													
15 *C. columbianus* Booners	93.8	100												
16 *C. columbianus* Kiger	96.0	97.0	100											
17 *C. platyrhynchus*	96.7	95.8	98.0	100										
18 *H. etowanum*	97.0	95.8	98.0	100	100									
19 *E. oblongus* NC	96.7	95.3	98.0	98.7	98.7	100								
20 *E. oblongus* AL	97.0	95.8	98.0	100	100	98.7	100							
21 *M. melanops*	97.0	95.8	98.0	100	100	98.7	100	100						
22 *T. rhothoeca*	97.0	95.8	98.0	100	100	98.7	100	100	100					
23 *Mox. macrolepidotum*	97.0	95.8	98.0	100	100	98.7	100	100	100	100				
24 *Mox. pappillosum*	97.0	95.8	98.0	100	100	98.7	100	100	100	100	100			
25 *Ca. carpio*	83.2	84.7	85.0	84.9	84.9	84.7	84.9	84.9	84.9	84.9	84.9	100		
26 *Ca. cyprinus*	83.2	84.7	85.0	84.9	84.9	84.7	84.9	84.9	84.9	84.9	84.9	99.5	100	
27 *Cy. elongatus*	97.0	95.8	100	100	100	98.7	100	100	100	100	100	84.9	84.9	100

Table 3.12: Locus 120 informative sites. Shaded cells are ancestral or equivocal states at the Catostomini level (two are equivocal). Upper Klamath Basin species in boldface; del represents gap associated with insertion/deletion polymorphisms. The *C. macrocheilus* group is Willamette, Hood Umpqua, Siuslaw, and Coquille.

Site(s) number Taxon	131	133	206	238	255	256-261	262	285	286	287	302	309	348	368	374
CATOSTOMAINI															
Ch. brevirostris	C	T	G	C	T	ATTCCC	C	del	del	del	A	T	C	C	T
Ch. cujui	A	T	G	C	T	ATTCCC	C	A	A	A	A	T	C	A	T
D. luxatus	C	T	G	C	T	ATTCCC	C	del	del	del	A	A	C	C	T
X. texanus	A	G	A	G	del	del	C	A	A	del	G	A	del	A	T
C. snyderi	C	T	G	C	T	ATTCCC	C	del	del	del	A	T	C	C	T
C. rimiculus	A	T	A	G	del	del	C	A	A	del	G	A	A	A	C
C. occidentalis	A	T	A	G	del	del	C	A	A	A	G	A	A	A	C
C. microps	A	T	G	G	T	ATTCCC	del	A	A	A	G	A	del	A	C
C.sp. Wall Canyon	A	T	A	G	del	del	C	A	A	A	G	A	del	A	T
C. warnerensis	A	T	A	G	T	ATTCCC	del	A	A	A	G	A	del	A	T
C. macrocheilus group	A	G	A	G	del	del	C	A	A	A	G	A	del	A	T
C. macrocheilus Woahink	A	G	A	G	del	del	C	A	A	del	G	A	del	A	T
C. macrocheilus Millicoma	A	T	A	C	del	del	C	A	A	A	G	A	del	A	T
C. commersoni	C	T	A	C	C	ATTCCC	C	A	del	del	A	T	C	C	A
C. columbianus Booners	A	G	A	G	del	del	C	A	A	A	G	A	C	A	T
C. columbianus Kiger	A	G	A	C	T	ATTCCC	C	A	A	A	A	A	C	C	T
C. platyrhynchus	C	T	G	C	T	ATTCCC	C	A	del	del	A	T	C	C	T
MOXOSTOMATINI															
H. etowanum	C	T	G	C	T	ATTCCC	C	A	del	del	A	T	C	C	T
E. oblongus NC	C	T	G	C	C	ATTCCC	C	A	del	del	A	A	C	C	T

Table 3.12: continued.

E. oblongus AL	C	T	G	C	T	ATTCCC	C	A	del	del	A	T	C	C	T
M. melanops	C	T	G	C	T	ATTCCC	C	A	del	del	A	T	C	C	T
T. rhothoeca	C	T	G	C	T	ATTCCC	C	A	del	del	A	T	C	C	T
Mox. macrolepidotum	C	T	G	C	T	ATTCCC	C	A	del	del	A	T	C	C	T
Mox. pappillosum	C	T	G	C	T	ATTCCC	C	A	del	del	A	T	C	C	T
ICTIOBINAE															
Ca. carpio	C	T	A	G	T	CTCAAG	A	A	A	A	A	A	del	del	A
Ca. cyprinus	C	T	A	G	T	CTCAAG	A	A	A	A	A	A	del	del	A
CYCLEPTINAE															
Cy. elongatus	C	T	G	C	T	ATTCCC	C	A	del	del	A	T	C	C	T

Table 3.13: Locus 184 allele sizes and autapomorphies listed as single nucleotide polymorphisms (SNP) and insertion/deletions (InDels). Upper Klamath Basin species in boldface. The *C. macrocheilus* group is Umpqua, Millicoma, and Coquille.

Taxon	Allele size (bps)	Autapomorphies SNP	Autapomorphies InDel (bps)	Site numbers
CATOSTOMINI				
Ch. brevirostris	362			
Ch. cujui	361			
D. luxatus	362			
X. texanus	358	4		145, 332, 340, 365
C. snyderi	362			
C. rimiculus	362			
C. occidentalis	356	1	6	149, 352-357
C. microps	359	1		338
C.sp. Wall Canyon	353		4	348-351
C. warnerensis	363	2		162, 163
C. macrocheilus 231 Hood	360			
C. macrocheilus Willamette	357	1	1	198, 357
C. macrocheilus group	359			
C. macrocheilus Woahink	360	2		36, 179
C. macrocheilus Siuslaw	356	2	2	136, 206, 366-367
C. commersoni	303	5	49	43, 85,143, 106, 267, 289-347
C. columbianus	362	1		241
C. platyrhynchus	349	14	13, 4	25, 26-38, 63, 80, 81, 82, 210, 236-239, 257, 261, 265, 279, 290, 321, 354, 363
MOXOSTOMATINI				
E. oblongus NC	358	17		20, 53, 150, 178, 190, 215, 234, 236, 246, 260, 269, 275, 305, 311, 315, 327, 354
T. rhothoeca	330	7	7	3, 34, 63, 101, 120, 279, 285-291, 322
M. melanops	357	4	1	53, 79,147, 194, 234
Mox. macrolepidotum	327			
Mox. duquesnei	327			
Mox. pappillosum	327			
Total		61	9	

Table 3.14: Identity matrix from locus 184 sequences.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12
1 *Ch. brevirostris*	100											
2 *Ch. cujui*	99.4	100										
3 *D. luxatus*	100	99.4	100									
4 *X. texanus*	97.2	97.2	97.2	100								
5 *C. snyderi*	100	99.4	100	97.2	100							
6 *C. occidentalis*	97.7	97.7	97.7	97.2	97.7	100						
7 *C. microps*	93.6	94.2	93.6	92.8	93.6	93.1	100					
8 *C. sp.* Wall Canyon	96.1	96.6	96.1	95.0	96.1	95.5	92.8	100				
9 *C. warnerensis*	98.3	98.3	98.3	96.1	98.3	96.6	93.6	96.6	100			
10 *C. macrocheilus* Hood 231	93.9	94.4	93.9	92.8	93.9	92.8	98.8	92.8	93.9	100		
11 *C. macrocheilus* Willamette	94.4	95.0	94.4	93.3	94.4	93.3	96.6	93.9	94.4	97.2	100	
12 *C. macrocheilus* Umpqua	95.3	95.3	95.3	94.2	95.3	94.2	97.5	94.1	95.3	98.0	98.6	100
13 *C. macrocheilus* Woahink	94.7	94.7	94.7	93.6	94.7	93.6	96.9	93.1	94.7	97.5	98.0	98.8
14 *C. macrocheilus* Siuslaw	94.2	94.2	94.2	93.6	94.2	93.1	96.3	92.5	94.2	96.9	97.2	98.3
15 *C. macrocheilus* Millicoma	95.5	95.5	95.5	94.4	95.5	94.4	97.7	93.9	95.5	98.3	98.3	99.7
16 *C. macrocheilus* Coquille	95.3	95.3	95.3	94.2	95.3	94.4	98.0	93.6	95.3	98.0	98.0	99.4
17 *C. commersoni*	81.7	81.7	81.7	80.1	81.7	80.1	77.7	79.5	80.7	77.7	77.9	78.7
18 *C. columbianus*	94.2	94.2	94.2	93.1	94.2	93.1	98.6	92.6	94.2	99.1	96.9	98.3
19 *C. platyrhynchus*	90.1	90.1	90.1	88.2	90.1	88.7	86.9	88.4	90.1	87.1	88.2	89.0
20 *E. oblongus* NC	90.0	90.6	90.0	88.9	90.0	90.3	87.3	89.1	89.8	88.4	88.6	88.6
21 *M. melanops*	93.1	93.6	93.1	92.7	93.1	93.0	91.1	93.3	93.3	90.9	91.9	92.2
22 *T. rhothoeca*	85.0	85.5	85.0	83.3	85.0	83.4	82.0	83.3	84.5	82.0	82.5	82.8
23 *Mox. macrolepidotum*	84.5	85.0	84.5	82.8	84.5	82.9	80.7	82.8	84.6	81.3	81.7	82.0
24 *Mox. duquesnei*	84.8	85.3	84.8	83.1	84.8	83.1	81.0	83.1	84.8	81.5	82.0	82.3
25 *Mox. pappillosum*	84.8	85.3	84.8	83.1	84.8	83.1	81.0	83.1	84.8	81.5	82.0	82.3

Table 3.14: continued.

Taxon	13	14	15	16	17	18	19	20	21	22	23	24	25
1. *Ch. brevirostris*													
2. *Ch. cujui*													
3. *D. luxatus*													
4. *X. texanus*													
5. *C. snyderi*													
6. *C. occidentalis*													
7. *C. microps*													
8. *C.sp.* 74 Wall Canyon													
9. *C. warnerensis*													
10. *C. macrocheilus* 231 Hood													
11. *C. macrocheilus* Willamette													
12. *C. macrocheilus* Umpqua													
13. *C. macrocheilus* Woahink	100												
14. *C. macrocheilus* Siuslaw	98.0	100											
15. *C. macrocheilus* Millicoma	99.1	98.6	100										
16. *C. macrocheilus* Coquille	98.8	98.3	99.7	100									
17. *C. commersoni*	78.2	77.6	79.0	78.7	100								
18. *C. columbianus* B & K	97.7	97.2	98.6	98.3	78.0	100							
19. *C. platyrhynchus*	88.2	87.4	88.7	88.5	73.7	87.5	100						
20. *E. oblongus* NC	87.6	87.0	88.4	88.1	74.3	87.6	82.7	100					
21. *M. melanops*	91.1	90.6	92.0	91.7	76.5	90.6	86.3	91.4	100				
22. *T. rhothoeca*	81.8	81.2	82.6	82.3	70.1	81.9	80.1	81.1	85.3	100			
23. *Mox. macrolepidotum*	81.0	80.4	81.8	81.5	68.8	80.8	78.5	80.9	83.9	90.5	100		
24. *Mox. duquesnei*	81.3	80.7	82.1	81.8	68.5	81.1	78.7	81.2	84.2	90.8	99.6	100	
25. *Mox. pappillosum*	81.3	80.7	82.1	81.8	68.5	81.1	78.7	81.2	84.2	90.8	99.0	99.3	100

Table 3.15: Locus 184 informative sites. Shaded cells are ancestral or equivocal states at the Catostomini level (seven are equivocal). Upper Klamath Basin species in boldface; del represents gap associated with insertion/deletion polymorphisms. The *C. macrocheilus* group is Willamette, Umpqua, Woahink, Siuslaw, Millicoma, and Coquille.

Site(s) number	5	17	24	43	50	64	73	78	86	99	109	117	132	139	144	151	160-164	165	166-170
Taxon																			
CATOSTOMINI																			
Ch. brevirostris	G	C	A	G	A	T	G	G	T	C	A	T	C	C	A	A	CTTCA	T	CTTAA
Ch. cujui	G	C	A	G	A	T	G	T	T	C	A	T	C	C	A	A	CTTCA	T	CTTAA
D. luxatus	G	C	A	G	A	T	G	G	T	C	A	T	C	C	A	A	CTTCA	T	CTTAA
X. texanus	G	C	A	G	A	T	G	T	T	C	C	T	C	C	A	A	CTTCA	T	CTTAA
C. snyderi	G	C	A	G	A	T	G	G	T	C	A	T	C	C	A	A	CTTCA	T	CTTAA
C. rimiculus	G	C	A	G	A	T	G	G	T	C	A	T	C	C	A	A	CTTCA	T	CTTAA
C. occidentalis	G	C	A	G	A	T	G	T	T	C	A	T	C	C	A	A	CTTCA	T	CTTAA
C. microps	G	C	A	G	A	T	G	T	T	T	C	T	T	A	A	A	CTTCA	G	CTTAA
C. sp. Wall Canyon	G	C	A	G	A	T	G	T	T	C	A	T	C	C	A	A	CTTCA	T	CTTAA
C. warnerensis	G	C	A	G	A	T	G	T	T	C	A	T	C	C	A	A	CTAAA	T	CTTAA
C. macrocheilus Hood 231	G	C	A	G	A	T	T	T	T	T	C	T	T	A	A	A	CTTCA	G	CTTAA
C. macrocheilus group	G	C	A	G	A	T	G	T	T	C	C	T	T	C	A	A	CTTCA	G	CTTAA
C. commersoni	G	C	A	C	A	T	G	T	T	C	A	T	C	C	A	A	CTTCA	T	CTTAA
C. columbianus	G	C	A	G	A	T	G	T	T	T	C	T	T	A	A	A	CTTCA	G	CTTAA
C. platyrhynchus	G	C	A	G	A	T	G	T	T	C	A	T	C	C	A	A	CTTCA	G	CTTAA
MOXOSTOMATINI																			
E. oblongus NC	G	G	A	A	A	C	T	T	A	T	A	T	C	C	G	A	CTTCA	T	CTTAA
M. melanops	G	G	A	A	A	C	G	T	A	C	A	T	C	C	G	A	CTTCA	G	CTTAA
T. rhothoeca	G	C	A	A	G	T	G	T	A	A	A	C	C	C	G	C	CTTCA	G	CTTAA
Mox. macrolepidotum	C	C	T	A	G	T	C	T	A	A	A	C	C	C	G	C	del	del	del

Table 3.15: continued.

Site(s) number	175	180	194	195-196	213	220	225-233	235	236	237-238	239	240	242-244	245	246-247
Mox. duquesnei	C	C	T	A	G	T	C	T	A	A	A	C	C	C	G	C	del	del	del
Mox. pappillosum	G	C	T	A	G	T	C	T	A	A	A	C	C	C	G	C	del	del	del
Taxon																			
CATOSTOMINI																			
Ch. brevirostris	G	T	T	TT	A	T	AAAAAAAAA	del	G	AA	A	del	GTT	G	GA
Ch. cujui	G	T	T	TT	A	T	AAAAAAAAA	del	G	AA	A	del	GTT	G	GA
D. luxatus	G	T	T	TT	A	T	AAAAAAAAA	del	G	AA	A	del	GTT	G	GA
X. texanus	G	T	T	TT	A	T	AAAAAAAAA	del	G	AA	A	del	GTT	G	GA
C. snyderi	G	T	T	TT	A	T	AAAAAAAAA	del	G	AA	A	del	GTT	G	GA
C. rimiculus	G	T	T	TT	A	T	AAAAAAAAA	del	G	AA	A	del	GTT	G	GA
C. occidentalis	G	T	T	TT	A	T	AAAAAAAAA	del	G	AA	A	del	GTT	G	GA
C. microps	G	A	del	del	G	A	AAAAAAAAA	A	A	AA	A	A	GTT	G	GA
C. sp. Wall Canyon	G	T	T	TT	A	A	AAAAAAAAA	A	G	AA	A	del	GTT	G	GA
C. warnerensis	G	T	T	TT	A	A	AAAAAAAAA	A	G	AA	A	del	GTT	G	GA
C. macrocheilus Hood 231	G	A	del	del	G	A	AAAAAAAAA	A	A	AA	A	A	GTT	G	GA
C. macrocheilus group	G	A	del	del	A	A	AAAAAAAAA	A	A	AA	A	del	GTT	G	GA
C. commersoni	G	A	T	TT	A	T	AAAAAAAAA	del	G	AA	A	del	GTT	G	GA
C. columbianus	G	A	T	TT	G	A	AAAAAAAAA	A	A	AA	A	A	GTT	G	GA
C. platyrhynchus	G	A	del	del	A	A	AAAAAAAAA	A	del	AA	del	del	GTT	G	GA
MOXOSTOMATINI															
E. oblongus NC	G	T	T	TT	A	A	AAAAAAAAA	T	T	AA	del	del	GTT	A	GA
M. melanops	G	T	A	TT	A	A	AAAAAAAAA	T	A	AA	del	del	GTT	G	GA
T. rhothoeca	A	T	T	TT	A	A	del	del	del	del	del	del	del	del	del

Table 3.15: continued.

Site(s) number	251-252	256	271	272	273	289	290	291	292	303	307	316	338	341	352	353	354	355
Mox. macrolepidotum	A	T	T	TT	A	A	del		del	del	del	del	del	del	del	del	del	
Mox. duquesnei	A	T	T	TT	A	A	del		del	del	del	del	del	del	del	del	del	
Mox. pappillosum	A	T	T	TT	A	A	del		del	del	del	del	del	del	del	del	del	
Taxon																		
CATOSTOMINI																		
Ch. brevirostris	AA	G	T	T	del	A	A	G	A	A	A	C	G	T	A	A	G	T
Ch. cujui	AA	G	T	del	del	A	A	G	T	A	A	C	G	T	A	A	G	T
D. luxatus	AA	G	T	T	del	A	A	G	A	A	A	C	G	T	A	A	G	T
X. texanus	AA	G	T	T	del	A	A	G	T	A	A	C	G	T	del	del	del	T
C. snyderi	AA	G	T	T	del	A	A	G	A	A	A	C	G	T	A	A	G	T
C. rimiculus	AA	G	T	T	del	A	A	G	A	A	A	C	G	T	A	A	G	T
C. occidentalis	AA	G	T	T	del	del	A	G	del	A	A	C	G	T	del	del	del	del
C. microps	AA	G	T	del	del	A	A	A	T	A	A	G	A	T	T	del	G	C
C. sp. Wall Canyon	AA	A	T	del	del	A	A	G	A	A	A	C	G	T	del	del	G	T
C. warnerensis	AA	A	T	T	del	A	A	G	A	A	A	C	G	T	A	A	G	T
C. macrocheilus Hood 231	AA	G	T	del	del	A	A	A	T	A	A	G	G	T	T	C	G	T
C. macrocheilus group	AA	G	T	T	del	A	A	G	T	A	A	G	G	T	T	C	G	C
C. commersoni	AA	G	T	T	del	A	del	G	del	A	A	del	del	T	A	A	G	T
C. columbianus	AA	G	T	T	del	A	A	A	T	A	A	G	G	T	T	C	G	T
C. platyrhynchus	AA	G	T	T	del	A	G	G	A	A	A	C	G	T	A	A	A	T
MOXOSTOMATINI																		
E. oblongus NC	AA	G	T	del	del	A	A	G	A	C	C	C	G	T	T	C	C	T
M. melanops	AA	A	T	del	del	A	A	G	A	C	C	C	T	T	del	del	del	T
T. rhothoeca	del	G	del	del	del	del	del	del	A	C	C	C	T	T	A	A	G	T

Table 3.15: continued.

