AN ABSTRACT OF THE THESIS OF

Stacy L. Remple for the degree Master of Science in Fisheries Science presented on March 15, 2013.
Title: Taxonomy and Systematic Relationships of Tui Chubs (*Siphateles*: Cyprinidae) from Oregon's Great Basin.

Abstract approved:

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There are three recognized species of *Siphateles* from the Great Basin; *S. alvordensis*, *S. boraxobius* and the tui chub, *S. bicolor*. One species, *S. boraxobius*, is endangered and one population of tui chub at Hutton Spring is threatened. Despite several morphological and molecular studies, the taxonomy and relationships of tui chubs are unclear. A recurrent theme in prior studies has been the possibility of translocation of tui chubs, especially into Summer Lake Basin, and probably by bait bucket introductions. I approached this problem by using cytochrome *b* (cyt *b*) sequences to define clades and constructed a neighbor-joining tree to examine relationships. Developmental ontogeny and adult meristic characters were used to corroborate clades, and microsatellites (nDNA) used to explore the possibility of hybridization among Summer Lake Basin fish and those from surrounding basins.

The cyt *b* tree recovered a basal polytomy containing a western clade from Sycan Marsh, an eastern clade from the Alvord Basin, and *S. bicolor*. The Sycan Marsh clade was represented by two fish and requires additional research. Within the Alvord Basin, *S. boraxobius* and *S. alvordensis* were well corroborated by morphological characters but

sequence divergence was only 0.37%. There were three major clades in *S. bicolor* – a basal *S. newarkensis* clade in Nevada, an Oregon Lakes *S. bicolor* clade, and, sister to it, a disjunct *S. obesa* clade in Nevada and the Oregon Lakes. In the Oregon Lakes, there were two clades within *S. bicolor*: *S. thalassinus* was sister to the remaining *S. bicolor* and there were two clades within *S. obesus*: *S. oregonensis* was sister to a "Summer Lake Basin" clade. There was some morphological corroboration for *S. oregonensis*, but no corroboration for the others. Clades were geographically disjunct or not confined to single basins. The *S. oregonensis* clade was sister to a Nevada polytomy and historical evidence implicates that at least one population of *S. oregonensis* in XL Spring was introduced in the late 1800's. Average sequence divergence with the Nevada clade, 0.62 - 0.88%, did not seem to support possible Miocene or Pliocene vicariance scenarios. Elsewhere, the *S. thalassinus* clade was found outside of Goose Lake in Summer Lake Basin and the "Summer Lake Basin" clade was found in Goose Lake Basin. Clustering of three microsatellite loci did not match cyt *b* clades, rather, individuals clustered based on sample location, suggesting that the cyt *b* patterns were due to introgression. In Summer Lake Basin, evidence of poisoning and subsequent transplants was consistent with these observations. These results suggest the presence of three or four tui chub taxa in the Oregon Lakes and Alvord Basin, however translocation and subsequent introgression appear to have been common in many populations, and will prove challenging for taxonomists and conservation managers.

Taxonomy and Systematic Relationships of Tui Chubs (*Siphateles*: Cyprinidae) from
Oregon's Great Basin.

by

Stacy L. Remple

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

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INTRODUCTION

The genus *Siphateles* (Cope, 1883), commonly known as tui chubs, is a widely distributed, polytypic minnow. It ranges from the Columbia River Basin in the north to the Mohave Desert in southern California and from the Klamath Basin in the west to the Lahontan drainages of Western Nevada (LaRiver, 1962). Tui chubs inhabit a multitude of environments including springs, streams, large, slow moving rivers, and large lake systems. Many of these bodies of water are contained within endorheic lake basins, which during the Pleistocene, were part of much larger pluvial lakes. During times of high water levels many of these ancient lakes were connected (Negrini, 2002) allowing for possible faunal exchange. However, as the Pleistocene climate warmed many of these connections were lost; isolating populations not only between, but within these basins.