Mox. macrolepidotum	del	G	A	del	A	A	C	G	A	C	C	C	G	C	A	A	G	T
Mox. duquesnei	del	G	A	del	A	A	C	G	A	C	C	C	G	C	A	A	G	T
Mox. pappillosum	del	G	A	del	A	A	C	G	A	C	C	C	G	C	A	A	G	T

Table 3.16: Average number of autapomorphies per species for Catostominae at five loci.

Locus	Moxostomatini		Catostomini (without Upper Klamath)		Upper Klamath species	
	Average	n	Average	n	Average	n
4	13.5	2	2.67	9	0	3
39	1.33	6	3.00	8	0	3
81	4.00	8	0.30	10	0	3
120	0.17	6	1.55	11	1.33	3
184	5.00	6	3.64	11	0	3
Weighted Average	3.50		2.21		0.27	

Table 3.17: Average number of derived informative sites per species for Catostominae at loci 4, 120 and 184.

Locus	Moxostomatini		Catostomini (less Upper Klamath)		Upper Klamath species	
	Average	n	Average	n	Average	n
4	0	2	4.33	9	9.00	3
120	0.33	6	8.55	11	1.00	3
184	13.67	6	6.91	11	4.00	3
Weighted average	6.00		6.74		4.67	

Discussion

The two non-coding loci (39 and 81) that were monomorphic in Upper Klamath Basin species were less conservative in other catostomids. Locus 39 had many SNPs, especially within *C. macrocheilus* and *C. columbianus*, but was otherwise fairly conservative. The Klamath character state at one of the three informative sites in locus 39 was only found in eastern moxostomatins, though its polarity was ambiguous. The entire Klamath sequence of locus 81 was identical to ictiobine and cycleptines. Locus 81 was

highly variable only in Moxostomatini which had most of the autapomorphies and informative sites at the locus, and differed from Catostomini by 21-30% (Table 3.9). The non-coding nature of these loci did not apparently affect their utility as species markers or phylogenetic tools, but their usefulness as species markers is clearly restricted. In the case of locus 39, most variation was between two species, (*C. macrocheilus* and *C. columbianus*) and in locus 81, most variation was restricted to one tribe (Moxostomatini) from one of three subfamilies.

The three coding loci (4, 120 and 184) consistently produced SNP and InDel autapomorphies in both eastern and western species and in some populations within species. Of the species surveyed at all three loci, two of the Upper Klamath Basin species (*Ch. brevirostris* and *C. snyderi*) and *Ch. cujui,* were the only species with no unique positions (Tables 3.2, 3.10 and 3.13). *Chasmistes, Catostomus* and *C.* (*Pantosteus*) were unsupported by synapomorphies at these loci, unlike the eastern genera, *Moxostoma* and *Carpiodes*. Harris and Mayden (2001) considered *Catostomus* potentially para- or polyphyletic and *Chasmistes* as a group that may not warrant recognition.

Informative sites at locus 4 distinguished the two Catostominae tribes and sites between 244 and 261 grouped Upper Klamath Basin species with *C. occidentalis*, an association that does not reflect current taxonomy. Upper Klamath Basin species also had 99.7% sequence identity with *C. occidentalis,* but only 95.2% with the lower Klamath *C. rimiculus.* Outgroup analysis suggests that the Upper Klamath Basin sequences were equivocal or advanced, but not ancestral. Informative sites at locus 120 distinguished Ictiobines (*Carpiodes*, 15 SNP, 2 indels) and Upper Klamath Basin species (1 SNP) but, otherwise, Upper Klamath Basin species were most like moxostomatines, sharing 10 of 14 sites with their sister group. Informative sites at locus 184 provided strong support for *Moxostoma* (11 SNP, 1 indel). Upper Klamath Basin species had minimal support (1 SNP) and shared 42 of 52 informative sites with at least one moxostomatin.

Because catostomids are tetraploids, different lineages could have different rates of diploidization (Ferris and Whitt, 1978) and we could have amplified paralogous genes. However, allopolyploids, like catostomids, are predicted to transition rapidly to disomic inheritance and duplicate genes diverged early (Ferris and Whitt, 1980). In part because

the polyploidization event was 50 million years ago, Ferris and Whitt (1980) concluded that catostomids "are exhibiting disomic inheritance like normal diploids, only with a higher than diploid level of duplicate genes." For us to amplify paralogous genes, primer regions would have had to remain unchanged while amplified regions mutated. We think it is most likely that we were examining orthologous genes.

In general, these loci provide support for the current taxonomy of subfamilies and tribes, although the topography of moxostomatin genera (Figure 3.2) is quite different from either Smith (1992) or Harris and Mayden (2001). There is some support for eastern genera but no support for western genera other than the species markers for the monotypic *Deltistes* and *Xyrauchen*. At the informative sites, there is no indication of site saturation since most sites only had two of five possible states. These loci also had autapomorphic sites that could distinguish species and/or populations within some species, but their discriminatory power was highly variable. As Smith (1992) found with morphological characters, a broad spectrum of characters differentiate clades and there is no correlation between a clade's antiquity and the characters that differentiate them. Using these five loci, allopatric populations of *C. macrocheilus* could be distinguished, but the three Upper Klamath Basin species could not. These loci suggest that a revision of western sucker taxonomy is needed, a view also held by Harris and Mayden (2001). Smith (1992) found that drainage vicariance was the dominant if not the only speciation mechanism in suckers and we found the Upper Klamath Basin species often shared informative sites with a variable group of western species, usually including *C. rimiculus*. *C occidentalis*, *Ch. cujui* and *X. texanus*. Of the thirteen derived states found in Upper Klamath Basin species at loci 4 and 184, *C. occidentalis* shared twelve.

Upper Klamath Basin species also consistently shared informative, usually ancestral or equivocal, sites with moxostomatins. However, they did not share the unique moxostomatin sites at locus 81 and locus 4 and, thus, we do not suggest that their classification in Catostomini is incorrect. Rather, the consistent presence of sequences shared by their sister group (Moxostomatini), or their more distant sister taxa (Ictiobinae and Cycleptinae), or other Catostomini suggests that the Upper Klamath Basin species largely retain the ancestral sequence for these loci.

Hybridization and massive introgression could also have produced sequence similarity in Upper Klamath Basin species. Because the Klamath hybridization is among three genera, this could have been effectively resolved if these loci had corroborated the taxonomy of western genera, but they did not. The best evidence in these loci for hybridization in Upper Klamath Basin species, especially *Ch. brevirostris* and *C. snyderi*, is the absence of autapomorphies (Table 3.16), perhaps suggesting swamping of one genome by another. However, for species surveyed at all loci, another lake sucker, *Ch. cujui*, had similarly low numbers of autapomorphies (Tables 3.2, 3.5, 3.8, 3.10, 3.13). On the other hand, derived sequences in Upper Klamath Basin species were often shared with *C. occidentalis* (Tables 3.4, 3.12, 3.15), which is allopatric and can not hybridize with Klamath species. Unlike Upper Klamath Basin species, *C. occidentalis* had many autapomorphies (Tables 3.5, 3.10, 3.13).

Smith (1992) has suggested that *Chasmistes* and *Deltistes* retain many primitive morphological characters and that late Miocene fossils are not more primitive than modern forms. Our data suggested that sequence similarity in Upper Klamath Basin may also reflect retention of ancestral sequences, while derived states suggested western clades that are currently unrecognized. At least with regard to these loci and western suckers, there may be a confusing mixture of phylogenetic and geographic signal. Do the similarities in derived states between *C. occidentalis* and Klamath species suggest a more recent geographic contact with the sharing of alleles or a real clade? Similarly, does the lack of similarity between the two *Chasmistes* mean they are not a clade or that much of the signal from their Miocene ancestor has been lost due to contact and the sharing of alleles with other clades? The case for or against Klamath sucker hybridization can not be answered with these loci, instead these loci suggest the equally parsimonious hypothesis that Upper Klamath Basin species have retained a largely ancestral genome at these loci. Thus, the failure of this technique to uncover significant variation in Upper Klamath Basin species may be a reflection of their plesiomorphic genome at these loci and not necessarily hybridization.

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Chapter 4

Conclusions

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The goals of this study were to: 1) isolate and characterize variable anonymous nuclear loci as species markers for Klamath Basin suckers; and 2) investigate the potential for hybridization among these species.

This study reported the findings of 28 anonymous nuclear loci (Wagman and Markle, 2003a and b). The sequences indicated that the Klamath/Rogue Basins catostomid genomes are conserved and highly related. Among the four species from three genera of Klamath Basin suckers, rare occurrences of three types of species markers were found among two polymorphic loci. Two species were distinguished by strictly diagnostic species markers (an allele or genotype that is only found in one species and identifies an individual to that species) represented by the eight fixed single nucleotide polymorphisms (SNPs) found in 10,421 bp. These autapomorphies were found in locus 120 in two Upper Klamath Lake *D. luxatus* (Table 3.10) and in four Topsy Reservoir *C. rimiculus* (Table 3.10). Topsy Reservoir *C. rimiculus* were further distinguished by autapomorphies in locus 4 (Table 3.2) (Wagman et. al, 2003b). These may represent fixed sequence species differences, but these alleles had small sample sizes and therefore have limited power to segregate Klamath Basin catostomids.

Eight of 28 loci were polymorphic and four of these had shared alleles common among the four species. Locus 142 was tetraploid with upwards of 12 alleles but no consistent species frequency pattern could be discerned (Table 2.4). Loci 4, 120 and 184 either contained operationally diagnostic species markers (allele or genotype fixed for one population of a species and with corroborative information, identify an individual to a species) or frequency dependent species markers (allelic or genotypic statistically significant difference in frequency between species and with corroborative information, can identify a population to a species) for some of these species. Significant allele usage between species is clearest in *C. rimiculus* from all sites (Tables 2. 2, 2.3, 2.5). The fixed homozygous BB genotype of Locus 4 was an operationally diagnostic species marker for Rogue River *C. rimiculus* in the (Table 2.2). Although the allele was widespread in the Lost River sub-basin and upper Williamson River of the UKL sub-basin and a single *C. rimiculus*, genotype AA has been identified, this allele (B) may be an autapomorphy for *C. rimiculus*. Locus 120, allele B was a frequency-dependent marker for *C. rimiculus*

(Table 2.3). In Rogue River fish, 72% were homozygotes, BB, for this locus. This suggests that genotypically, *C. rimiculus* are BB and have significantly lower allele A frequencies for this locus. *Chasmistes brevirostris, C. snyderi*, and *D. luxatus* have AA genotypes. Loci 4, 120 and 184 exhibited different allele frequencies between species; indicating three distinct groups, *D. luxatus. C. rimiculus, and Ch. brevirostris/ C. snyderi.* (Tables 2.2, 2.3, 2.5). The sequence similarity of random loci and low levels of polymorphism across the four species does not support current classification of these species in three genera.

These data suggested either hybridization is common throughout the history of the Klamath and Rogue Basin species or they share an ancestral genome. Loci 4 and 120 genotypic patterns suggest either ancestral polymorphisms and/or upstream hybridization gene flow. The latter requires gene movement up river from the Lower Klamath sub-basin to the Upper Klamath and Lost River sub-basins. Infrequent recent exchange between Lost River and Klamath River water (Rykbost and Todd, 2002) suggests that the movement is ancient. Sharing of a plesiomorphic genome or ancestral polymorphism was tested by including genetic samples from more members of the Catostomidae.

Chapter 3 (Wagman and Markle, 2003b) examined phylogenetic patterns in, three polymorphic coding loci (4, 120, and 184) and two monomorphic non-coding loci (39 and 81) in 25 species representing 11 of 13 catostomid genera to determine if the homogeneity in the Upper Klamath Basin was due to massive hybridization and introgression or to retention of ancestral sequences. Loci contained evidence of support for various subfamilies and tribes; loci 4 and 184 supported Catostomini (Tables 3.4, 3.15), loci 81 and 184 supported Moxostomatini (Table 3.6, Figure 4.1), and locus 120 supported Ictobinae (Table 3.10). Generic level evidence gave support for eastern genera, the polytypic *Moxostoma* (Tables 3.13 and 3.15) and *Carpiodes* (Table 3.10 and 3.12) and little support for western genera, the monotypic *Deltistes* (Table 3.10) and *Xyrauchen* (Tables 3.2, 3.5, 3.8, 3.10, 3.13). *Chasmistes, Catostomus* and *C.* (*Pantosteus*) were unsupported by synapomorphies at these loci. Each locus was a combination of species or population markers, sometimes providing discriminatory power for allopatric populations of a species. Autapomorphies were found for all other western and eastern

species and some populations of *C. macrocheilus, C. columbianus* and *E. elongatus,* except *Ch. brevirostris, C. snyderi, Cycleptus elongatus* and *Hypentelium etowanum.* The eastern species proved difficult to amplify or sequence for two and three of the five loci (Table 3.1) respectively.

Monomorphic, non-coding loci 39 and 81 were found to be variable in other catostomids. Locus 39 had many SNPs, especially within *C. macrocheilus* and *C. columbianus*, but was otherwise fairly conservative. Locus 81 contained only three variable sites in western species but was highly variable in Moxostomatini, which had many autapomorphies and synapomorphies among members. The non-coding nature of these loci did not limit their utility as species markers, but their usefulness as species markers is clearly restricted. Phylogenetically, only locus 81 contained sufficient information to make a well-supported consensus tree but this contained only a partial list (8 of 11 surveyed) of the eastern Moxostomatini. The three polymorphic coding loci (4, 120 and 184) consistently contained autapomorphies in both eastern and western species and in some populations within species (Tables 4.2, 4.10, 4.13) and gave conflicting phylogenetic patterns.

Informative sites involving Klamath species were mostly shared. Locus 4 contained shared sites between upper Klamath Basin species and *C. occidentalis* (Table 3.4). This grouping does not reflect current taxonomy. Upper Klamath Basin species also had 99.7% sequence identity with *C. occidentalis,* but only 95.2% with the lower Klamath *C. rimiculus* (Table 3.3). Outgroup analysis suggested that the Upper Klamath Basin sequences were not ancestral, but polarity was ambiguous. The upper Klamath species shared three informative locus 39 sites with at least two eastern moxostomatins. The Klamath sequence of locus 81 was identical to that of the ictiobines and cycleptine. An informative site at locus 120 distinguished Upper Klamath Basin species (Table 3.12) otherwise, they were most like moxostomatines, (Table 3.12) sharing many sites. Informative sites at locus 184 had minimal support (Table 3.15) for upper Klamath Basin species and shared 80% of the informative sites with at least one moxostomatin (Table 3.15). Locus 184 contained four shared sites between upper Klamath Basin species and *C. occidentalis* (Table 3.15). Upper Klamath Basin species lacked autapomorphies (Table

3.16), but had similar numbers of derived informative sites as other catostomins (Table 3.17). These species consistently shared ancestral or equivocal informative sites either with moxostomatins or a variable group of western species and shared derived sites with other western species, especially *C. occidentalis*. These loci suggest that Upper Klamath Basin species have retained a largely ancestral genome at these loci. Thus, the failure of this technique to uncover significant variation in Upper Klamath Basin species may be a reflection of their plesiomorphic genome at these loci and not necessarily hybridization.

The inability to find loci which were more variable in western catostomids and have more phylogenetic information within the family could be due to bias in the anonymous nuclear loci technique toward coding loci. The endonuclease, Sau3A1, used for the restriction of the genomic DNA was chosen because it compliments the BamH1 cloning site in pUC18. Dowling et al. (1996) list 55 commonly used restriction endonucleases, which have a range of 0–72 restriction sites in *Homo sapiens* mitochondria DNA (mtDNA). This represents an average of 12 restriction sites per 16.5 kilo bases (kbps)(Avise, 1994) in *Homo sapiens* mtDNA. Although Sau3A1 was not among these, its compliment, BamH1, was found to cut human mt DNA once. Possibly the Sau3A1 enzyme also has a low number of restriction sites within the sucker nuclear genome. As a rough estimate, if *Homo sapiens* DNA have 3 billion bp (diploid 2n = 46 chromosomes) and suckers have more than twice this amount of DNA (i e. 6.5 billion bps, tetraploid 4n = 100 chromosomes, Ferris and Whitt 1978) and this enzyme cuts once every 16.5 kbps then there are 394,000 restriction sites in the nuclear genome. Normally an average enzyme should generate 4.7 million restriction cuts among 6.5 billion bases. Since bacterial endonucleases are antiviral mechanisms there is selection against these sequences at least in bacteria. Therefore selection to remove this sequence would be high in all DNA, but coding regions might be forced to use this sequence due to functional constraints of the protein. This bias would increase the number of non-variable coding regions in our sample of DNA fragments used to make the genomic library, increasing the number of coding loci and decreasing the amount of variation. In support of this type of bias, 75% (21) of the 28 loci screened, contained open reading frames. Also within Klamath Basin, the most variable locus was a non-coding, tetraploid (142) locus. This

locus had a large amount of variation but it did not corroborate species identifications. Non-coding loci used for outgroup comparisons (39 and 81) were highly variable among eastern sucker taxa as were the coding loci (4, 120 and 184). So it is only in western suckers, primarily within Klamath Basin, we found a lack of variation either in coding or non-coding loci.

Another potential error involves the use of small genomic clones. Genomic clones have been shown to be less variable than cDNA clones (Dowling et. al., 1996). Genomic libraries are simpler to make than cDNA libraries because they do not involved the isolation of mRNA from tissue. The largest number of variable loci from genomic clones was 50% reported by Karl (et. al., 1992). We found eight variable loci in Klamath Basin or 27% of the loci but only four loci (14%) were commonly polymorphic. Theoretically the number of variable loci, would expand using taxa from outside of Klamath Basin. Dowling (et. al., 1996) reports there appears to be no correlation between clone size and the level of polymorphism. A size range of between 300 and 600 bp was chosen for ease of sequencing. Karl (et. al., 1992) screened clones between 500 and 2000 bp for restriction site polymorphisms. Small clone sizes made it necessary to screen more loci, a difficult and tedious task

Contributing to the low amount of variation in Klamath Basin species was the choice of electrophoretic methods used to detect variation within a locus. A clear example of this is seen with loci 4 and 120. Within both of these loci, autapomorphic sequences were later realized for two of the Klamath Basin sucker species (*D. luxatus* and *C. rimiculus*). During the search for polymorphic loci, it was determined from sequencing, that these loci had size variant alleles because of the deletions they contained. These loci were only tested with denaturing PAGE technique to determine frequencies of the alleles for the loci. The eight SNPs found in loci 4 and 120 were initially believed to be sequencing artifacts and/or were shared among all the Klamath Basin species. Single strand conformational polymorphism electrophoresis of these loci would have determined if these variable sites were present in other Klamath Basin samples or if other sequence variation existed. This bias was re-enforced through my own bias that species in three separate genera would have large sequence differences, so

these SNPs were initially ignored. It was only when more than one individual per species was sequenced that it was determined that these SNPs constituted autapomorphies for these two species.

A potential source of error was the choice of *C. rimiculus* individuals for sequencing. The study was centered on Klamath Basin species, and therefore Rogue River samples were not sequenced and only Klamath River *C. rimiculus* samples were. An examination of carcasses of these fish, found no obvious signs that they were misidentified. But three of the Klamath River *C. rimiculus* used for sequencing were not *C. rimiculus* and were re-identified as *Ch. brevirostris* (Markle, et. al, in preparation). In hindsight any fish from the Klamath River could be more likely of hybrid origin as previous authors have noted due to their sympatric situation with extant individuals from the upstream Klamath sub-basins.

A final bias in my approach was not including non-Klamath outgroup taxa from the beginning of the study. The assumption that the three genera in Klamath Basin would be outgroups to one another was obviously erroneous. The inclusion of good outgroups (other non- Klamath Basin western and eastern taxa) sooner would have shown that the SNPs in loci 4 and 120 were autapomorphies early in the process and loci that were monomorphic in Klamath Basin were polymorphic elsewhere, directing a different approach to the Klamath species. Although outgroup analysis was used, only five loci were chosen, three because they were polymorphic in the Klamath Basin (4, 120, 184) and two monomorphic loci (39 and 81) were arbitrarily chosen from among non-coding loci. These loci had limited phylogenetic information. If outgroups had been used from the beginning more loci could have been identified which had more variation and potential phylogenetic information across the family. Outgroup taxa proved to be difficult to amplify and sequence for the five loci used in chapter 4 (Wagman, 2003b). Many outgroup taxa did not amplify or they would not sequence or the sequences were very confusing. This could imply that the primer regions for these loci had changed in these species, suggesting that eastern taxa have diverged at a higher rate than western sucker taxa.

Smith (1992) found that the upper Klamath species have retained many primitive or ancestral morphological characters. The genetic data presented by this study implies a plesiomorphic genome in Klamath species but also to some extent within western catostomids. Eastern catostomids, including *C. commersoni* exhibit more autapomorphies (Table 3.16) and Moxostomatini has more synapomorphic sequences than the western species (Table 3.17). Interestingly, the data suggested taxonomic relationships between *C. macrocheilus* and *C. rimiculus,* a finding not suggested before. Also a strong relationship found between Klamath species and *C. occidentalis,* supported by 12 of 14 derived characters (Tables 3.4, 3.10, 3.15) and the high degree of sequence identities (Tables 3.3, 3.6, 3.14), suggests a relationship not documented before. This study suggested a Great Basin connection between Klamath species and *C. warnerensis* (Tables 3.6, 3.9, 3.11, 3.14). The data also found four autapomorphies that may support a new species designation for an un-identified species of *Catostomus* from Wall Canyon, Nevada (Tables 3.10, 3.13).

This study cannot fully relate these new relationships to present taxonomy of the family. It does call into question taxonomic relations within the tribe Catostomini a finding suggested by Harris and Mayden (2001). The only well supported cladogram produced by this study (locus 81) differs from previous authors' (Smith, 1992; Harris and Mayden, 2001) in the placement of *Minytrema* and *Erimyzon*. This locus could not find synapomorphic sites between two *Hypentelium* species (Table 3.8).

Although there is limited success in genetically identifying Klamath Basin suckers, this study has shown that the Catostomidae is quite diverse and that western species are less diverse than eastern species. Our study furthers the need for research and revision of this family.

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Appendices

Appendix 1: Klamath Basin adults amplified for four polymorphic loci. Fish are listed by location, Genus, species OS catalogue number, alpha, Klamgen sample number, and tag number. Amplified samples for a locus are marked by X.

Drainage	Genus species	Catalog Number	Alpha	Klamgen number	Tag number	Loci 4	120	142	184
Sprague River									
	Catostomus snyderi	OS 015895	A	200	BR00962	X		X	X
			B	201	BR00966	X	X	X	X
			C	202	BR00968	X	X	X	
			D	203	BR00965	X		X	
			E	204	BR00963	X	X	X	X
			F	205	BR00964	X	X	X	X
			G	206	BR00967	X	X	X	
		OS 015893	A	308	BR00413	X	X	X	X
			B	309	BR00414	X	X	X	X
			C	310	BR00415	X		X	
			D	311	BR00416	X	X	X	X
			E	312	BR00418	X	X	X	X
			G	314	BR00420	X	X	X	X
			I	317	BR00423	X			
			J	315	BR00421	X	X	X	
		OS 015894	A	318	BR00424	X	X	X	X
			B	319	BR00425	X	X	X	
			C	320	BR00576	X	X	X	X
			E	322	BR00579	X	X	X	X
			G	324	BR00581	X	X	X	X
			H	325	BR00582	X	X	X	X
			J	327	BR00584	X	X	X	X
			M	330	BR00587	X	X	X	X
			O	332	BR00589	X	X	X	X
	Chasmistes brevirostris	OS 015956	A	016	GF01549	X	X	X	X
			B	018	GF01601	X	X	X	X
			C	019	GF01602	X	X	X	X
		OS 015957	A	027	BR00244	X	X	X	X
			B	030	BR00242	X	X	X	X
	Deltistes luxatus	OS 015923	A	020	GF01603	X	X	X	X
			B	017	GF01600	X	X		X
		OS 015924	A	021	GF01604	X	X	X	X
			B	022	GF01605	X	X	X	X
		OS 015925	A	029	BR00241	X	X	X	X
Sycan River									

Catostomus snyderi	OS 015897	A	077	GF01630	X	X	X	X
		B	078	GF01629	X	X	X	X
	OS 015898	A	120	GF01792	X	X	X	X
		B	121	GF01790	X	X	X	X
		C	122	GF01789	X	X	X	X
		D	123	GF01788	X	X	X	X
	OS 015968		124	GF01787	X	X	X	X
Upper Williamson River: Rocky Ford								
Catostomus snyderi	OS 015899		056	GF01622	X	X	X	X
	OS 015900	A	051	GF01617		X		X
		B	052	GF01618	X	X	X	X
		C	053	GF01619	X	X	X	
		D	054	GF01620			X	
		E	055	GF01621	X	X	X	X
		F	057	GF01623	X	X	X	X
		G	058	GF01649	X	X	X	
		H	059	GF01648	X	X	X	X
		I	060	GF01647	X	X	X	X
		J	061	GF01646	X		X	
	OS 015901	A	062	GF01645				X
		B	063	GF01644	X	X	X	
		C	064	GF01643	X		X	X
		D	065	GF01642	X	X	X	X
		E	066	GF01641	X	X	X	
		F	067	GF01640	X	X	X	X
		G	068	GF01639	X		X	
		H	069	GF01638	X	X	X	X
		I	070	GF01637	X	X	X	X
		K	072	GF01635	X	X	X	X
		L	073	GF01632	X	X	X	X
		M	074	GF01626	X	X	X	X
		N	075	GF01650	X	X	X	X
Lower W illiamson River								
Catostomus snyderi	OS 015896		082	GF01631	X	X	X	X
Chasmistes brevirostris	OS 015959	A	035	GF01609	X	X	X	X
		B	036	GF01611	X	X	X	X
		C	038	GF01610	X			X
	OS 015963	A	040	BR00247		X	X	X
		B	041	BR00248	X	X	X	X
		C	042	BR00249	X	X	X	X

		D	043	BR00250	X	X	X	X
	OS 015964	A	044	?01825	X	X	X	X
		B	045	?01826	X	X	X	X
		C	046	?01827	X	X	X	X
	OS 015965	A	031	GF01612	X	X	X	X
		B	032	GF01613	X	X	X	X
		C	037	GF01614	X	X	X	X
	OS 017480		047	?01828	X	X	X	X
Deltistes luxatus	OS 015930		039	GF01615	X	X	X	X
	OS 015931	B	048	?01829	X	X	X	X
Upper Klamath Lake								
Catostomus rimiculus	OS 017490		289	BR00708	X	X	X	X
Chasmistes brevirostris	OS 015952		013	GF01546	X	X	X	X
	OS 015953	A	014	GF01547	X	X	X	X
		B	015	GF01548	X	X	X	X
	OS 015954	A	001	GF01540	X	X	X	X
		B	004	GF01543	X	X		
	OS 015960		005	GF01544	X	X	X	X
	OS 015961	A	287	BR00704	X	X	X	X
		B	288	BR00706	X	X	X	X
		D	290	BR00711	X	X	X	X
		F	296	BR00713	X		X	X
		G	300	BR00715	X	X	X	X
	OS 015962		307	BR00719	X		X	
	OS 017479		295	BR00712	X	X	X	X
Deltistes luxatus	OS 015922		002	GF01541	X	X	X	X
	OS 015926		006	GF01545	X	X	X	X
	OS 015927		003	GF01542	X	X	X	X
	OS 015928	A	291	BR00702	X			
		B	292	BR00703	X	X	X	X
		C	293	BR00705	X	X	X	X
		D	294	BR00707	X	X	X	X
		F	297	BR00709	X	X	X	X
		G	298	BR00710	X	X	X	X
		H	299	BR00714	X	X	X	X
	OS 015929	A	302	BR00720	X		X	X
		B	303	BR00721	X	X	X	X
		C	304	BR00722	X	X	X	X
		D	305	BR00724	X	X	X	X
		E	306	BR00725	X	X	X	X
	OS 017491		301	BR00716	X	X	X	X
Lost River								
Chasmistes	nonlethal		008	NA	X	X	X	X

brevirostris								
Deltistes luxatus	nonlethal		007	NA	X	X	X	X
	nonlethal		009	GF5595	X	X	X	X
	nonlethal		010	NA	X	X	X	X
	OS 017478		012	7F7B1F3269	X		X	
Lost River: Gerber Reservoir								
Catostomus snyderi	OS 015892	A	079	NA	X		X	
		B	080	GF01627	X	X	X	X
Chasmistes brevirostris	OS 015943	B	188	BR00953	X		X	
	OS 015946	A	197	BR00969	X	X	X	X
		B	198	BR00970	X	X	X	X
		C	199	BR00971	X	X	X	X
	OS 015947	A	207	BR00972	X	X	X	X
		B	208	BR00973	X	X	X	X
		C	209	BR00974	X		X	X
		D	210	BR00975	X		X	
		E	211	GF01800	X	X	X	X
		F	212	GF01801	X	X	X	X
		G	213	GF01802	X	X	X	X
		H	214	GF01803	X	X	X	X
		I	215	GF01804	X	X	X	X
		J	216	GF01805	X	X	X	X
	OS 015948	A	217	GF01806	X	X	X	X
		B	218	GF01807	X	X	X	X
	OS 015949	A	189	BR00954	X	X	X	X
		C	191	BR00956	X	X	X	X
		D	192	BR00957	X	X	X	X
		E	193	BR00958	X	X	X	X
		F	194	BR00959	X	X	X	X
		G	195	BR00960	X	X	X	X
		H	196	BR00961	X	X	X	X
Lost River: Clear Lake Reservoir								
Chasmistes brevirostris	OS 015933	A	258	BR00767	X	X	X	X
		B	259	BR00768	X		X	
		C	260	BR00769	X	X	X	X
		D	261	BR00770	X			
		E	262	BR00771	X	X	X	X
	OS 015934	A	125	GR01775	X	X	X	X
		B	126	GR01776	X	X	X	X

	C	127	GR01777	X	X	X	X
	D	128	GR01778	X	X	X	X
	E	129	GR01779	X	X		
	F	130	GR01780	X	X	X	X
	G	131	GR01781	X	X	X	X
	H	132	GR01782	X	X	X	X
	I	133	GR01783	X	X	X	X
	K	135	GR01785	X	X	X	X
	L	136	GR01786	X	X	X	X
	N	163	BR01000	X	X		X
	O	164	BR00999	X	X	X	X
	P	165	BR00998	X	X	X	X
	Q	166	BR00997	X	X	X	X
	R	167	BR00996	X	X	X	X
	S	168	BR00995	X		X	
	T	169	BR00994	X	X	X	X
	U	170	BR00993	X	X	X	X
	W	172	BR00990	X	X	X	X
OS 015936	A	263	BR00773	X	X	X	X
	B	264	BR00774	X	X		
	C	265	GF07924	X	X	X	X
	D	268	GF01922	X	X	X	X
OS 015937	B	251	BR00760	X	X	X	X
	C	252	BR00761	X	X	X	X
	D	253	BR00762	X	X	X	X
	E	254	BR00763	X	X	X	X
OS 015939	A	242	BR00746	X	X	X	X
	B	243	BR00752	X	X	X	X
	C	244	BR00753	X	X	X	
	D	245	BR00754	X	X		
	E	246	BR00755	X	X	X	X
OS 015966	A	086	GF01652	X	X	X	X
	B	087	GF01653	X	X	X	X
	C	088	GF01654	X	X	X	X
	D	089	GF01655	X	X	X	X
	E	090	GF01656	X	X	X	X
	F	091	GF01657	X	X	X	X
	G	092	GF01658	X	X	X	X
	H	093	GF01659	X	X	X	X
	I	094	GF01660	X	X	X	X
	J	095	GF01661	X	X	X	X
	K	096	GF01662	X	X	X	X
	L	097	GF01663	X	X	X	X
	M	098	GF01664	X	X	X	X
	N	099	GF01665	X	X	X	X

	OS 015967	A	114	GF01794	X	X	X	X
		B	115	GF01799	X	X	X	X
		C	116	GF01798	X	X	X	X
		D	117	GF01797	X	X	X	X
		E	118	GF01796	X	X		X
		F	119	GF01795	X	X	X	X
	OS 015969	A	100	GF01667	X	X	X	X
		B	101	GF01668	X	X	X	X
		C	102	GF01669	X	X	X	X
		D	103	GF01670	X	X	X	X
		E	107	GF01673	X	X	X	X
		F	108	GF01674	X	X		
		G	109	GF01676	X	X	X	X
		H	110	GF01677	X	X	X	X
		I	111	GF01678	X	X	X	X
		J	112	GF01679	X	X	X	X
		K	113	GF01680	X	X	X	X
	OS 017477		139	GF01877		X		
Deltistes luxatus	OS 015915	A	247	BR00756	X	X	X	X
		B	248	BR00757	X	X	X	X
		C	249	BR00758	X	X		
	OS 015916	A	138	GF01876	X	X	X	X
		C	140	GF01878	X	X	X	
		D	141	GF01879	X	X	X	X
		E	142	GF01880	X	X		
		F	174	BR00989	X			
		G	175	BR00988	X	X	X	X
		H	176	BR00987	X	X	X	X
	OS 015917		270	GF01923	X	X	X	
	OS 015920	A	255	BR00764	X	X	X	X
		B	256	BR00765	X	X		X
		C	257	BR00766	X	X	X	X
	OS 017489		134	GF01784	X	X	X	X
Klamath River: Topsy Reservoir								
Catostomus rimiculus	OS 015906	B	237	BR00742	X	X	X	X
	OS 015908	A	104	GF01671	X	X	X	X
		B	105	GF01672	X	X	X	X
		C	106	GF01675	X	X	X	X
		D	076	GF01633	X	X	X	X
	OS 015909	A	271	BR00311	X	X	X	X
		B	272	BR00312	X	X	X	
		C	273	BR00313	X	X	X	X
		D	274	BR00314	X	X	X	

		E	275	BR00315	X	X	X	X
		F	276	BR00316	X	X	X	X
		G	277	BR00317	X	X	X	X
		H	278	BR00318	X	X	X	X
		I	279	BR00319	X	X	X	X
		J	280	BR00320	X	X		
		K	281	BR00321	X		X	X
		L	282	BR00322	X	X	X	X
		M	283	BR00323	X	X	X	X
		N	284	BR00324	X	X	X	X
		O	285	BR00325	X	X	X	X
		P	286	BR00701	X	X	X	X
	OS 015911		266	BR00718	X	X		X
	OS 017487	A	238	BR00743	X	X	X	X
		B	239	BR00741	X	X	X	
Catostomus snyderi	OS 017476	A	267	BR00717	X	X	X	X
Chasmistes brevirostris	OS 017487	C	240	BR00744	X	X	X	X
		D	241	BR00745	X	X	X	X
Klamath River: Copco Reservoir								
Chasmistes brevirostris	OS 015940	A	219	GF01808	X	X	X	X
		B	220	GF01809	X	X	X	X
		C	221	GF01810	X	X	X	X
		D	222	GF01811	X	X	X	X
		E	223	BR00727	X	X	X	X
Rogue River								
Catostomus rimiculus	OS 015913	A	143	GF01881	X	X	X	X
		B	144	GF01882	X	X	X	X
		C	145	GF01883	X	X	X	X
		D	146	GF01884	X	X	X	X
		E	147	GF01885	X	X	X	X
		F	148	GF01886	X	X	X	X
		G	149	GF01887	X	X	X	X
		H	150	GF01888	X	X		X
		I	151	GF01889	X	X		X
		J	152	GF01892	X	X		X
		K	153	GF01893	X	X	X	X
		L	154	GF01895	X	X		
		M	155	GF01890	X	X	X	X
		N	156	GF01891	X	X	X	X
		O	157	GF01894	X	X	X	X

		P	158	GF01896	X	X	X	X
		Q	159	GF01897	X	X	X	X
		R	160	GF01899	X	X	X	X
		S	161	GF01898	X	X	X	X
		T	162	BR00976	X	X	X	X
		U	173	BR00978	X	X	X	X
		V	178	BR00979	X	X	X	X
		W	179	BR00977	X	X	X	X
		X	181	BR00981	X	X	X	X
		Z	183	BR00983	X	X	X	X
		Z1	184	BR00984	X	X	X	X
		Z2	180	BR00980	X	X	X	X
		Z3	185	BR00985	X	X	X	X
		Z4	186	BR00986	X	X	X	X
Klamath Tribe Hatchery								
Chasmistes brevirostris	OS 015950		081	GF01651	X	X	X	X

Appendix 2: Each locus was assigned a locus number, which corresponds to the clone isolated from a Chasmistes brevirostris library. The GenBank accession number is given for each locus and a statement about how complete the search was for a species marker, the original clone size (bp), Primers (+/-) designed for amplification, PCR product size (bp), the concentration of MgCl2 in the PCR reaction and annealing temperature (0C) used. The clone sequence is reported with the primer sites underlined. The results of a GenBank Blastx search (amino acid identity) and a Blastn search (nucleic acid identity) are reported.

Locus 001: Accession # AY351333 PCR amplification in all Klamath species results in a strong single band. Sequence alignment showed that the first 70 bp were identical and then the sequences became confused as if there were other DNA types present. Possibly a tetraploid locus.
Clone size: 334 bp. PCR product size: 254 bp.
Primers: 1R+: 5' ACA ACA CTC CCA ATC CTT ATT CTT T 3'
1R-: 5' TCA CTG TAA ACT GAT AGC CCA AAC A 3'
Mg++ Concentration: 2mM Annealing Temp: 52 ^{0}C
Reverse Sequence:
AAGTATACGTTCATTTAGAAGGGAAAGCAGATTTAAGATTCACTGTAAACTG
ATAGCCCAAACAGGGACATCAACCCTGTACCCTCAGATTAAAAGTTTCATGC
GTTACTTGCTGAGCTACCCAGGCTCCTGAAAGCTTTGACACCCCCGTGAATAT
AAAGACAGTCCAGCCAATATGTGCACTTGACCTGTTGACTAAGTTAATTTCAA
ATCAAATCAAATCACTTTATTGTCACACTACCATTTTTTAATTTGCAATGAAC
TCTACAAAGAATAAGGATTGGGAGTGTTGTCGTGCTCTTGGCAGGAAGGGAA
TGCTAAAAAAAAAGTGTGA

Blastx Search: No significant amino acid sequence similarity found in GenBank.

Blastn search: emb AL021808| HS24O18 Human DNA sequence from clone 24o18 on chromosome 6p21.31-22.2; zinc finger protein pseudogene. 60/70 bp (85% identity), 24% overall identity.