Currently, there are three recognized species: *S. alvordensis* (Hubbs & Miller, 1972) Alvord chub, the endangered *S. boraxobius* (Williams & Bond, 1980), Borax chub, and *S. bicolor* (Girard, 1856), tui chub, which includes the threatened Hutton Spring tui chub (*S. bicolor spp*.). In 1985 the United States Fish and Wildlife Service (USFWS) listed the Hutton Spring Tui Chub as endangered, yet undescribed, subspecies of *S. bicolor*. LaRivers (1994) examined the taxonomic history of this group and reported

at least 10 generic names and 11 specific names have been variously ascribed to these over the years. Depending on definitions (species or subspecies, Bills 1978; Williams 1985; Harris 2000), the number of taxa present within this complex varies.

Morphological differences amongst tui chubs from these endorheic basins and subbasins have long been recognized (Cope 1883; Snyder 1908; Hubbs and Miller, 1948; Bills, 1977). However, different authors' interpret this diversity as either warranting species designation (Harris 2000; Chen 2008) subspecies designation (Bills, 1977) or interpreted this variation as intraspecific phenotypic variation (Bailey and Uyeno, 1964).

Girard (1856) described three new species, one each from Klamath Lake, the Humbolt, the Merced and Mohave rivers respectively. They were all classified under the genus *Algansea* Girard 1857 and named *A. bicolor, A. obesa*, and *A. formosa*, respectively. The characteristic used to establish this classification was a single row of pharyngeal teeth with a dentition pattern of 5-5, 5-4 or 4-4. In 1883, Cope described three related species from Pyramid Lake, Nevada; *Leucus olivaceus*, *L. dimidiatus* and *Siphateles vittatus*. Snyder (1908) described five tui chub species from Oregon and placed them under a single genus, *Rutilus* Rafinesque, 1820. In a review of cyprinid osteology Bailey and Uyeno (1964) included *Siphateles* as a subgenus of *Gila* Baird & Giard, 1853, due to a lack of definable characters between the two genera; concluding pharyngeal dentition was a trophic modification and thus a homoplastic character.

Taxonomic studies within the last 40 years (Hubbs and Miller, 1974; Bills, 1977) suggest that much of the morphological diversity described within *Siphateles*, especially from the Oregon Lakes region, could be interpreted as response to environmental

conditions. However, Bills (1977) further concluded that individuals could be correctly assigned to locality based on visual inspection, suggesting distinct population differences. Similar conclusions were reached by Hubbs and Miller (1974). Harris (2000), using mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*), found *Siphateles* formed a monophyletic genus, with at least nine allopatric species within the *S. bicolor* complex alone.

Analysis of mtDNA cyt *b* sequences indicated five, well established, clades; *S. alvordensis + S. boraxobius, S. movhavensis, S. obesa, S. bicolor* and *S. isolatus* (Harris, 2000). Three of these clades, *S. alvordensis+S. boraxobius*, *S. bicolor* and *S. obesa* are represented in Oregon, with *S. mohavensis* occurring in southern California and *S. isolatus* occurring in northwestern Nevada. There were two puzzling patterns within the cyt *b* phylogeny; a disjunct north-south distribution of the *S. obesa* clade and the presence of both *S. bicolor* and *S. obesa* mtDNA cyt *b* haplotypes within Summer Basin (Harris, 2000). *Siphateles obesa* is primarily found in Nevada and California, but an *S. obesa* Oregon population has been identified from the Oregon Lakes Region. This Oregon population of *S. obesus* was first described by Snyder (1908) from the Oregon Lake's region and named *Rutilus oregonensis*. He described fish from Abert Basin (the holotype is from XL Spring, OR.), Warner Basin, Summer Basin, Alkali Basin and the Silver Basin. A subsequent morphological study of tui chubs from Summer Basin, Warner Basin, Abert Basin, and Alkali Basin (Bills 1977) reassigned *S. bicolor oregonensis* as a subspecies of the Klamath Basin *S. bicolor* and restricted the definition to fish from Abert Basin (XL Spring and the Chewaucan River). Tui chubs from Ana Reservoir, in Summer