Locus 002: Accession # AY351334 All Klamath catostomids species are identical at this locus.
Clone size: 619 bp. PCR Product size: 501 bp.
Primers: 2R+: 5' TTG TCG GAT GCA GTG AAA AGT CAG C3'
2R-: 5' GAT TAAGTT GGG TAA CGC CAGGTT T 3'
Mg++ Concentration: 1mM Annealing Temp: 52 ^{0}C
forward Sequence:
GCTGACTTTTCACTGCATCCGACAAAAACTGTCTTTTTGGTTGCCATGATATT
AATGCCTGTCATTATCCATGTTATCATTTTACATAGTCATGGCAATATTATTG
AGAAGTGAGCTTGATTTTCCTCTGGTGAAAGTTACATGGAGCCACATAATTA
GGAACATCCCATTACAAAAATATATGGTTGTTCAAAAACCAAGTGAGCTGCT
TCCAGATAGACAGCTGCCTTGTGTGGCAGTATCCTAATCATCATGGAACTTCA
TAAATGACTGATTTAAAACACTACGTAAGCAGTAACTTTGCCACACAAATAG

CTCTCCTCGTGTGCAGTGCACGGGAGACTCGCAGTTGATTTGCGAGGTGCAA
GAGGATAGGATGTGATAATTTTCTCATAATTTACTCAAGCTGACTTTTCACTG
CATCCGACAAACAACTGAGAATAGGCCAGAATCAGTCATTTTCAATGGAGAN
TTGGATGGGGACTGTGTTGTTCCGACCATTTGCCGATGTGGAATTTT

Blastx search: gb|AAD10590.1| (U01771) unknown *Mycoplasma genitalium*
Identities = 20/22 (90%), Positives = 20/22 (90%)

Blastn search: emb|X99258.1|MTMOB *M.tuberculosis* genomic sequence containing 4 ORF's Identities = 74/76 (97%)

Locus 003: Accession # AY351335 PCR amplification in all Klamath species resulted in 3 product bands. Attempts to optimize PCR reaction failed. Testing with this locus was discontinued. Clone size: 580 bp. PCR Product size: 444 bp.
Primers: 3F+: 5' CCA AAG GTG CTT CAA CAA AGT ATT G 3'
3F-: 5' ATT TCA TTG CAG ATG TCA GGC AGA C 3'
Mg++ Concentration: 1mM Annealing Temp: 52 ^{0}C
Forward Sequence:
TGAAAATGGCTGTGTACCGACGCTCCCCATCCAACCTGATGGAGCTTGAGAG
GTCCTGCAAAGAAGAATGAGAGAAGCTGCCCAAAAATAGGTGTGCCAAGCTT
GTAGCATCATACACAAGACTTGAGGCTGTAATTGGTGCCAAAGGTGCTTCAA
CAAAGTATTGAGCAAAGGCTGTGAATATTTATGTACCTTTTATATATATATAT
ATATATAAATGCATTTGCAAAGATTTCAAACAAACGTCTTTCACGTTGTCATT
ATGGGGTATTGTTTGTAGAATTTAATCCATTGTGGAATAAGGCTGTAACATGA
AAAAATTTGGAAAAAGTGAAGCACTGTGAATACTTTCCGGATGCACTGGATA
TTGCAAAAAGTATTTTTGTCTTTTTTCTAGTGAAAATATCTAAACATTCTTAAA
ATGTGATTTGTTTACTTGACAAACTGAAAACTGGCATAAGANTGTAAGTCTTG
TTTTTAGAGAAATCTAATCAAATTTATTGAACTTTAGACATNAAACAAAAAA
AAAACCAATCTGCCATGGGGTAAACAACTGTCTGCCTGACATCTGCAATGAA
ATCCCCGGTACCGANCTCAAATCTC

Blastx search: emb|CAB51372.1| (AJ249085) transposase *Pleuronectes platessa*
Identities = 35/54 (64%), Positives = 40/54 (73%), Gaps = 1/54 (1%)

Blastn search: gb|U51230.1|DRU51230 *Danio rerio* transposon Tzf.49 Identities = 147/170 (86%), Gaps = 3/170 (1%)

Locus 004: Accession # AF362135, AF362136 Polymorphic locus, Rogue River *C. rimiculus* are fixed for the B allele, all other species are homozygous AA or AB. The B allele contains a deletion at the site of the bold sequence. Possible species marker. *C. rimiculus* has three autapomorphic bases for this locus. Clone size: 540 bp. PCR Product: 454 bp.
Primers: 4F+: 5' GAG TCG CAA TCT GAC ACC TAC CTG T 3'
4F-: 5' CAC CAG CCT CTG AAA CCT GCC ATT T 3'
Mg++ Concentration: 1mM Annealing Temp: 52 ^{0}C

Forward Sequence: Bold nucleotides are the deleted bases.
ACCATAAAAATGAATGAGGAGAGTCGCAATCTGACACCTACCTGTCGCAACA
TTGTCAGCAGGGATGGATTACCAACCGGGCCAAAGGGGCCAGTGCCCAGGG
GCCCTTGACTACCAGGGGCCCTTGACTGCCCATCAACAACTTCAGTTGTGTGA
GTTGTGTGCTATATTCCAAGCCTTCTGAAGTACACTGTGCTAATACAGATGCA
ACTCCATAAGAGTTTGCAATTTATGTCACACAGGATGCAAAATGTCACCTCA
AAACTCATACACTAAAATGATAATTACTTTATCAGAAAGAATTTTCTTTATAT
CAGAATTGTCAGATGAAAGAGTCTGTTCATTAAACATAAATATTTTCTCACTT
AAGCTATAAGGCTTGGAGAGTCTACATGGAGACTGTCTGTATGTTTGCCATTG
TGTATGAATATTCACATTGTGACAGTTGTAAATGGCAGGTTTCAGAGGCTGGT
GAGTGCTCACTGTTTGTGGGCCCGGCCAGGCAAAAAGAACAGTGTTGTGTGC
CCAATCCCATCATG

Blastx search: pir||S31521 collagen COLF1 - freshwater sponge *Ephydatia muelleri* >gi|9300|emb|CAA49472| (X69818) Emf1 alpha *Ephydatia muelleri* Identities = 16/27 (59%), Positives = 20/27 (73%), Gaps = 1/27 (3%)

Blastn search: gb|AF222686.1|AF222686 *Homo sapiens* chromosome X PAC K6166 map Xp11.23, Identities = 20/20 (100%)

Locus 006: Accession # AY351336 PCR difficulties, amplified in all Klamath species but was never a single band.
Clone size: 478 bp. PCR Product size: 415 bp.
Primers: 6F+: 5' TAG GCA GCT TTT TAG AGT CGT ATG T 3'
6F-: 5' GCT ATC TTC ACA ATA ATG ACA GTT C 3'
Mg++ Concentration: 2-3mMAnnealing Temp: 48-49 ^{0}C
Forward Sequence:
ATGGCCAAAAAACACTTTTTAATATTAGGCAGCTTTTTAGAGTCGTATGTCAC
CAAGATTAACTACTTTTTCAATAAATATGACTTTTCAGTATTTTCACATGAAT
GCAGCTATGAAGTGCATGAGGGGACAAAATGAATCCTTGCAACATGTTTGCA
TGCATTATGAGAGATGTAAAGTCATTATAATGAAGAAATATGTGTTTTAAGA
AAGAAATGTGCAAACTTCTTAATACAGCTCTCAAAGGCCCAAATTCAACTTTT
TATTATTGGACTGCTGTTTGGAGTCATATCTCATTTAAATTATCAACTTTAAA
AAAAAAAGGGCATTTTAGATTTTCACATTAAAGTAGCTATGAAGTACATGAG
AGTTCAAAATGCATCATTACAACATGTGTGCATGCGTTATAAGAGATGAACT
GTCATTATTGTGAAGATAGCAGTGTATTGTAAGAAATGTCAAACATCT

Blastx search: emb|CAB12544.1| (Z99107) alternate gene name: yfnJ; similar to cytochrome P450 /NADPH-cytochrome P450 reductase *Bacillus subtilis* >gi|2116974|dbj|BAA20123.1| (D87979) YfnJ *Bacillus subtilis* Identities = 19/61 (31%), Positives = 32/61 (52%), Gaps = 4/61 (6%)

Blastn search: gb|AC006001.2|AC006001 *Homo sapiens* clone DJ0756H11, Identities = 21/21 (100%)

Locus 008: Accession # AY351337 All Klamath catostomids species are identical at this locus. The primers for this locus amplifiy a region larger than the clone sequence.
Clone size: 341bp. PCR Product size: 543 bp
Primers: 008F+: 5'GCTGTCGTCCGATAGAGGGTGGAGGAGTGG3'
008F-: 5' TGATTTGAAAAACAGTGTTATCTTAGGAAG3'
Mg++ Concentration: 2mM Annealing Temp: 49 ^{0}C
Forward sequence:
CTGTCGTCCGATAGAGGGTGGAGGAGTGGCAGAGGAACAAGCTACGGCATA
TTGGAGAACTGGCGAGTAACAGTTTGTGTCTTCTGTTTTAGTTTATTATTAAA
ATATTATTTATGTTGAAAAGCCGGTTCTCACCTCCTCCTTTCCATTAACCTCTT
TACAGTGAGCCAAACCATTCGGACAATTTCGTGTGATTTCACCATATAATTTG
TTGCACGTTACAAGCTAAGATACATTGTTGCTAGTTCAGATTGGTTTTAATTT
GTGGAGAGAAATAATTCAACAGAATCTAGACTTCCTAAGATAACACTGTTTT
TCAAATCA

Blastx search: gi|3414805 (AF060882) NADH dehydrogenase subunit 8 *Crithdia oncopelti* 14/36 (38% identity)

Blastn search: gb|AC006971.2|AC006971 *Homo sapiens* PAC clone RP4-791C19 from 7p11.2-q11.21 Identities = 29/31 (93%)

Locus 009: Accession # AY351338 All Klamath catostomids species are identical at this locus.
Clone size: 528 bp. PCR Product size: 375bp.
Primers: 9F+: 5' TCC TGT AAA TTT GCT TAT TGG 3'
9F-: 5' ACA TAT ATG CCT GGT GAA AAA 3'
Mg++ Concentration: 2mM Annealing Temp: 49-51 ^{0}C
Forward sequence:
TTGCATGCCTGCAGGTCGACTCTAGAGATCAATTTAATGAGCTCACCAAGAG
ACAAAAACTTTATGTTTTGAGCTACATCAGACATCAGTATAACTGAAATATAC
TCAAATCATTGGAATTTAAATTCCTGTAAATTTGTCTATTGGGACGTTTTATTG
TTTTTGTCTATAAGTTTTTCTGCTTTACAATATTACTAACATTACTTTATTAGG
TAACACTCACATTGCTGAAAAGTGCATGTCAATTTTATTATACAACTGAGGGT
TGAAGCTTTTATAACATCTACCAACTTGGTTTTAATTAAGTCTTTTTAGGAAA
AATAGGAGGTTAATTTCTAAATTATAGCCTACAATTATTTTCATTGGGGTCTC
TGGGACTTAACACAGGTCTATATGTCAATACATGAGTGTAGAATATGCGGGG
GTATTGGGGACATGTCCCCCTCACTTTTGATAAAACCAAAGTCCGTCCACTTC
ACTTTTTTCACCAGGCATATATGTTTTTGTTGTATATTTCAGTGTATATGTGTA
AATAAAGATGTCAAAATTCTGAAACTGGATCCCCGGGTACCGAACTCNAATT
CGTAATCATGTCATA

Blastx search: sp|P40584|YIW0 Yeast Hypothetical 13.0 KD Protein in HYR 3' region *Saccharomyces cerevisiae* 22/74 (29% identity), 31/74 (41% similar)

Blastn search: gb|M80338|CORDTXRAA *Corynebacterium diphtheriae* diphtheria toxin repressor (dtxR) gene, Identities = 39/41 (95%)

Locus 010: Accession # AY351339 PCR difficulties, amplified in all Klamath species but was never a strong single band. Clone size: 488 bp. PCR Product size: 421 bp.
Primers: 10R+: 5' TTT ACA CTA AAC ACA ACT AAT AA 3'
10R-: 5' AAG GCT AAT ATA CAT CAA TAG AT 3'
Mg++ Concentration: 8mM Annealing Temp: 57 ^{0}C
Reverse Sequence:
ATTTTACACTAAACACAACTAATAAATATAAATTAAATAAAACTAGAAACAC
CCTTATTTTATAACT?ATT?ATAGATAAAAAGTACTATATAAAAATATAGTAAT
AAACAGCCACATTGAACAATATATTTTGCTACAAAAAGATGTCAAATGTCAC
TGAAACATTTGTAACATCAATTTATCTGACTTCCCAAAACTAATTTTGAGGCA
GGACAGTTTAGAACATTTTACATGTGTTTTTTGATTGTTGGGTCAGCTCACTC
AGCTGTGTGTGCATAACAGCGTAACATTAAAAACAGTGAAGTTTTGTCACAT
TTCACCACTATTTATTATGAAAATGAGCAAGTGTTGTCAAGTTAGCAGCATTG
CTAGTTTTAGTTAAACCTCAATGATAGTTATTTATCTATTGATGTATATTAGCC
TTCATACTGCTGTATCACCTCTCAAATACTCCTTCAAAACAGCTAATAGCCAC
TTGTTTCTG

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: gb|AC005039 CLONE NH0512E16 *Homo sapiens* 23/23 (100% identity) overall 4.7% identical

Locus 012: Accession # AY351340 Amplified in all Klamath species, appeared to be a single band on agarose gel, but sequencing results were always confusing. Sequencing results impled that another DNA was present in the sample. Clone size: 456 bp. PCR Product size: 456 bp.
Primers: 12F+: 5' TGA AAC TTC ACA GAC GCA TTC TGG A 3'
12F-: 5' AGG TCA TAC AGG GGT AAT TCT TAT T 3'
Mg++ Concentration: 2mM Annealing Temp: 51 ^{0}C
TGAAACTTCACAGACGCATTCTGGAGACACCTGAGACTTATATTACATCTTGT
GAAAAGGGGCATAATAGGTCTCCTTTAACGGTAACAAGATGATATTAATGAG
CTTGACTCGGCACAGGTTTTATCTAATGATTGCCAGACCTACTTACCTGCTAC
TAAAATGTNACGTCCCTGACCATGCACATTAAAAGTGAACAGCATCATATCG
AAATGCTTTGATACTTAAAAGTAATGTAGTTGGGCTGACATGACTGTTAGACT
CATGAGCTTTTAAATTTGGAAAATTGATAAAGCCCCAATGTTTGAAGGTCCCC
ATAGATTGAAACTTGGTCTGAACAAAGATGAGTGAATGATTTATGAAGACAT
ACAATGCAAATTTCTGCACTTTAATGATGCCTGAATGAGTAATAAAATGTNA
ATTTGCCGTGAAATAAGAATTACCCCTGTATGACCT

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: emb|AL023878|HS618F1 sequence from clone 618f1 chromososme Xq25 *Homo sapiens,* Identities = 24/25 (96%)

Locus 013: Accession # AY351341 All Klamath catostomids species are identical at this locus.
Clone size: 499 bp. PCR Product size: 320 bp.
Primers: 13F+: 5' AAT GTC ATT TAC TGG ACC ATA CT 3'
13F-: 5' TTA TTT AAA ATA CGA TTG TGA AT 3'
Mg++ Concentration: 1-2mM Annealing Temp: 53 ^{0}C
Forward Sequence:
AGTTAAGATACGGTATGATTCTGGACTGTACAGAGTATGTTGTCAATACGTG
ATACAGTAAGAATTGTGTAACAAATACAGTAATGTACACTGTAATGTCATTT
ACTGGACCATACTGTACCGTACTCTAACAGTACAGTATGAATGAACTAACAG
TTTACCTATTCAGATTGGGGTGAGAAATTACATTTACTTTTTGTCATTTAGCA
GACACTAATGGTAACCAAATTGACTTAAAAATGAGAGACTCCCAAATTTGTC
ACAGTCCTGTATCTTGCATTACAGTGGTTAAGCCTTTGTTTATTGTAACACAT
GGTGGACATTGACATTGATTGTGTACCTTGCCACAGTTGCCTTCGGCTTGCTC
ACTGGGGTTATAAATACAATTATTATTTAAATACTTATTTTTAAAAACAATTC
ACAATCGTATTTTAAATAATTACACAATGATGACTCATAGACATTATAGACAT
TACAGTTTTATCTTCTGTTAATGCCT

Blastx search: pir||S64826 probable membrane protein YLR004c *Saccharomyces cerevisiae* 17/32 (53% identity), Positives = 21/32 (65%)

Blastn search: emb|X91894.1|MBMTBAGEN *M.barkeri* mtbA gene
Identities = 28/30 (93%)

Locus 019: Accession # AY351342 *Ch brevirostris.*, *D. luxatus.*, *C. snyderi* are identical at this locus, *C. rimiculus* never produced clean sequences.
Clone size: 526 bp. PCR Product size: 421 bp.
Primers: 19R+: 5' CGT CAA GCT AAC ACA GTG ATG TCC T 3'
19F-: 5' GTG GAG CGG CAA TGA AAG CAA GAG A 3'
Mg++ Concentration: 2mM Annealing Temp: 47 ^{0}C
Forward sequence:
AATTCTTACTGTTTCCATAAAGCAAATTCGAAGGGGCAACAGGAGAGGTGGA
GCGGCAATGAAAGCAAGAGAGAACACTCTCCAATTACAAGTGTTCGTACAGT
AATTCTGTCCTCCCAGAGGAAAATTTTACAGCTCTTTCCTGGCTCAGGACACT
TATGAAACTTTTCCACTGCACGTTACAGTTCGACTCGACTTGACACTGCTCAC
TTTACTTGTCTGAGCTTGCTTTCCCAATGCAGGCTGATCGTCATAGTTGTGCA
GCCTCTACTGCTGTGACATCATTGTAAACGTAACACAAACACACAACAAAGG
AGCAATGGAGGATTTCGAAGGAATGGTGTTCTTGATTCTCGGCGTGTGGCTGT
TTTTCACAGCAAGACAAGACAAGACAATCTTTGTTTCAAAAGAGCTGATGGC
ATCATACACCAAAGCGCAGACAAGGACATCACTGTGTTAGCTTGACGACACA
TGGGGTTAAGTTAACCTGCCATCTAGCTTATAGACGCTCCCTGACCAATCAGT

Blastx search: ref|NP_003150.1|PSTK9| serine/threonine kinase 9 protein *Homo sapiens,* Identities = 40/125 (32%), Positives = 56/125 (44%), Gaps = 21/125 (16%)

Blastn search: gb|AF037352.1|AF037352 *Mus musculus* T cell receptor gamma locus, TCR gamma 1 and gamma 3 gene clusters Identities = 21/21 (100%)

Locus 021: Accession # AY351343 Weak PCR amplification in all Klamath species, never sequenced in each species. Clone size: 462 bp. PCR Product size: 414bp.
Primers: 21F+: 5' TAA CAC AGC AGA ATG TCA GGG TAG C 3'
21F-: 5' ATA AAT AAA AGG TTT GGT AAC ACT T 3'
Mg++ Concentration: 1mM Annealing Temp: 49 ^{0}C
Forward Sequence:
CAAATATGTACTGTATAATGGGAGCTGGCACTTGTGAGTAACACAGCAGAAT
GTCAGGGTAGCCTACTGGAACATTCCGTAGTAACATGTCGACTTCCTGTGTGA
AAGATATGACTGCAGTCTTCATCTCATTTAGTTAATGCATAAGGTATCATTAA
CAAACAATGGACAATATATTTATTGCAGCATTTTTTAAGCTTTGTTACTGTTA
GCTAATAAAATTACAATCGTTCATAGTTAGTTAATGTTAGTTTATATTGTTAG
TTTATAACTAATGTTAACATATACCACTACATTTTAAAAATGTATTAGTTAAT
GTTGTAATTAACATTAACAAACATTAATAAATGCTTTAAAAGTATTGTTAATT
GTTAGGTCATGTTAACTAATGTTGATAACCAATGTTAACAAATGGCACATGA
CTGTAAAGTGTTACCAAACCTTTTATTTATTTTCAGAAAT

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: gb|U31357|NCU31357 *Notropis chrysoleucas* ependymin (sh) precursor gene,
32/36 (88% Identity)

Locus 026: Accession # AY351344 PCR amplification in all Klamath species but never as a single product. Not sequenced in each species. Clone size: 595 bp. PCR Product size: 493 bp.
Primers: 26R+: 5' GTC TCC CCA TGT CTA AGG AAG TGA C 3'
26F-: 5' CCA AAA ATG AAA AAT CTG TTA TCT T 3'
Mg++ Concentration:1-4mM Annealing Temp: 48-53 ^{0}C
Reverse Sequence:
TTTTACTCACATGACACATCCTTTTGCTCATCTGTCTGTGTCTGGTCTCCCCAT
GTCTAAGGAAGTGACATAATAACCCAGCCATTCTGTCCCACAGATGCTGTCT
GCAACCGCCACGCTGGCTTCTTATTGGCCTGCTGATGGGCCCACAGTGGCTCA
ACTCAGACCTCCATGTGCCGGAGGTTGTTGGGTTCAACTCATTCAAAAAGAT
GCAAAGCCTAAGCAACAGTACACAACAAACAGAGAACAGTCAAACATTTAG
GCCAGAAAACACACTCCTCAGATGGCCTTAAAGAGATTGTTCCCCCAAAAAG
GACAATTCTGTCACATTTACTCACTCTCATGTTGTCCCAAAGCTCTATGACTTC
TTTATATCGTGGAACACAAAAAGATATTAAGCTGTATGTTAATCTCAGTCACC
ATTCACTTTCAGAGCATATGAAAGTAACTGACCCTGTTAAAGTGCATAAAAA
GAAAATCACATTGATTTGGAATAACTTTAGGGTGAGTTAAAGATAACAGATT

TTTCATTTTTGGGCAAAACTGGTCCCTTTAAAAAAAAGTGGTGTACTTACAAA
AAACACACTTTATACAGTGG

Blastx search: gb|AAD38587.1|AF145612_1 (AF145612) BcDNA.GH02976 *Drosophila melanogaster*, Identities = 15/40 (37%), Positives = 22/40 (54%), Gaps = 1/40 (2%)

Blastn search: dbj|AB009335|AB009335 *Carassius auratus* mRNA for brain aromatase 35/39 (89% Identity)

Locus 039: Accession # AY351345 All Klamath catostomids species are identical at this locus.
Clone size: 459 bp. PCRProduct size: 338 bp.
Primers: 39F+: 5' TCG ACT TCC TGT GTG AAA GAT 3'
39F-: 5' TAC AGT CAT GTG CCA TTT GTT 3'
Mg++ Concentration: 2mM Annealing Temp: 56^{0}C
Forward Sequence:
AAATATGTACTGTATAATGGGAGCTGGCACTTGTGAGTAACACAGCAGAATG
TCAGGGTAGCCTACTGGAACATTCCGTAGTAACATGTCGACTTCCTGTGTGAA
AGATATGACTGCAGTCTTCATCTCATTTAGTTAATGCATAAGGTATCATTAAC
AAACAATGGACAATATATTTATTGCAGCATTTTTTAAGCTTTGTTACTGTTAG
CTAATAAAATTACAATCGTTCATAGTTAGTTAATGTTAGTTTATATTGTTAGTT
TATAACTAATGTTAACATATACCACTACATTTTAAAAATGTATTAGTTAATGT
TGTAATTAACATTAACAAACATTAATAAATGCTTTAAAAGTATTGTTAATTGT
TAGGTCATGTTAACTAATGTTGATAACCAATGTTAACAAATGGCACATGACT
GTAAAGTGTTACCAAACCTTTATTTATTTTCAGAAA

Blastx: No significant amino acid sequence similarity found in GenBank.

Blastn search: gb|AC004831|AC004831 *Homo sapiens* PAC clone DJ0538P11 from 7p11.2-p13,
23/24 (95% Identity)

Locus 054: Accession # AY351346 All Klamath catostomids species are identical at this locus.
Clone size: 446 bp. PCR Product size: 286bp.
Primers: 54F+: 5' TAG GGA GAC ATG CTT TTG TTT 3'
54F-: 5' GCT ACT GGC ACC CAC ATC TTA 3'
Mg++ Concentration: 2mM Annealing Temp: 56 ^{0}C
Forward sequence:
TAGGGAGACATGCTTTTGTTTGACATTGATAGTGGAAGTTATCCACAACAGTG
GGACCATGCTTTGGTCCAGCGAGGACTGACACAGCAAGTGGAAGACCACACT
CAGGTCAGTAAAAACGGGCATTCAACATCTGATTGTGGGGCTTTTTGGATTTC
AGTTGTCTTAACACATCCAAGGGTTTAGGCCTTATGTAATTTTAGCTACCTCT
CCTGCTAATTGCCATAATGTGACTGCTGCAATAACTATGTCACTTTAAAAATG
AATCATCTGCTAAAACATTAATTAAGATGTGGGTGCCAGTAGC

Blastx search: pir||H64168 hypothetical protein HI1195 - *Haemophilus influenzae*
Identities = 13/39 (33%), Positives = 22/39 (56%)

Blastn search: gb|L14561|HUMCAATPX Homo sapiens plasma membrane calcium ATPase isoform 1 (ATP2B1) gene, 20/20 (100% Identity)

Locus 061: Accession # AY351347 All Klamath catostomids species are identical at this locus.
Clone size: 375 bp. PCR Product size: 215bp.
Primers: 61F+: 5' ACC TGT CGT ACA TCG GAG AGG 3'
61F-: 5' AAC CAT TAG GGA AAT ACT CCA 3'
Mg++ Concentration:1-3mM Annealing Temp: 48^0C
Forward Sequence:
AAAAAGTACTTTGAAAAATGTTAGACCCTGAATCAACTTGTCTATTATTGCCA
ATACACATTTAAAACCATTAGGGAAATACTCCAAAGTCCACAGTACGCATTA
CAAGGAAAAGTGCATGCAACTGGTTTCAAGACAAGCAATAGTAGGCAAGAC
TAGGGCTGGTGCACAGCTCTGTGATTGAATGAACAAACCCGTAAGAGAAAAG
AAAACATTATTACAATTCTAGGAAAAACAACGGAAACAATGCGGAAGCTGGT
GGTAAGTATAACAGCATCTTACCAAACCATGCCGTATGCACCCTCTCCGATGT
ACGACAGGTTACTGTAGCGCGGCCCGACATCGAAAGCTTGACCACGGACCAT
CTCGGCACC

Blastx search: sp|P26696|ERK2_xenla mitogen-activated protein kinase (myelin xp42 protein kinase) *Xenopus laevis,* Identities = 28/30 (93%), Positives = 29/30 (96%)

Blastn search: dbj|D87264.1|D87264S1 *Mus musculus* DNA for ERK2, exon 1
75/88 (85% Identity)

Locus 063: Accession # AY351348 All Klamath catostomids species are identical at this locus.
Clone size: 338bp. PCR Product size: 278 bp.
Primers: 63F+: 5' TTT CAA AGG GAG GGT CTC TGA TTT A 3'
63F-: 5' CCA AAG GGC ACT TTG AAG ATT GCT T 3'
Mg++ Concentration:1-4mM Annealing Temp: 53^0C
Forward sequence:
TACAGTCTAAACTGGATAAATCGCACTCCAAAGGGCACTTTGAAGATTGCTT
CAAACAGAATTTCCATTAAAATTGAATTGTGAGTTCCGAATGACCATTATTTT
TGAGCCCCAAACCTTTTAGCCCTCCAAAGTTCTCACAGTGGATGGTTAAGTCA
GTACACGTATGCTAATAAAATAGGCCTAAAAAAGCACGCAATTTGTAGCACC
TTTTGACTTTTTGCGCTTTATTATCATGCAGTAACACTTATGTAGTGATTGATG
TAGAGTATGTCGTAATTAAATCAGAGACCCTCCCTTTGAAATCCAAACACAA
GTGGCCAGTAGAGAAGCATATTATGAT

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: emb|Z48717|CET10B9 *Caenorhabditis elegans* cosmid T10B9 23/24 (95% Identity)

Locus 067: Accession # AY351349 All Klamath catostomids are identical at this locus.
Clone size: 427 bp. PCR Product size: 305 bp.
Primers: 67F+: 5' TTG CAA TAA GTT ATG TGC TGT 3'
67F-: 5' TCT GCT AGA GAT GGA AAA ATA 3'
Mg++ Concentration: 0.5-2mM Annealing Temp: 56^0C
Forward Sequence:
TGCTCTTCTTCAATGGTATTGGTCTATAATTTGACATATGTAGCACTTTATGAA
TTAGTTGTTTGAAGCTGATTTGCAATAAGTTATGTGCTGTATCTGTTCTTTTGC
ATCACAGATGATTCAGGGAGGAGAGAGGATGTTGAGGATAGTACTAGAGTG
GAAGATTTAGGAACTGTAGAAACAGGCCCAGCCAGAGCAAAACTCAAAGAA
TACCCTCTCACCAGCTTTGGACTACAGGGAAGTGGTTGCTAGTGTGAAAATA
AAATGGTCTGGGCTAAGCCCCGGATGTCCTTCAATGCTGGAAACGCCTCTGC
ATCTGATGATAAATTACAATTTAAAAGTGGATTGTTTTGTGCATATTTTTCCA
TCTCTAGCAGATGAGTGAGGTCTGACACAACACAAATCCAGTTTAAGCCCTA
TAGTCTAA

Blastx search: gi|2745729 (AF016192) potassium channel *Rattus norvegicus* 17/49 (34% Identity), Positives = 27/49 (54%), Gaps = 2/49 (4%)

Blastn search: gb|AC006067.3|AC006067 *Arabidopsis thaliana* chromosome II section 83 of 255 of the complete sequence Identities = 22/22 (100%)

Locus 076: Accession # AY351350 All Klamath catostomid species are identical at this locus.
Clone size: 260bp. PCR Product size: 190 bp.
Primers: 76F+: 5' TGA AGA GTA AAA TAC AAC CAG 3'
76F-: 5' TGT GGA CTC GTT TGA AAG ATA 3'
Mg++ Concentration:1-3mM Annealing Temp: 48^0C
Forward sequence:
TCTTCATCTGATTATCTTGTGTGTGGACTCGTTTGAAAGATAATCACAGACTC
TAAATAATGATATGTGATATTTTATGTTCCGTTTAATTTGCATGAAGTTATTA
GCAAAAATGCGGCATATAAGCGACATCAACATACTTGTCAAAAGACATATAT
TTAGCTGTTACTCGCTATATTTTCATCTGTGAGTAAAACAAATAATCTGGTTG
TATTTTACTCTTCAAGAGATTTCCAACAACATATGACACATGACTATTT

Blastx search: dbj|BAA20793.1| (AB002333) KIAA0335 *Homo sapiens* Identities = 12/38 (31%), Positives = 21/38 (54%)

Blastn search: gb|AC005880|AC005880 *Homo sapiens* chromosome 10 clone CIT987SK-1143A11 map 10q25 20/20 (100% Identity)

Locus 079: Accession # AY351351 All Klamath catostomid species are identical at this locus.
Clone size: 328bp. PCR Product size: 249 bp.
Primers: 79R+: 5' CGT CCT CTT TCT GTC TTG TGA 3'
79R-: 5' TCC CAT TAA ATT GAT TGG AAT 3'
Mg++ Concentration: 0.5-2mM Annealing Temp: 56^{0}C
Reverse Sequence:
TCAGCTCTGGACAGACTTTTTGTGCTCAAACCTCGTGTTTCTGTGCTTCTCCAA
GCCGTCCTCTTTCTGTCTTGTGAGTTTCAAGAAAATCTGAGGAAGTCGAAATT
TGCGCGCGCAAATCCTTAAAGGGGTACGCACGAAATTTTCGACATCCTCCGA
TCTTAATGAAATTTATACCATAGAAAGAGGAGGCTTGGAGATGAACAGAAAT
ACAAGTTTCAGCTTCCCAAGTCTGTCTAGAGCTGAGATATGCACATCCCAAA
AATTTAATGTGGGTAGTCCCAATTCCAATCAATTTAATGGGACAAATTGTACA
TGGGATGTTCCA

Blastx search: gb|AAD35361.1|AE001709_7 (AE001709) pyruvate,orthophosphate dikinase *Thermotoga maritima* Identities = 16/40 (40%), Positives = 26/40 (65%)

Blastn search: gb|AF086906.1|AF086906 *Arabidopsis thaliana* root gravitropism control protein (PIN2) mRNA,Identities = 21/21 (100%)

Locus 081: Accession # AY351352 All Klamath catostomid species are identical at this locus.
Clone size: 402 bp. PCR Product size: 261 bp.
Primers: 081F+: 5' ACT ATG GGA AAC GTC TCT GTA 3'
081F-: 5' TGG GGA ACA CCG CAA GGT AAC 3'
Mg++ Concentration: 1mM Annealing Temp: 56^{0}C
Forward sequence:
TCATTGAAATGATGTGACTGTGGTGACAGTGGTTCTGTTCCAAACATTCTTTA
GGAATTCTTCTCACTATGGGAAACGTCTCTGTACGAAGGGCAGACCAATTAC
AGCTTGTTATAAGAGCCCGCCTCCTCCCTAAAATACCTGCTGTAATGCTCTGA
AAAGACACTTTTCCTCAGCACCAGAACTTCACTATTTAACTCTTTGCAGGCGT
CACCCAGCATAGTCACAGTATTACTCTGCTATTTATTAAGCTGCTTGTCTGCTT
TTCACTAAGCAGTGTTTTGTAACTGAAAATTTGTTTGGAGGTTACCTTGCGGT
GTTCCCCATGGCTTCGACAAAAGTTATCATAGACAAGAATAAAGATGCCTCG
TCTCACCTGTGTCTGGCTGCCTGTGGATTTAA

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: gb|AF047826|AF047826 *Homo sapiens* cadherin-7 (CDH7) mRNA, Identities = 20/20 (100%)

Locus 082: Accession # AY351353 All Klamath catostomid species are identical for this locus.
Clone size: 316 bp. PCR Product size: 262 bp.

Primers: 82F+: 5' TCC CAG TTG CAA CAT TTT GAG 3'
82F-: 5' ACT CTG TGC TCC CTC CCT CTC 3'
Mg++ Concentration: 2-3mM Annealing Temp: 53^0C
Forward Sequence:
ACGGTGCATGCCTGCAGGTCGACTCTAGAGGATCCCATTAAATTAATTGTTAA
TATTTGCAAATAGCTTTCCCAGTTGCAACATTTTGAGCATTGGAGAGCAATAG
GAAAAAATAAGGAAATAAAAAAAGTGAAAGAAAGGAGAAACTTTTTTGCCA
ACCTGCACAGTACAATCAGAGGTGTGCAGTCTGCTGGTTTGCTTTGTGACAGC
CTCTTTCCCTGCCTCACTCTCTCCCTGACGTTCTCTGTCTCAATGAAAACCTCA
TGCATGTGTGCTCACTGCCGTCCCACTCTGAAAGAGAGAGCAAGAGAGGGAG
GGAGCACAGAGTAGGAGAGAGAATCACATCATCAGAG
Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: gb|M58040|RATTRFR Rat transferrin receptor mRNA, 3' end. Identities = 34/34 (100%)

Locus 083: Accession # AY351354 Klamath species are identical but differ from the clone sequence. Clone size: 513 bp. PCR Product size: 451 bp.
Primers: 83F+: 5' CAT TTG TTC CCC ATT CTG ATG 3'
83F-: 5' CCA CAA CAT TAA AAC CAC CTG 3'
Mg++ Concentration: 2-4mM Annealing Temp: 53^0C
Reverse Sequence:
AAAACAATGGAATATATAAATATACATATATATACATATACATACACACACA
AACTTATCAGCCACAACATTAAAACCACCTGCCTAATATTGTGTAGGTTCCCC
TTGTGCCACCAAAACAGCGCCAACCTGCATCTCAGAATAGCATTCTTCTTACC
TCAATTGTACAGAGTGGTTATCTGAGTTACGGTAGACTTTGTCAGTTCGAACT
AGTCTGGCCATTCTCTTTTGACCTCTCTCATCAACAAGACATTTCCGTCCACA
GAACTGATGCTCACTGGATGTTTTTTGTTTTTGGCACCATTCGGAGTAAACTC
CTAGAGACTGTTGTGGGTGAAAATCCCAGGAGCAGTTACAGAAATACTCAAA
CCAGCCCATCTGGCACCAACAATCATAAATGAGATTATCTAATCAGCCAATT
GTGTGGCAGCAGTGCAGTGCATAAAATCATGCAGATGCGGTTGAAGA-
CTTCAGTTAATGTTCACATCAGCCATCAGAATGGGGAACAAATGT

Blastx search: gi|1022896 (U37772) WI-1 adhesin *Ajellomyces dermatitidis*
Identities = 14/45 (31%), Positives = 22/45 (48%), Gaps = 1/45 (2%)

Blastn search: emb|AJ009633.1|DRAJ9633 Danio rerio spermine synthase gene
Identities = 70/81 (86%)

Locus 088: Accession # AY351355 All Klamath catostomid species are identical for this locus.
Clone size:538 bp PCR Product size: 482 bp
Primers: 88F +: 5'TGTGAAACTGTATCGCCAAACATTA3'
88F- : 5'AGCTAGCTTGCTTCCCTCTCTGTGTA3'
Mg++ Concentration: 1-4mM Annealing Temp: 53^0C

Forward Sequence:
AATGGACTGTGAAACTGTATCGCCAAACATTAAGACTGGTAGGCAGAGCCAG
GGTCTAAAAACAGGTCCATGAAGAGCTAATTTGGGCAAAACACTGAGCAGA
GAGATTTGTGACTCCACAAGAGGGCACTGCACTCATTTGTTGTATGTGATGAA
ATATTCTTAATTCTCTCCCATTCCTATTCTTTCATTCTCTCCCTCCCTCACACAC
ACACACATTGACACCCACACCCACATAGGAACTGAATCAAACAGACAGACTG
GTTGTCTACAGCATGTGGGAGGCCCTTTCAACCAAGAGAACATCTCCAGGTT
AATCTAGCTCTAACTCTTTCTATCTTTATCTGTCATTCCAAATCCCACTTAAGG
GATTCATTGTAAACAGTCCCAAATTCATAATTCATTCAAAATAATGGAATTAT
CTTGCAGAATTGCATTTCAACTAAAATATTTCAGTGATGTACACAGAGAGGG
AAGCAAGCTAGCTTGGCATTGCCTGCTAGAGTTTAACACTGCATAGCAACAG
TCAGTGTATTA

Blastx search: emb|CAA17249.1| (AL021899) hypothetical protein Rv2035 *Mycobacterium tuberculosis* Identities = 11/28 (39%), Positives = 19/28 (67%)

Blastn search: gb|AC004866|AC004866 *Homo sapiens* PAC clone DJ0728H09 from 7q11.23-q21.1, complete sequence Identities = 22/22 (100%)

Locus 090: Accession # AY351356 PCR difficulties, *C. rimiculus* amplified very weakly at different temperature but other species never amplified. Clone size: 517 bp
PCR Product size: 455 bp
Primers: 90F+: 5'AGAACGGAAGAGTAAGACGAGGTCA3'
90F- : 5'CTCTACAGAGCCCTACCATCAATGA3'
Mg++ Concentration: 1-4mM Annealing Temp: 48-56^{0}C
Forward Sequence:
CAAACCAAGAACTGTGAGCTGCAGCACAGCAGAGTGCAGCTGATGCCCTGG
ACTCCAGCAGAACGGAAGAGTAAGACGAGGTCATTAACCTCTGTGACTAAAA
TGCACTCAGATGGCTCCCTGGTCTGCAGGCTGATGACAAGCGTCTGTGTTCTT
TTCAACAACTTGTTGAGCTGTCTCCACCCCCACCTCTGAAACCAACACCACCT
ATCCCCATCTCAGGAATGTAACCCAAGCCCCTCTCCTCTCCAATCACTTTGAG
AACAGCCCACTGAAGGTGTAAGACAGCTGTTGCTGTACTCTTTCCTTTTGTGC
GTTTATGTATTTTCTCCACAACTGCCTCTTGTATTTCCTCTGTTTCACTCTCTCG
CTGTGGCACTGCACAATAATTCATGATGCACTGGACCTGAGTGCAGTACAGT
CGACTTGACTGACGGGAATCGAGCGGCGTGTCTCTATAATGGTGAAAGTGCT
TTGGTAATGGCTATGTCATTGATGGTAGGGCTCTGTAGAGGGA