Basin, were problematic for Bills. Meristic counts taken from Ana Reservoir tui chubs suggested a decrease in the mean number of scales over time, especially along the caudal peduncle and in predorsal scales counts. Harris (2000) using tui chubs collected pre-1958, 1975-1985, and 1993 found similar results. Predorsal scale counts from tui chubs in Ana Reservoir had decreased from an average of 27.8 ± 2.73 pre-1958 to 25.9 ± 1.97 in tui chubs from 1975-1985 and to 24.7 ± 1.41 in 1993. Harris (2000) examined tui chubs scale counts, not only from Summer Basin, but surrounding basins. Tui chubs from Summer Basin were the only populations to exhibit decreases in all scale counts examined. Further, Harris (2000) examined correlation coefficients between mean scale counts and summer temperatures over a five year period and found no correlation between scale counts and temperature for this time period. Bills had concluded these discrepancies were possibly due to multiple "rough fish" eradication attempts in 1957, 1961 and 1970 with corresponding reintroductions and subsequent hybridization via "bait bucket" with tui chubs from one of the neighboring basins. Due to the uncertain heritage of fish from Ana Reservoir they were excluded from further analysis. Bills further concluded that the other Oregon Lake Basins (Alkali (Hutton Spring), Warner and Silver) each contained a unique subspecies.

Harris (2000) using mtDNA to examine the phylogenetic relationships in tui chubs, encountered two mtDNA cyt *b* haplotypes co-occuring in Summer Basin; *S. obesa* and *S. thalassinus*, the latter is confined within the *S. bicolor* clade. Harris also concluded that introduction and introgression by tui chubs from a neighboring basin had occurred. He suggested an introduction from either the Goose Lake Basin or Pit River Basin, due to

similarities between *S. thalassinus* mtDNA cyt *b* sequences from Ana Reservoir and *S. thalassinus* from the Goose Lake-Pit River Basins. According to cyt *b* data *S. thalassinus* occur in the Warner Basin, Cow Head Lake Basin, Goose Lake Basin and Pit River Basin. A Factorial Component Analysis (FCA) of microsatellite data indicated Summer Basin fish were distinct; however Summer Basin fish appeared to cluster nearest the Goose Lake-Pit River Basins and Warner Basin (Chen et al. 2008). Bayesian analysis of microsatellite data (Chen 2006) indicated a relatively small number of Summer Basin fish had a high probability of placement into either Goose Lake Basin, Pit River Basin or Warner Basin. Results from morphometric, meristic, mtDNA cyt *b,* and microsatellites all suggest either the taxa occur in sympatry or there is some degree of gene-flow (via introduction or allopatric speciation and dispersal) between these hydrologically disconnected basins.

Despite both morphologocial and genetic work, the status and identity of tui chubs from Oregon remains unsettled. Studies utilizing data sets from various life stages might possibly shed light on these questions. Until these questions regarding the relationship of tui chub from the Great Basin are answered, any efforts to conserve or protect this biodiversity will be hampered.

Developmental features provide a wealth of characters, such as the timing and rate of development, pigmentation patterns and osteological development all of which are useful in separating closely related species (Moser et al., 1984). Systematists have long recognized the importance of larval characters and in using "ontogeny to reconstruct the phylogeny of fishes" (Cohen, 1984). Studies utilizing developmental information along

with other life history data, such as, adult characters or genetic datasets have successfully described species that were previously difficult to differentiate (Orr and Matarese, 2000; Roje, 2010)

Study Area

During the Miocene and Pliocene the Great Basin encountered extreme tectonics in the form of east-west crustal stretching, which lead to thinning of the crust and allowed for magma to rise to the surface in form of lava flows and volcanos (Orr and Orr, 2000). These events lead to the current horst-graben topology (mountains with intermountain basins) that characterize Oregon's Great Basin.

Oregon's Great Basin contains seven of these major horst-grabens, which are bordered by the Cascade Mountains in the west, the Brother Fault Zone roughly on the northern border and Idaho in the east. Basins are as follows: from west to east; Klamath Basin, Goose Lake Basin, Warner Basin, Summer Basin, Abert Basin, Alkali Basin, Guano Basin, Catlow Basin, Alvord Basin, and McDerrmitt Basin. Typcially, these basins are considered endorheic, however in Oregon two basins have current connections with the sea, they are the Klamath in the west and the Owyhee located on the Oregon-Idaho border, which drains into the Snake River. During levels of lake maxima Goose Lake connects with the Pit River, draining into the Pacific. The last recorded connection between Goose Lake and the Pit River was an overflow event in 1881 (ODFW, 2008)