Blastx search: gi|203227| pir||B44173 calcitonin gene-related peptide alpha *precursor Rattus norvegicus* (M11597) alpha-type calcitonin gene-related peptide
Identities = 27/89 (30%), Positives = 37/89 (41%)

Blastn search: gi|3777528|gb|AF060228|AF060228 *Homo sapiens* retinoic acid receptor responder (tazarotene induced) Identities = 24/25 (96%)

Locus 094: Accession # AY351357 All Klamath catostomids species are identical for this locus.
Clone size: 473 bp PCR Product size: 372 bp
Primers: 94F+ : 5'TATGACCTCACTATTTATTCC3'
94F- : 5'CATTTAATCGTAGAAAACATT3'
Mg++ Concentration: 1-4mM Annealing Temp: 50^0C
Forward Sequence:
CTTGTTGTTTATCACTTGTGGTAAATATTTATGAAATTTTTACATTTTATGACC
TCACTATTTATTCCCATCGTAAATTTGTTATATGTTTGTTATATGTTTTTAATTT
CTGTAACCTTGTTAATTGTGCTGCTGCCTGTCTTGGCTAGGACGCTCTTGGAA
AATAGATTTTTAATCTCAATGAGTTTTCTTTCTGGTTAAATTAAAGGTAAAAA
AAATTTTAAATACAAATTTGACTCAAGTTAAACTATTTAAAGGCAATGCCACC
AAATACTGACAAAGTGTATGTAAACTTCTGACCCACTGAGAATGTGATGAAA
GAAATAAAAGCTGAAATAAATCATTCTCTCTACTATTATTCTGACATTTCACA
TTCTTAAAATAAAGCAAAGATGGGGAATGTTTTCTACGATTAAATGTCAGGA
ATTGTGAAAAACTGAGTTTTAATGTATTTGGCTAAGGTGGTGTAAAGG
Blastx search: gi|1513298 (U66526) AbcA *Dictyostelium discoideum*
Identities = 23/64 (35%), Positives = 32/64 (49%), Gaps = 5/64 (7%)

Blastn search: gb|AF017232|AF017232 *Salvelinus namaycush* transposon Tsn1-3 transposase (Tsn1) pseudogene, Identities = 147/156 (94%)

Locus 107: Accession # AY351358 All Klamath catostomids species are identical for this locus.
Clone size: 593 bp PCR Product size: 543 bp
Primers: 107R+ : 5'ACCCCATACTCAACACTCAATCA 3'
107R- : 5'TATCTTTTGGCCTGCTGCTTCAG 3'
Mg++ Concentration: 1-2mM Annealing Temp: 55^0C
Reverse Sequence:
TTGTTGTGGGAGAGCCCTTGAGGCTATCTTTTGGCCTGCTGCTTCAGCTTCAA
ACCCTTTGGCAGTTTGTATGTCCTACTGGTCAGCTCTAGCTTTCTATGTTCTGA
TTGGCCAGTCTCAAACCTTTAGGCAGCTTTTTCAACTAAATTAAGTCCCTGCA
GCTAAAACTGTCCTGCTTTTATTGGTTAGTAATGGTCTCTATGCCATTTGCTCA
CTGTGATTGGTAGATATTGATGCCCAAGGCAGAATTGGTGTTATTTGTTTGGC
GTGTTCCCCAAGGCGGTGTTTTGTTCTGATTGGATGGCCTAGGTAAGGGCATG
CACTGCCCTGGAGCATGTACACAGACCTCTACACAACCCCCAATCGAATGAC
TGTATGAATAACATTTACAAATCAAAAAAAGGATAGAAACACAATCATTTGA
CAACTTTTCTGATTTTAACATTTTTGGAACATTACGTGATAAAGAACTTCGTA
CAAAACTCCTCGAAATACAGCTTGTCTCAATGTCTTGATTGTGTATCTAGCAT
CGAAAACCTCTATATAGCATGATTGAGTGTTGAGTATGGGGTCTTGCTTCATG
CAGAAAGTCA

Blastx search: emb|CAB38989.1| (AL034558) predicted using hexExon; MAL3P2.2 (PFC0165w), Hypothetical protein, len: 1676 aa *Plasmodium falciparum* Identities = 23/85 (27%), Positives = 44/85 (51%), Gaps = 1/85 (1%), gb|AAD31534.1|AF148447_1

(AF148447) ubiquitin C-terminal hydrolase UCH37 *Mus musculus* Identities = 29/80 (36%), Positives = 42/80 (52%), Gaps = 6/80 (7%)

Blastn search: emb|Z97195|HS106H8 Human DNA sequence from PAC 106H8 on chromosome 1q24. Identities = 21/21 (100%)

Locus 117: Accession # AY351359 All Klamath catostomids species are identical for this locus. This locus is polymorphic with a transversion of C to T at position 123 of the *C. rimiculus* sequence. This polymorphism was found in 2 of 150 (1.3%) individuals tested.
Clone size: 580 bp PCR Product size: 498 bp
Primers: 117R+ : 5'TTATTTCGACTTATTGAACCATAGA3'
117R- : 5'TTAAACAAATCCCGCAACCAAAACA3'
Mg++ Concentration: 1-4mM Annealing Temp: 50^0C
Reverse Sequence:
CGCTTTTCTGTCGGCCGTTTTGAACGGTGATTCGCAAATTAATCACTATGATG
CTGGTACATACACTTAATTTAAACAAATCCCGCAACCAAAACAATCCTATCGT
TTGCCCGCGAAAGGATGTGCAGCTTTTGAACGACCTGTTTCAGGTAAAGGCA
TTTTTCAACTAAAAGAGTACCACAACACTAACAGTAGATGAACGCAACTTTC
AGTTTATATTTTGAAGTAGTTCAAATGACCTGAAATGGTTTTTATGAACCATT
ATATTGATTAGCTCAAAAATGACCTCTTTTCTAAAAATAAAAAAACCATCAGT
GAGGCACTTACAATGGAAGTGAATAAAAGCCTACTCACTGTTTCAAAAGCAT
AGCCACAACACGTAAACAATATGTGTGTAAACTAAAAATTTCCGTGATTATA
GCCAATATTACTTCGCTGTCATTACGATGGAATGCCAACAAACCCTAAAACC
ACTTTTTGCTCTTTTGTTTTTAAACCATTAATAAAGCAACGTTCCACAACGTTA
TTTTGAATTTAAGCAATATCTATGGTTCAATAAGTCGAAATAAATGTTTATGG
T

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: emb|Z82194|HS272J12 Human DNA sequence from PAC 272J12 on chromosome 22q12-qter contains ESTs Identities = 21/21 (100%)

Locus 119: Accession # AY351360 All Klamath catostomids species are identical for this locus. This locus is polymorphic with a transversion of G to A at position 361 of the clone sequence. This polymorphism was found in 2 of 150 (1.3%) individuals tested.
Clone size: 524 bp PCR Product size: 460 bp
Primers: 119R+ : 5'TCTTCAGCTTCACTACTGGTA3'
119R- : 5'GTTCAATCTTGCCGTAAACTG3'
Mg++ Concentration: 1-4 mM Annealing Temp: 51^0C
Reverse Sequence:
TTGGGATACCATCTTATCAGTTCAATCTTGCCGTAAACTGAGGGTGTGCAGTA
AAAAGTTTTCACAAGATTTTGTTTGTACAATGAACTTAAGTACTGTGTGTTTG
TAAATTAAAAATTGGAACAGAAAAAAGGCATTCTGGTAAAACTGTATAGTTA
GAATGCATTCCATATTTGAACAGTACTTCTGATATACAACATACCTTGAATCC

AAAACATTATGGATGTTGTTGCAGCTGGACGTGGTCGAGTGCCCGTCTGGAG
AGATAAAGCGTTAAGGGTGCTTGCACCTGAGCTGAATTATGTCTTAACACCT
GTCTCTAATTCCATTGAGCATGGGGAGAGCAGCATATAAATGGTCATATCAC
AGCCAGACGAGAGAGAGAATGACACGAGTGACCAGTGACTGGTGTGTCTTAT
GTTATTGTGAAGCTGAAGACTCAGAGAAGTTTATGTTGACAAGTACCAGTAG
TGAAGCTGAAGACTCAGAAGCTGAGTTTAAGTTGACAAAGTTTGTGTTACAC
T

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: gb|AC007436.1|AC007436 *Homo sapiens* 12p13 BAC RPCI11-
Identities = 19/19 (100%)

Locus 120: Accession # AF362137 and AF362138 Polymorphic locus resulting from a 4 bp deletion at clone position 300 (bold sequence). *C. rimiculus* (1 base) and *D. luxatus* (4 bases) have unique autapomorphic alleles for this locus. Clone size: 511 bp
PCR Product size: 430 bp
Primers: 120R+ : 5'GCGATTGTCTTCTGTCTTTCTTTGA3'
120R- : 5'TACTGCCTTTATGTGCTTTTCTGAG3'
Mg++ Concentration: 2 mM Annealing Temp: 51^0C
Reverse Sequence: Bold nucleotides are the site of the 4 bp deletion
CTTTTTGAGACTTTCAGCGATTGTCTTCTGTCTTTCTTTGACAAAACGCAGCAG
GTTCTTCAGCTGGGTAATTCTGTACTCAAGTGATTTGGTCCGACCTGTGAGGA
AGGCCTTCCTGGCATGCTCTACTGCTTTTTGCTCACGAGACATACTGTGAAAG
AGGAAGTCATTCATTTCACTCGGGTTACACTTTTGTCACATTTACGTACATTTT
ACCCATACATTATGAATGAGAGTCAAGTAGGGGGATTTATAGTGGATTGATA
TCTAAGCCCTTACAGAGTGAATATTGAAATATTCCCCTATGAAAATTCTCTCA
TCGTTTTCTCACCCTCATATCATCACAGATGTTTTTACTTTTTTCCTTCTGCAG
AACACAAATTACGATTTTTAAGCTCTGTTAGTCCTCACAATGCTAGTGAATGG
GTGCCAGAACTTTGAAGCTCAGAAAAGCACATAAAGGCAGTATAAAATAATT
AATTAAGCAATATGATAGGTGTGGATGAGAAAC

Blastx search: sp|P11883|DHAP_RAT aldehyde dehydrogenase, dimeric nadp-preferring (aldh class 3),tumor-associated aldehyde dehydrogenase (htc-aldh) >gi|91936|pir||A30149 aldehyde dehydrogenase (NADP+) (EC1.2.1.4) 3, tumor-associated - rat>gi|202833|gb|AAA40713.1| (J03637) aldehyde dehydrogenase *Rattus norvegicus,* Identities = 17/37 (45%), Positives = 26/37 (69%)

Blastn search: gb|AF132287.1|AF132287 Cyprinus carpio pituitary specific transCription factor Pit-1 (PIT-1)gene, Identities = 23/24 (95%)

Locus 126: Accession # AY351361 All Klamath catostomids species are identical for this locus. This locus does exhibit 4 single base pair positions that are heterozygous found in *D. luxatus* and *C. rimiculus.*
Clone size: 453 bp PCR Product size: 306 bp

Primers: 126F + : 5'TCAGCGTGGCAGTTTTGGAAT3'
126F- : 5'GTTGAGGGAGAGGTTGTGCTC3'
Mg++ Concentration: 1 mM Annealing Temp: 51^{0}C
Forward Sequence:
CGTCCATCCTTATCTCAAGCTTTCTGTCCTAAGAAGTTAAAAGACACTTTGTG
ATGATTGGAAATTGTTAAGCCACAATGATATTTAGTAGGAACATAATCAGCG
TGGCAGTTTTGGAATGATGGCAGGATTTATCCGGCATTGGCCCTTTGCCGTAT
ACAGCACATTCTTCCAGTAAGTGCCAGTCAGAGCTGGGCACTAACTGAAGGC
CAAATACCTAAGAAAGTATTACTCAAGTGAAAAAACATCTTGTTTTGTGCTG
AAATGTGAAAATGCTTTTCGGTACTTAAACTCTCAACCTTGATTGTGTGACTA
GGCAATATTTCCATTTACTGAAATAAAAAACAGTGAATAAGTAGCAAGAGAA
ATATCACCTGGTGTCAGGAGCACAACCTCTCCCTCAACGTCAGTAAAACCAA
GGAGCTTGTTGTGGACTTGAGGAAGAAAGACAGA

Blastx search: gi|1127551 (U18939) orf2 *Battrachocottus baikalensis* Identities = 16/26 (61%), Positives = 19/26 (72%)

Blastn search: dbj|D86995|D86995 Human (gene 1) DNA for phosphatase 2C motif, Identities = 20/20 (100%)

Locus 139: Accession # AY351362 PCR amplifications were very weak and each species tended to work better in different Mg++, sequence was confusing, implying that more than one type of DNA was present.
Clone size: 518 bp PCR Product size: 431 bp
Primers: 139R+ : 5'AATTGAAACATAAAACATTCCACTA3'
139R- : 5'AGCCTACACTATTGGTTGAGTCAGT3'
Mg++ Concentration: 1,2,4 mM Annealing Temp: 56^{0}C
Reverse Sequence:
TCTTGAACACTCCCCTAGTCTTCCATTTGACCACCCCAAACATAGCCTACACT
ATTGGTTGAGTCAGTGTGGCTGGTCAGTAGAGCTGGGCGATATGGCTACAAA
AATTGTATCTTGATATTTTTCAGCTAATTGACGTTATTTAATATATATCTTGAT
AATTATGTTTCCTCTGATTTGACATAAAAGTTATTAGTTTTTGTTTTCAGAGGA
ACCATTATCTCTGCTAAATAGACATAGAGCTCTGTTTTCTTGAGTTCGAGCAA
AAAGTGCAATTTGAAGTTGCTTAAAGCGAAATTTGCTGTTTTCCTAAGAAATA
GACATAGCTTTAAAACATCTTCGTGCAATGACCTGTTTTCAGCATAGTGCCTA
AATTCAGTTTCTAAATTTGCAGTTTGGAAATTCCATCAGCAGGTGGTAATAAA
GTTCAAGTCCAAACTCTGAAAATATAGTGGAATGTTTTATGTTTCAATTATTA
TTATTATTATTATTATGTTTATTTATACAGTTGTATTTCT

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: gb|L10232|CHKMSLT *Gallus gallus* miCrosatellite DNA sequence. Identities = 20/20 (100%)

Locus 140: Accession # AY351363 Sequence over laps with Locus 139. No testing was done.
Clone size: 518 bp PCR Product size: bp
Primers: 140R+ : 5'ATTGAAACATAAAACATTCCACTAT3'
140R- : 5'AGCCTACACTATTGGTTGAGTCAGT3'
Mg++ Concentration: 1-4mM Annealing Temp: 50.4^{0}C
Reverse Sequence:
TTGAACACTCCCCTAGTCTTCCATTTGACCACCCCAAACATAGCCTACACTAT
TGGTTGAGTCAGTGTGGCTGGTCAGTAGAGCTGGGCGATATGGCTACAAAAA
TTGTATCTTGATATTTTTCAGCTAATTGACGTTATTTAATATATATCTTGATAA
TTATGTTTCCTCTGATTTGACATAAAAGTTATTAGTTTTTGTTATCAGAGGAAC
CATTATCTCTGCTAAATAGACATAGAGCTCTGTTTTCTTGAGTTCGAGCAAAA
AGTGCAATTTGAAGTTGCTTAAAGCGAAATTTGCTGTTTTCCTAAGAAATAGA
CATAGCTTTAACACATCTTCGTGCACATGACCTGTTTTCAGGATAGTGCCTAA
ATTCAGTTTCTAAATTTGCAGTTTGGAAATTCCATCAGCAGGTGGTAATAAAG
TTCAAGTCCGAACTCTGAAAATATAGTGGAATGTTTTATGTTTCAATTATTAT
TATTATTATTATTATGTTTATTTATACAGTTGTATTTCT

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: emb|Y15279.1|GFP450III *Gibberella fujikuroi* P450III gene
Identities = 23/24 (95%)

Locus 142: Accession # AY351364 Tetraploid locus containing 11 alleles and 8 genotypes throughout the Klamath basin across all species. Clone size: 513 bp. PCR Product size: 361 bp
Primers: 142R+ : 5'TCTCATTATATGGAAAAGAGC3'
142R- : 5'TTTGTTCTAGTCGGTAATCTT3'
Mg++ Concentration: 1-4mM Annealing Temp: 50^{0}C
Reverse Sequence:
TGTAAATAAAACTGTCATTTTAAAAAGAACATTATTATTAACTTTTTTTTTTT
TTTTGTTCTAGTCGGTAATCTTTAGGAAAGGAGGCTGCAGAACTTCTAAACCG
GAAAAAAAAAAGTTTTTACAGTTTTTGCTCAGAACTAACCGAAATTAAAAAC
ATTTTGTTTTTAAGCTTTTCTAGCATTTCAAATGTCAACTTGGCTCAGACTTTC
TGATGCAGGATTTACAGGTTATGGCCTAAACTACAGTTGTTATTTATGGGATA
TTGGAAGCCTAATGATTAAAGGGAGTATTCACCCAAACATTTTACTCGCCCTC
ATGTTGTTCCAAACATATATGCCATATTTCTCCAGTTTAACACAAAATGAGAT
GTTTAGCAGAATTTCCATGCTGCTCTTTTCCATATAATGAGAGTAAATAAGGG
ACCAGGGCCGGATTCATTAATTTTCTTAAGAAACAACTTCATCTACCATTTTT
TTCTTATTATTTTCAAACTTAAGAATATTTTAGAA

Blastx search: No significant amino acid sequence similarity found in GenBank.