During the Pleistocene the landscape of the Great Basin looked quite different from today. Instead of an arid high desert, water ruled the landscape. Many of the basins,

within these horst-graben systems, contained large Pluvial Lakes varying in size. The largest being lake Bonneville in Utah, at depths of 1,100 feet, covering 20,000 square miles and stretching over 500 miles (north-south), which overflowed into the drainages systems of both the Columbia and Snake Rivers (Madsen et al., 2002). In northwestern Nevada and northeastern California was Lake Lahontan, which at a depth of 700 feet, covered 8,500 square miles and throughout this period maintained connections with multiple sub-basins (Bishop, 2006). Oregon contained nine of these Pluvial lake systems.

There is much speculation regarding the hydrological connections not only between the large Pluvial Lakes once present in Oregon, but also between these ancient lakes and areas boarding the Great Basin. Some of these hypothesized connections included: Pluvial Lake Lahontan with Pluvial Lake Modoc, currently Klamath Basin, via northwestern Nevada and south-central Oregon (Hubbs and Miller, 1948), Pluvial Lake Catlow spilled into Pluvial Lake Malheur (Minckely, 1986), connection between Deschutes River system with Pluvial Lake Fort Rock (Allison, 1979) and Pluvial Lake Fort Rock with Pluvial Lake Modoc, via the Sycan Marsh (Hubbs and Miller, 1948). Fossils of ancestral tui chubs suggest a late Miocene or early Pliocene origin (Smith et al., 2002). Many of these connections cannot be confirmed using geological evidence; so one must use extinct and extant faunal distributions to establish these hypothesized links.

MATERIALS and METHODS

Table 1. gives a list of species delineations, collection locality, Samples of tui chubs were collected from all the major basins present with Oregon (Fig. 1).

<u>Data Collection</u>

DNA isolation

DNA was recovered from fin clips taken from the upper caudal fin lobe for all specimens and preserved in 95% ethanol. Specimens were either obtained from the Oregon State University Ichthyological Collection or fin clips were obtained in the field.

Genomic DNA was extracted following the methods of Ivanova et al., (2006). In brief, small amounts of caudal fin clips were mixed with 50 µl of vertebrate lysis mix (1 M NaCl, 1 M Tris Hcl, 0.5 M EDTA, 1.0 g SDA and 0.5 ml Proteinase K) in 96-well PCR trays using a Applied Biosystem thermocycler. Fin clip extracts were incubated overnight at 56°C to allow for digestion.

A 100 µl of binding mix (50 ml binding buffer and 50 ml 96% ethanol) was added to each well and centrifuged. Roughly, 150 µl of final product was removed, transferred to new wells and centrifuged to facilitate binding of the DNA to the glass fiber membrane.

Two washes were used: 1) 180 µl of protein wash buffer (26 ml binding buffer and 70 ml 96% ethanol) and 2) 750 µl wash buffer (300 ml 96% ethanol, 1 M NaCl, 1 M Tris-HCl and 0.5 EDTA). DNA was collected and stored at -20°C until PCR was performed.

Mitochondrial PCR amplification and sequencing

Amplification of an 800 base pair (bp) portion of the cyt *b* gene, using primers L14724 (5'-gtgacttgaaaaaccaccgttg-3'; Schmidt and Gold, 1993) and H15669 (5'agtcctcgttgtttttgaggtgtg-3'; Harris, 2000) were performed. The PCR mixture contained

the following; 2.0 µl genomic DNA, 2.0 µl 10x buffer (Promega), 0.2 µl dNTP, 0.5 µl of a 10 µM solution of both primers, 0.1 µl *Taq* polymerase and ddH$_2$O for a final volume of 10 µl. Samples were denatured initially at 95°C for 5 min and then consisted of 35 cycles at 95°C (30 sec) denature, 55°C (45 sec) annealing, 72°C (1 min) elongation and a final elongation cycle at 72°C for 10 min.

PCR amplification was checked visually using ethidium bromide on 1% agarose gels. Bi-directional sequencing was performed with ABI Big Dye chemistry using ABI 3100 capillary system. Sequences obtained for this study have been deposited in Genbank.