Blastn search: gb|AC003954|AC003954 *Homo sapiens* chromosome 5, Pac clone 162o17 (LBNL H147) Identities = 21/21 (100%)

Locus 146: Accession # AY351365 All Klamath catostomids species are identical for this locus.
Clone size: 528 bp PCR Product size: 381 bp
Primers: 146R+ : 5'AGACATGGTGACGGCTTGATTTCAT3'
146R- : 5'ATACTGACCTATGGGATTTTTGGAG3'
Mg++ Concentration: 1mM Annealing Temp: 53^{0}C
Reverse Sequence:
TGGGTTTCTCACCCAAACCTATCAAATCACTTCTGAAGACATTGATTTAACCG
CTGGAGTCATATGGACCACTTTTATACTGACCTATGGGATTTTTGGAGCTTCA
AAATTTTGGCAACCATTCACTTGCATTTTATGGACCAACAGAGTTGAAATATT
CTTCTAAATCTTTATTTGTGTTCTGCTGAAGAAAGAAAGTTATATACATCTGG
GATGGCATAAGTGTTAGTAAATGATGATAATTTTTGGGTGAACTATCCCTTTA
AGTGTTATAGAAGCACCACAAAATGACAAGATTTCCTTCAAACTATTGGTGA
AATTAGTTGACCTGAAAGAAAACCTTACAGTAGAAAAAAACAGTTTGAAAAA
ATTCCAGCTACAGTTTTATTATAGCATTGTGTACTGTGCTGTGTCCCGTAATGT
TGTTTTGCCATGAAATCAAGCCGTCACCATGTCTAGGTTTAGTAAGTCTGCAG
CACTTGGCTTAATAAAGACATCTCAGTGTTACTCTGAGAGCAAAGACATTCA

Blastx search: gi|3790700 (AF099914) No definition line found *Caenorhabditis elegans* Identities = 12/33 (36%), Positives = 23/33 (69%)

Blastn search: gb|U70479|DRU70479 Danio rerio no arches (nar) mRNA,Identities = 26/26 (100%)

Locus 166: Accession # AY351366 PCR amplifications were very weak and involved several other bands. No further testing was done. Clone size: 529 bp PCR Product size: 475 bp
Primers: 166R+ : 5'GAAACGTTATCTTTTTGTTATTTT3'
166R- : 5'TTTTATGCTGCCTTTATGTGCTTTT3'
Mg++ Concentration: 1-4 mM Annealing Temp: 40^{0}C
Reverse Sequence:
AAACCACTGAAGTCGTGTGGATTACTTTTATGCTGCCTTTATGTGCTTTTTGG
AGCTTCAAAGTTCTGGCCACCATTCACTTGCATTATATGGACCTACAGAGCTG
AGATATTCTACTAAAAATCTTCATTTGTGTCCCGCAGAAGAAATAAGTCATAC
ACATCTGGGATGGCAGGAGGGTGAGTAAATGATGAAAGAATTTACATTTTGG
GGTGAATTATTCTTTTTAATAAGGTGGTGTAACCAAAATGCTGGTNGCTGTTT
TGTATACGTGAAGTAAAAAAAAAGGTGAAATTTGAAATTTTTGTGAAAGGCA
CATTTTGTTCAGTTTAAATGGACAAACTCTAATTGTGATTGCATTCAAAGAAA
AATATGTTAATGCCTTTTAATACTTGATGTTACACCATTTGACATTTTTAAAGT
CATTAAGTAAATTGTTTGTAGGAAATGCTATAAAAAGTGAAATATTTTCAAA
CAAAATAACAAAAAGATAACGTTTCAACAAACTTGACACGTGGTTTTTAAAC
CA

Blastx search: emb|CAA19433| (AL023816) T05G11.6 *Caenorhabditis elegans*

Identities = 18/73 (24%), Positives = 31/73 (41%)

Blastn search: emb|X60419|CIGH *C.idellus* gene encoding growth hormone
Identities = 41/46 (89%)

Locus 169 : Accession # AY351367 PCR amplifications were very weak and involved several other bands. No further testing was done. Clone size: 504 bp PCR Product size: 413 bp
Primers: 169R+ : 5'TCAGAGTTGGGGTGTTCCTCCTAAA3'
169R- : 5'ATATACTCCAGAATAAGGGCAAGAT3'
Mg++ Concentration: 2-3mM Annealing Temp: 40^0C
Reverse Sequence:
CTGAATGACTCCACTGCTGCCACAGTGCTCATTAGAATGGTGAGGGTAGTCA
GAGTTGGGGTGTTCCTCCTAAAGTCCACAATCATCTCCATAGTTTTGAGCATG
TTAAGCATGTTAAGCATGTTAAGCTCCAGGTTGCTCCAGGTTGTTTTGACTGC
ACCAGACAGCCAGCTGTTTAACCTCCCTTCTGTATGCAGACTCATCGTTATCT
CGGATGAGGCCAATGACAGTGGTGTCATCTGAAAAATTCAGGAGCTTGACAG
AGGTGTCCTTGGTGATGCAGGCATTGGTGTAGAGGAAGAAGACTAGTGGGGA
GAGCACAACTCCCCTGGGGGGACACCAGTTTTGATTGTACAGGTGCTGGAAT
TGAATNTCCCCTGTCTCACAAGCTGCTGCCTGTAATCCACTGACAGATAGATG
TTGGGAACAGAGAGTTGATCTTGCCCTTATTCTGGAGTATATCTGGGATTATG
GTGTTGAAAGCCAAACTGAAGTCCACATAAA

Blastx search: gi|1127550 (U18939) orf1 *Battrachocottus baikalensis*
Identities = 53/111 (47%), Positives = 65/111 (57%)

Blastn search: gb|U18939.1|BBU18939 *Battrachocottus baikalensis* orf1 and orf2 genes, Identities = 33/36 (91%)

Locus 172: Accession # AY351368 PCR amplified very well in all Klamath species, samples are purified, but never sequenced. Clone size: 464 bp PCR Product size: 400 bp
Primers: 172R+ : 5'TGCTCCTATCGACACAGCCCTCCAA3'
172R- : 5'CAGTCCCCCTTTTCGTCAGGTGGTG3'
Mg++ Concentration: 1-4mM Annealing Temp: 50^0C
Reverse Sequence:
CTCCGCCATACCTTACAGTCCCCCTTTTCGTCAGGTGGTGTCCCTGTTGGAGA
CATCATCGGACGACAATCATCACAATGGCGGATGGCAGGTGAATCAGGAGG
GTGTTGTAGTAGGTGGTTATTCGGACGGAGGGCTCAATTCGATGAGGTTCAA
AGTGGTCTCTGTTGTGGTCCCCTCTTTCCGGGGCTTCCGTCGGAAACCTACGG
ACATGGCTTTCGTGAGCTTGGCTGTGGATGTTTGCATCTCGTTGAGCCTCCTG
TACAGGATGAAGGCCAGGCCTAGTGTCAGGAAGTGGCTGATTCCCTTAGAGA
TACACAGCGCAGTGTCCGCTGCTGTCCAGGCTCGGACAACAGGTGAGGTGGT
CGAGATGGGCAATCGAGACAAGGCTTGGAGGGCTGTGTCGATAGGAGCAAT
GTCAATTAACTCGGAGCCCTCTCTTTCGATCCATAGTTCCAGTTCTG

Blastx search: gi|1078547|pir||S55100 hypothetical protein YMR218c yeast *Saccharomyces cerevisiae* and gi|854470|emb|CAA89933| (Z49809) unknown *Saccharomyces cerevisiae* Identities = 20/48 (41%), Positives = 26/48 (53%), Gaps = 1/48 (2%)

Blastn search: gb|L78073.1|L78073 *Homo sapiens* clone 16513.1 HLA-G cell surface glycoprotein (MHC-G)gene, Identities = 22/23 (95%)

Locus 176 : Accession # AY351369 Amplified well in all Klamath species, one individual from each species run on SSCP gel and showed no variation between them.
Clone size: 467 bp PCR Product size: 401 bp
Primers: 176F+: 5' TAACATCAACTCCCCTTTCAT 3'
176F-: 5' TTAACCTCAACCCAAGTGGAA 3'
Mg++ Concentration: 3 mM Annealing Temp: 50^0C
Forward Sequence:
ATGATTTTCATATCATAATAACCATCACATCACGTTAATAACATCAACTCCCC
TTTCATGTTTCTCTCTTTTTTGAGAAAGCTTGAGTAATAGTTATTTTACGACCC
TATCAGAGCAGAGCTGATAAAGCAAGATTCTTATCTTATTTGAAAAAATGTA
TGGCATTCAAGTTATTTTAAGCACAAGATTGGTATGAAACATAGAATTTGAA
AGTAACATCATACAAACATTTTGATGCAATACAACAATCTGCCATATTGTAA
AGCATAAGAACAATATGCATGTATTGCAAGATTCTTGCAAATACTGCCCATA
TGAGATACTGTACTATACTGACTGTACTCAGTGCAGGCACAGGCAATTTGTGT
AGGAGTGTAGCCGGAAAATTACAACCTGGCACACTCTCGCCAATGGGACTTT
CCACTTGGGTTGAGGTTAACGAAGCTTAAGAAACTTTATTTTTATAA

Blastx search: gi|1074769|pir||A64029 hypothetical protein HI1418 - *Haemophilus influenzae* (strain Rd KW20) >gi|1574254 (U32821) *H. influenzae* predicted coding region HI1418 [*Haemophilus influenzae* Rd] Identities = 11/23 (47%), Positives = 15/23 (64%)

Blastn search: emb|Z69718.1|CEW06D11 *Caenorhabditis elegans* cosmid W06D11, Identities = 24/24 (100%)

Locus 181: Accession # AY351370 PCR amplifications were weak and included many other bands. No further testing done. Clone size: 525 bp PCR Product size: 346 bp
Primers: 181F+ : 5'TTCACACTAATGCTAATCCAG3'
181F- : 5'AATATAAAAGGAGACTTCAGG3'
Mg++ Concentration: 1-4mM Annealing Temp: 47^0C
Forward Sequence:
TTTCTAGAAGAAAAATGCATTTTAGTCAAAAGCCATTTTAGTGTGAATGTCGA
CCATCTTGTATACATTCAGTGTACATTTTTTTTTTTTACTTTAATCTTCACACTA
ATGCTAATCCAGTCAAACTAACGGTGTACAAACAAAATTATTAAACCTCTGC
ATCAGTATGAATCTTACAGTATGAAGGATAACACCGTTTTTGTGCTTAAATAT

CTTGTACATAATTTAAAGGGATAGTTAAGCCACAAATAAAAATTGTCATACA
TGTTTCACCTATATGTTGTTCCAAAAATGTATGACTTATTTTTGTCTGTGGAAC
ATAAAAGCTGATGTTAGGCAGAATGTTAGTCTCCGTGACCATTCATTTTCATT
GTATGGAAAAAAGATGCAGTGAAACTGAATGGTGACTGAGGCTAACATTCTG
CCTGAAGTCTCCTTTTATATTCCACATAAGTCAGTCATACGTGTTTGGAGCAA
CATGAGAAAATTATGACAGATGCTTCAAATTTAGATGAACTATCATTT

Blastx search: gb|AAC61662.1| (U67083) KRAB-zinc finger protein KZF-2 *Rattus norvegicus* Identities = 14/40 (35%), Positives = 20/40 (50%)

Blastn search: emb|Z35595.1|CEC01G6 *Caenorhabditis elegans* cosmid C01G6, Identities = 21/21 (100%)

Locus 182 : Accession # AY351371 Amplified well in all Klamath species, one individual from each species run on SSCP gel and showed no variation between them.
Clone size: 489 bp PCR Product size: 337 bp
Primers: 182F+ : 5'GGAACTGTTCGTCATTTGT3'
182F- : 5'AGCTTCACTACCTCGTCAA3'
Mg++ Concentration: 1mM Annealing Temp: 50^0C
Forward Sequence:
AACACAAGAAAAAGGTGCAAAAAGGAGAAATAAATTTCAGTCTCTCCCTGTT
CTGCACATACTTCTGCCATTTACAGCCATGATATCAAAATTCTTACGGAACTG
TTCGTCATTTGTCCTGCTGTTTCCTCTTCTCCAATTACTGCATTTGGAAATGTT
CAGTGCTGCCCCCTCTGGCCCAGAGTCTGACACTTGCTCCACCCTGGTCCAGA
GTCGTTTCTTTGGTTTCTTTCTCTCATCCTCTGTATTTGCCAGCACACCTTGCA
CCTGGACCCTGCAGAACCCTGACCCACGGCGCTACACCATCTTCATTAAGGT
CACAAAGCCAATCAGAGACTGCATTCCCCGACAGCACCGGACCTTCCAGTTC
GACTCCTTCCTGGAGACAACACGAACCTTCCTTGGAATGGAGAGCTTTGACG
AGGTAGTGAAGCTTTGTGATGCCTCCACACATGTTGCCTTCCTGGAGGCAGG
GAAACAATTCCTGCA

Blastx search: ref|NP_001693.1|| brain-specific angiogenesis inhibitor 1 precursor>gi|2653432|dbj|BAA23647.1| (AB005297) BAI 1 *Homo sapiens*

Identities = 63/109 (57%), Positives = 88/109 (79%), Gaps = 3/109 (2%)

Blastn search: ref|NM_001702.1|| *Homo sapiens* brain-specific angiogenesis inhibitor 1 (BAI1) mRNA Identities = 57/68 (83%)

Locus 184 : Accession # AY351372 and AY366162 Two alleles differ by a single base. Both are found in all Klamath catostomids. Clone size: 530 bp PCR Product size: 434 bp
Primers: 184R+ : 5'GAGGGCCTGAGAGCATAAGAT3'
184R- : 5'CAGTCCTGGGACCATACCATA3'
Mg++ Concentration: 1,3 mM Annealing Temp: 50^0C

Reverse Sequence:
AGCCCCAAAGTGCGTCAGCCCACCGGGAAAATGCCGGTATGCCAGATTACCT
GTCCAGTCCTGGGACCATACCATATGTTGCAGGACTTCTTCTATTCTATTCTAT
TTGCACAAGGAATGTTTGGATGAAAAATAAATAATCCATGAGTCTTTATCAA
TTACAAAAGAAAAAATATTCCTCACTCCTTTTATCCAACTTTCTTTTTTTTTTC
ATTAGATTTTTGTGACAGGGGAAATCTAAAATCGATACTTCCCACTGACACAC
TTAAGATGAAGATATAAAATAACCAGTTTAAGAGAAATGTGAAACATTGACA
CATTTATTGTGCCGAAAACGTTTGCACAGTTCAATGCACAAATCTCTCTGAAA
CCTTAATTATCTTTGCTTGACCAGAAAAGGGCAATCTGCTGCATTAGTTTTCT
AACTTCTCTGAAAAAATATGTACTAAAATATTCTCATAGTTTTTCATCTTATG
CTCTCAGGCCCTCAAAGTCCCTGTGAAAGAACAGACCTTCGAATGGCTATTGT
GGCA

Blastx search: pir||A55575 ankyrin 3, long form - human >gi|608025 (U13616) ankyrin G *Homo sapiens,* Identities = 16/18 (88%), Positives = 18/18 (99%)

Blastn search: gb|AC004834.2|AC004834 *Homo sapiens* clone DJ0550A13, Identities = 20/20 (100%)

Locus 187: Accession # AY351373 Amplified well in all Klamath species, one individual from each species run on SSCP gel and showed 2 allele types. *D. luxatus* and *Ch. brevirostris* were fixed for one allele while *C. rimiculus* and *C. snyderi* have heterozygous alleles. No other testing done. Clone size: 482 bp PCR Product size: 382 bp
Primers: 187F +: 5'TGACAATTTAAGAAAGTTGACAAGC 3'
187F-: 5'TGGAAAATGTGGATAATCTCGTGAA 3'
Mg++ Concentration: 1 mM Annealing Temp: 47^0C
Forward Sequence:
ATTTTAAATGAAATGGCATACATTAATTGTAAAAGAATCTTACATGTTTCCGT
TTAAAGTCCTTTCACAAATGACAATTTAAGAAAGTTGACAAGCCCCCTCTGGT
GGGTTGGGGTTTAATCCCACGGTCAAGATTATTTCACAACATCTTAGGAAGTA
TGTGTGTTTTTAAAGTATTATTTAAACATTTACCTGTAGCGCTGGCAAAATGT
CTTTAAAATGTCTTTGAAACATTTGAAAATGTGTTTTAAACAAAATGTTTCAA
GTTGGCAAACACAACAAAGTCACTCTAAAAATTATAAAAAACTTTGACAACA
TTCACTAGTTTTGTGTTTTGAGTTATATTACTAAAACTCGATAAAACATTTCAC
GTAATTTTAGATGTATTTTTAACACCTGTTGTATTGCATTTTTCAGTTCAAAGT
CCTTTCACGAGATTATCCACATTTTCCAAAAAGACTTTAAAGCGATAAGGTAT
TTTT

Blastx search: gi|424235 (L21255) envelope glycoprotein [Human immunodeficiency virus type 1] Identities = 35/122 (28%), Positives = 50/122 (40%), dbj|BAA00448| (D00570) open reading frame (196 AA) *Mus musculus,* Identities = 15/49 (30%), Positives = 26/49 (52%)

Blastn search: gb|AC005410.1|AC005410 *Homo sapiens* chromosome 17, clone hRPK.1096_G_20, Identities = 23/23 (100%)

Locus 190: Accession # AY351374 Primers were never made for this locus, not tested.
Clone size: 526 bp PCR Product size: 350 bp
Primers Designed: 190F+ : 5'CTCGGGCAGTAAAACAAATGT3'
190F- : 5'TTATGAATTGGACAAGAGAGC3'
Mg++ Concentration: mM Annealing Temp: 51^0C
Forward Sequence:
TAGAGCATCCACATAACTGTCCCGAAGGTGTGTTCCTCCACAAAACAAGCTC
CAGACACTAGCGGAACCAGCAAAAACAAATTCAGTTTCCTCGGGCAGTAAAA
CAAATGTTCAGTGAGTCGGCTGTAATATGAGGGCAGCAGATGACCTAATCAC
CCCAATGTTTTGCAAGAAATATTCCACAAAACAAACTCCAGCCTCTATACGG
AACCAACACAAAAAGAAACAAAAAGGCGCTCAGTTTCCTCGGACAGTCAAG
TGAATGTTCAGTGAGTCAGGCGCACTCGGATGTTACATGAGTGCAACAAATT
CCCAAATCACTCCAATGTTTTGCAAGAAATATTCCACTAAACAAATTCCAACC
AAAAGGAGGCATAAGCAAAAATAACGTGCTTATTTGATTGCCTGATTATTTG
ATAGCTCTCTTGTCCAATTCATAAAGTCTGGTAACAAGAAAATGCTGTGGTTC
TTCCAAGAAAGAGTCCCTTTACTCCTTGCCTTGTGTAACATGAGATCTTTATC
GGAT

Blastx search: gb|AAC17659.1| (AF067943) F59B1.8 gene product *Caenorhabditis elegans* Identities = 17/41 (41%), Positives = 20/41 (48%)

Blastn search: emb|AL110485.1|CEY46G5A *Caenorhabditis elegans* cosmid Y46G5A, Identities = 21/21 (100%)

Appendix 3: Protocol for pouring and staining acrylamide gels.

Introduction

Laemmli (1970) first introduced gel electrophoresis to the biological sciences and it has subsequently been used for protein, RNA and DNA separations (Sambrook, et al., 1989). Many polyacrylamide gel electrophoresis (PAGE) techniques often rely on large (45 x 35 cm) vertical gel formats. These include denaturing PAGE, non-denaturing or native PAGE, such as SSCP (single strand conformation polymorphism, Orita, et al, 1989; Marklund, et al., 1995; Sunnucks, et. al., 2000) and sequencing of nucleic acids. Large gels are cumbersome and difficult to pour. There are many steps and precautions involved in pouring gels which contributes to the failure of this technique. One of the commonest reasons for failure or reduced productivity and increased cost of a gel is the presence of air bubbles trapped in the gel matrix. Bubbles can reduce the number of samples on a gel or result in unusable results due to band distortions caused by the change in the matrix's resistance as bubbles are encountered during the electrophoretic run. Gel pouring and manipulation are moderately difficult techniques to learn and in some labs seen as an art form. The lab catalogues hawk tools to help simplify this technique. Most items are only moderately useful or specific to only one gel technique and more useful items are costly such as Hoeffers Gel Pouring Stand. Large gel formats are also difficult to stain because of their sheer size. Many techniques require some measure of soaking of a gel to develop or fix the electrophoretic pattern. These techniques require large volumes of buffer that can become a biological waste hazard and lead to failure because the gel floats off of the glass plate supporting it. I describe three inexpensive tools which can greatly facilitate pouring and staining (or denaturing sequence) large gels. They include; a gel pouring apparatus (GPA), a bubble hammer, and a gel staining gasket (GSG). The GPA holds the gel mold at a constant angle for uniform poring. The bubble hammer reduces surface tension between the glass plates and air bubbles and releases they thereby maximizing the size of the gel. The GSG

allows staining of large format gels with as little as 300 ml of stain. A typical gel poring and staining procedure is included to illustrate the use of the three described tools.

Construction of the Gel Pouring Apparatus (GPA)

The GPA is a modified Styrofoam shipping container (Figure 1), such as the 28 cm (w) x 27.5 cm (h) x 28 cm (d) box typically used by scientific supply companies. The cardboard liner and top were removed and recycled. Cut the Styrofoam box as in Figure 1 beginning 3 cm. from the top left-hand of corner 1, cutting vertically down approximately half way, then cutting horizontally through corner 2 around to a point, 3 cm from corner 3, at which point a vertical cut parallel to corner 3 will allow removal of a piece of Styrofoam. For a Styrofoam container with 6 cm thick walls, remove approximately 3 cm of the interior wall from the two sides that were cut (Figure 1). For right-handed persons, place the gel mold with its lower left-hand corner in the front corner (Figure 1, corner 2) of the GPA. The left-hand side of the gel mold can be tucked remaining overhang in corner 1. The gel mold sat at approximately 45 $^{\circ}$ angle with a left-hand tilt. Left handed persons should reverse the tilt direction to the right side, i.e. by placing the right lower corner of the gel mold into the front corner (2) of the GPA and tilt it to the right hand side of the GPA (corner 3). The gel mold was free standing in the GPA and does not need to be supported by the researcher allowing both hands to be free to pour and manipulate the gel.

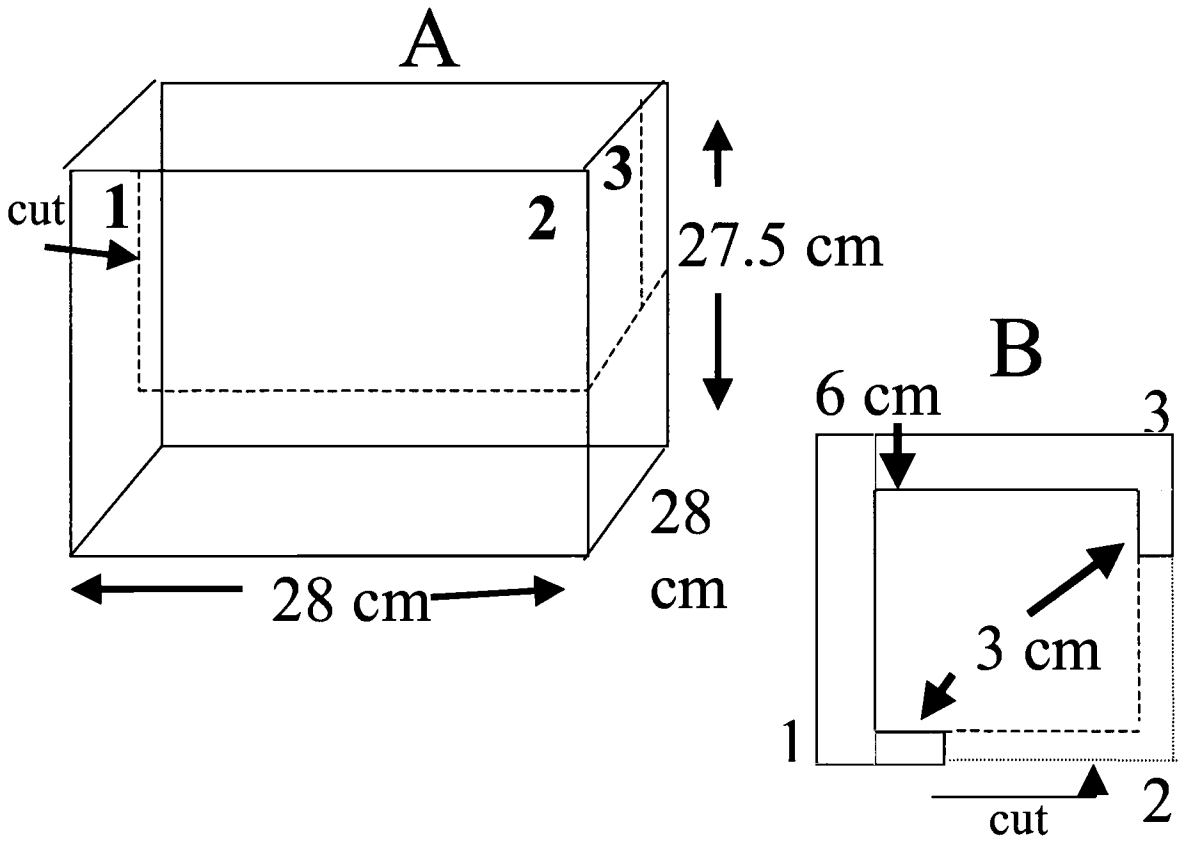

Figure 3.1: Line diagram of gel pouring apparatus, A: side view, B: top view looking down. Corners are numbered

Construction of the Bubble Hammer

A standard black rubber stopper (number 11) was used (Figure 2). A hole was drilled through the center of the stopper. The stopper was held so that the widest edge is down and a new un-sharpened pencil was inserted into the hole, so that the eraser end is distal to the stopper.

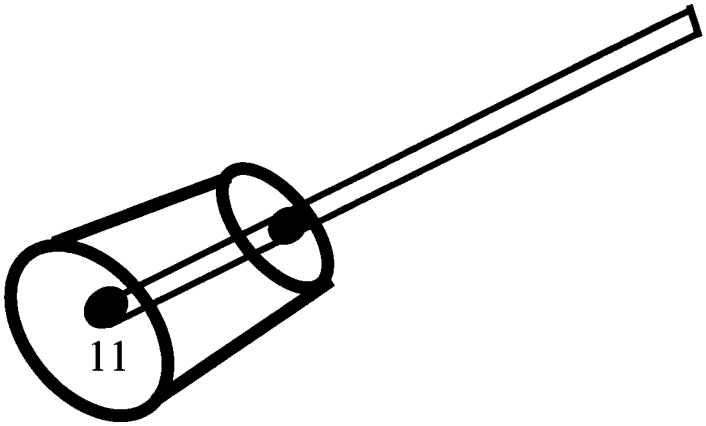

Figure 3.2: Bubble hammer, made from a new number 2 pencil and a black rubber stopper #11

Construction of the Gel Staining Gasket (GSG)

The gel staining gasket was made from a new, tractor trailer truck mud flap. The gasket was made by placing the mud flap (5 mm thick) on a counter top and overlaying it with the long glass plate from a sequencing apparatus (Figure 3). The rubber was cut with a razor to the dimensions of the glass plate. Side spacers (typically 10 mm wide) from the sequencing apparatus were used to outline the gasket on all four sides. Two-mm were added to this dimension so the gasket was wider than the spacers. The gasket was completed by cutting along the inside axis on all four sides (Figure 3).

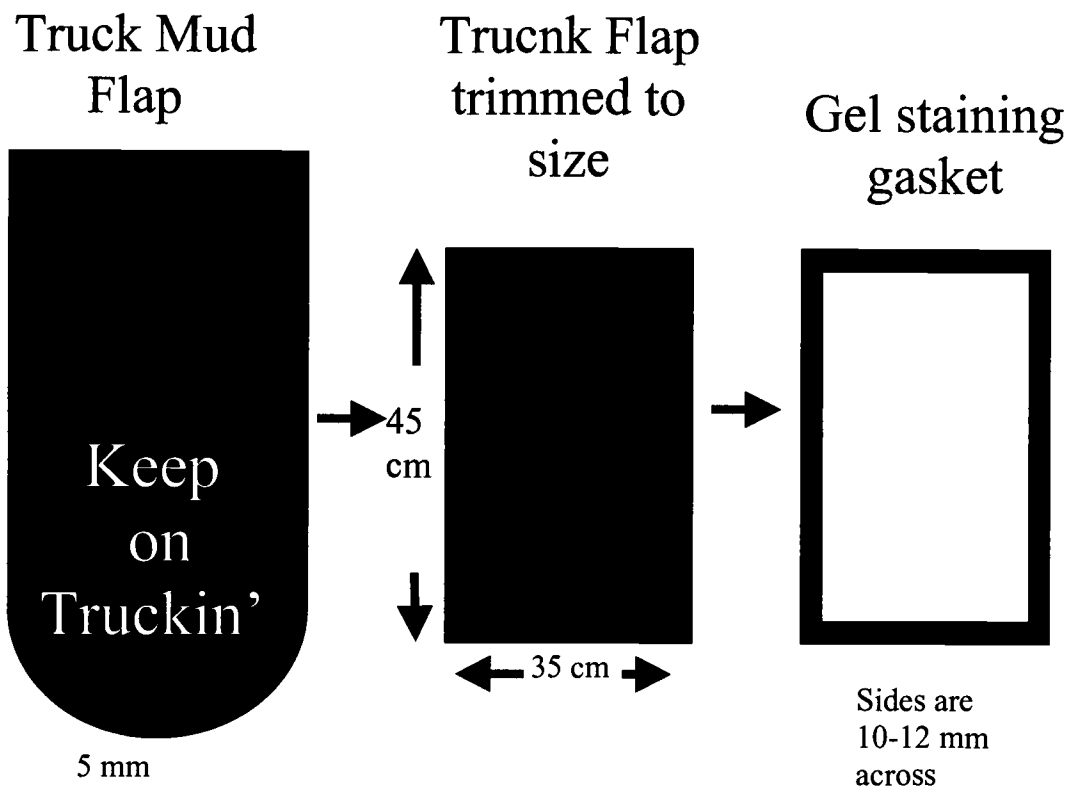

Figure 3.3: Construction of a Gel Staining Gasket.

Typical Polyacrylamide Gel Pouring and Staining Procedure

Additional equipment

Additional equipment and supplies that were needed: one 60 ml syringe for delivery of the gel solution into the gel mold; a roll of 3M electrical tape (#MT 56, 4 cm wide) for sealing the bottom and side edges of the gel mold, this is cheaper than gel casting tape and has better properties for cold runs; acetone and 70% ethanol for cleaning the plates; a siliconizing agent such as RainX™; two flat supports approximately 23 L. x

23 W. x 5 H. cm to use as supports for the plates of the gel mold, and large binder clips, enough to go completely around the outside of the glass plate to secure the GSG to the gel plate.

Making the Gel Mold

Standard lab safety should be used at all times, including the wearing of (powder free) latex gloves, lab coat and safety glasses. Powder from gloves can transferred to the glass plates and cause bubbles in the gel matrix.

Electrophoretic plates work best if they are paired. Always run a trial run to test plates for warps. Plates were labeled with a diamond etcher in the top right hand corner on the outside each plate. Plates were placed on flat Styrofoam supports on a clean bench top with the inside of the mold surface facing up. Each plate was cleaned thoroughly with 70% ethanol, wiping in a circular motion with high quality paper towels or Kimwipes. Edges and the first 3 cm of the flat side around the plates were cleaned. The cleaning procedure was repeated with acetone to remove any oils and fingerprints and to drive off any remaining water and ethanol.

The short (smaller) plate was siliconized with 3-5 ml of RainX. It is important not to siliconize the longer plate, to ensure that the gel will later adhere to this plate. The RainX was spread with a circular motion over the entire inside surface of the plate. Caution should be used to avoid getting the RainX on the edges and flat sides of the plates, it becomes difficult to seal the plates with 3M electrical tape. It is recommended to siliconize each time a short plate is used. Once the RainX had dried, the cleaning procedure was repeated to remove any excess RainX. Spacers were cleaned in the same way as the plates.

The side spacers were used and placed along the outer edges of the long plate. The short plate was inverted and carefully dropped on to the spacers and the long plate. Plates and spacers were clamped together with two large binder clamps along the sides of the glass gel mold.

3M electrical tape was used to seal the gel mold. The bottom of the gel mold was sealed first, by running tape along the edge of the mold starting 6 cm from the bottom and

continuing along the bottom and along the other edge for 6 cm. The whole mold was sealed starting at the top, the edge of the gel mold was centered on the tape and pulled entirely around the edge of the gel mold in a continuous piece, removing the clamps as they were encountered. The tape was then pushed down to seal the plates together starting from the outside edge of the plate inward to push any air out from under the tape. The corners of the tape were folded flat to prevent leaks during the pouring of the gel. The gel mold was flipped and the tape was seal on the other side. A 0.5 cm overhang of tape was left at each corner at the top of the gel mold.

Gel Pouring Procedure

The sealed gel mold was placed in the GPA, with the short plate facing upward, by placing the lower left corner of the mold into front corner (corner 2) of the GPA. The acrylamide solution was made in a beaker and swirled to mix and care was taken not to shake the acrylamide solution because the addition of air can be problematic. Fresh APS was added. The acrylamide solution was slowly drawn up into a 60 ml syringe carefully to avoid the addition of air into the syringe. The syringe is inverted and any air is evacuated. The full syringe was in my left hand with the nozzle in the top left edge of the gel mold. Holding the bubble hammer in the right hand by the pencil handle, the gel was pour with a slow and continuous flow of the solution down the inside edge of the plates. It is important to try to do this as a continuous pour to avoid bubbles.

If a bubble occurred in the lower half of the gel hitting the glass plates with the Bubble Hammer at the point of the bubble can release the bubble. If the bubble persisted the pour was stopped and the gel mold was picked up and tilted so the solution moved away from the bubble. As the solution moved back toward the bubble the glass was struck repeatedly with the bubble hammer as the solution crept back over the area of the bubble. Once the bubble was eliminated the pour continued. If bubbles occurred in the top half of the gel, you may strike the gel mold with the bubble hammer or wait until all the solution has been poured. Once the solution was completely in the gel mold, the gel mold could be tilted and the solution moved away from any bubbles that occurred in the

top half of the gel. As before, the gel mold was struck with the bubble hammer as the solution returned to the area. This was repeated many times if bubbles persist. Once the gel was bubble free, it was removed from the GPA and placed over a flat support, starting with the lower edge of the gel at a 45 ^{o} angle. The comb of choice was placed into the top of the gel mold. The gel mold was then lowered so the top edge was flat against the support. The comb was clamped into place using three binder clips, one in the middle of the comb and one on each side overlapping the plates. The best gels were poured a day before they are used. Typically, gels were poured as the last task of the day so they could be run first thing in the morning. To prevent gels from drying out overnight, they were hydrated by placing wet folded brown paper towels over the comb, clamps and the upper part of the gel. The top quarter of the gel mold was wrapped in plastic wrap.

Running and staining the gel:

After the acrylamide had polymerized, the plastic wrap and toweling were removed and discarded. A sharp razor blade cut the inside edge of the electrical tape at the bottom of the gel. All tape from the bottom of the gel was removed. The tape along the sides was left to prevent electrophoresis buffer from short-circuiting the electrophoresis by running down the sides. The sample lanes were marked on the outside of the large glass plate with a sharpie pen to add in loading and photography of the gel. The gel mold was then placed into the electrophoretic apparatus, loaded and the gel was run. Once the gel was finished, it was removed from the apparatus and laid flat on a support with the short plate on top. At the top of the gel, the overhang of tape was grabbed and pulled down the length of each side and removed. The short plate is loosened from the gel by inserting a flat wedge between the plates and removed it along with the side spacers. The gel was now exposed and overlaid with the gel staining gasket (GSG). The gasket is wider than the spacers are so it will overlap the gel along the sides and fully across the bottom. Beginning with the right hand bottom corner of the gel, the gasket was clamped in place by placing the bottom edge of the binder clip on the glass plate and the top edge on the gasket. The clip squeezed the acrylamide to form a

watertight seal with the gasket. Clips were placed next to each other and continued around the entire gel (Figure 4). The gel was ready for staining or sequence denaturing.

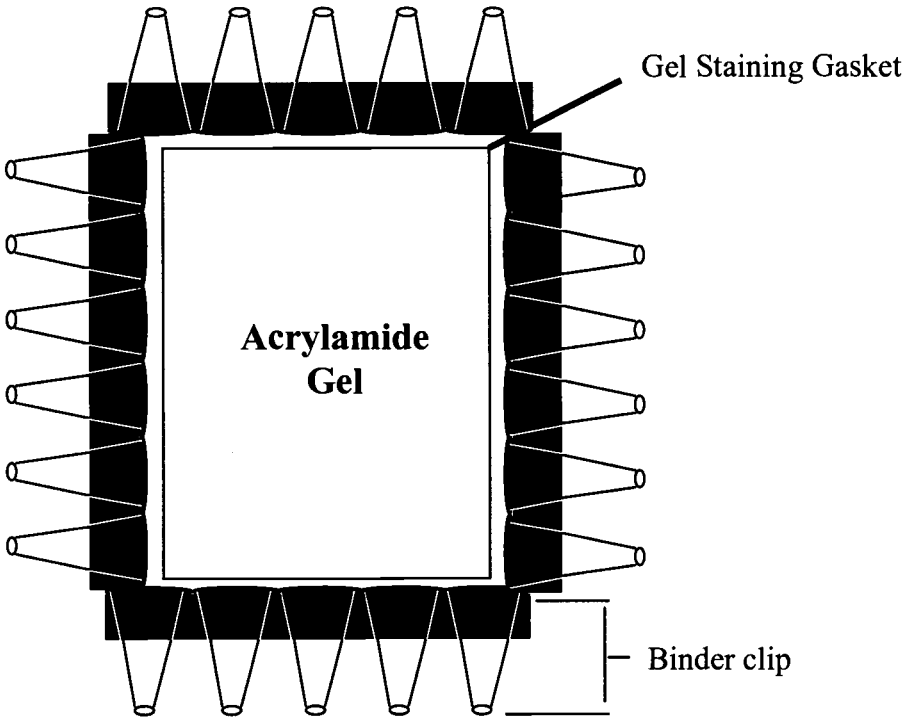

Figure 3.4: Gel staining gasket clamped to a gel.

The above technique was devised for SSCP and staining with Sybr Gold (Molecular Probes) but can be used for all types of PAGE, manual sequencing gels or staining with ethidium bromide or another nucleic acid stain.

Sybr Gold is a general nucleic acid stain that is light sensitive and sticks to glass. Gels were typically stained in the dark. This stain was mixed according to the manufacture's directions. The gasket created a dammed rectangular area, which held up to 600 ml of solution for staining and prevented most of the stain from coming into contact with the glass plate. As little as 300 ml can cover a gel completely. The gasket also prevented the gel from becoming unattached to the long glass plate. Particularly during the denaturing

step in manual sequencing, gels float off of the plate into large volumes of methanol and acetic acid and are usually lost. Once staining was complete (approximately 30 minutes) the stain was poured off and collected. The gasket, gel and plate were turned gel side down and placed on an UV light source without the removal of the clamps. The gasket assisted with keeping the gel in place on the glass plate. Photographs were taken through the glass plate. The gasket was cleaned with soap and water.

Discussion

The described procedure was used successfully with SSCP gels and denaturing nucleic acid PAGE (Wagman and Markle, 2003). The three tools, GPA, bubble hammer and GSG are inexpensive and useful for the success of gel electrophoresis.

The GPA and bubble hammer help to insure bubble free gels that can be used for the maximum number of sample. The GPA allows for easier pouring of the gel and allows the mold to be manipulated for the release of trapped air within the gel matrix. The bubble hammer is a necessary tool for releasing stubborn air bubbles. These tools have greatly increased the total number of successful gels I have used in my research.

The gel staining gasket is a preferred technique for staining gels because it secures the gel to the glass plate preventing any accidental slippage or distortion due to stretching of the gel during staining and photography. The gasket limits the total volume of the stain used, and prevents the glass from contact with stain, which can cause high background staining. The small volume of stain needed reduces wastes and decreases disposal difficulty when using biohazard stains such as ethidium bromide. The gasket technique limits the amount of stain that is necessary. This is both a cost savings and reduces safety concerns.

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