Mitochondrial DNA Analysis

The 800 bp sequences recovered were added to those from Harris 2000, which were obtained from Genbank (AF370115.1 – AF370041.1). A total of 117 sequences were imported into Bioedit v7.13 (Hall 2011) and manually aligned and edited. Some samples produced the original 1140 bp sequence as Harris (2000). However, an 800 bp sequence cut-off was used to maximize the number of useable sequences. Harris's original data was shortened from 1140 bp to a corresponding 800 bp sequence and analyzed using the same criteria as the data from this study. It was determined that the loss of 340 bp from the original data did not drastically alter the topology of his tree and therefore allowed for the combining of sequences from this study. The combined data were examined in Molecular Evolutionary G A (MEGA 5.0; (Tamura et al., 2011). Due to the possible presence of hybrid populations, a NJ tree was used to evaluate sequence data due to the lack of rigid requirements regarding specific information on rates of

evolution (Hillis et al., 1996) and report similar topologies as those trees which use explicit phylogenetic methods (McDade, 1997). McDade (1997) evaluated the placement of hybrids on a phylogenetic tree using multiple methods and found the NJ algorithm placed hybrids basal to one or the other parent populations, much like results using parsimony. The NJ model compared the number of differences in Transitions and Transversions, allowed for complete deletion of missing or gap data and equally weighted all codon positions. To evaluate node support the methods of (Felsenstein, 1985) were followed. Bootstraping with 1000 replications (implemented in MEGA 5.0) was used. To indicate relative support for internal branching 50 % and greater bootstrap values were retained; 50 % values indicate informative patterns and 95 % values are usually considered "correct". Kimura's two-parameter distances (Kimura, 1980) were estimated using MEGA 5. Autapomorphies were also derived from MEGA 5.0.

Microsatellite PCR amplification and scoring

Four microsatellite loci (Gbi-G3, Gbi-G13, Gbi-G38, and Gbi-G79) developed by Meredith and May (2002) were used in this study. Originally Gbi-G10 and Gbi-G87 were attempted, however due to the imperfect results of both loci we were forced to drop them from the study. All 10 µl PCR reactions contained the following 20 mM Tris-HCl, pH 8.4, 2.5 mM $MgCl_2$ (2.0 Gbi-G13) 3.0 mM dNTPs (0.175 Gbi-G13, 0.20 Gbi-G79), 0.5 µM fluorescently labeled primers forward and reverse (0.4 for both Gbi-G13 and Gbi-G79), and 0.025 units *Taq* polyermase (Promega). Mixtures were amplified using the following conditions: Gbi-G3 and Gbi-G79 both had initial denaturing phase 95°C (3 min) followed by 36 cycles of denaturing at 95°C (30 sec), annealing phase with the

following cycles 50 at 62°C, 4 at 60°C, 2 at 56°C and 25 at 54°C all at 20 sec then 36

cycles of the elongation phase at 72°C (30 sec). Gbi-G13 initial denaturing phase 95°C (3

min) followed by 35 cycles of denaturing at 95°C (45 sec), annealing phase with the

following cycles 5 at 61°C (30 sec), 30 at 58°C (30 sec) both cycles ended with an initial

elongation phase at 72°C (40 sec). Gbi-G38 had an initial denaturing phase of 95°C (5

min) followed by 35 cycles of denaturing at 95°C (45 sec), annealing phase at 55°C (30

sec), elongation phase at 72°C (40 sec). The only exception was Gbi-G38 with an

annealing phase which occurred at 51°C (30 sec). All reactions were exposed to a final

elongation at 72°C for 10 min on an Applied Biosystem thermocycler. Final PCR

products were then separated via polyacrylamide gel elecrophorsis on an ABI 3730XL

geneotyper and alleles were scored according to size using the program GeneMapper

4.1.1 (Applied Biosystem).

Microsatellites Analysis

 Allele frequencies, numbers of alleles per locus and estimates of genetic distance

(F_{st}; Wright 1951) were computed in GENETIX 4.04 based on 273 individuals and four

microsatellite loci. The F_{st} statistic is a relatively useful measure of genetic divergence

(Neigel 2002) and was calculated between samples when more than one sample was used

per basin. These pairwise F_{st} values were used to determine genetic similarities between

samples before combining locations by basin.

 Observed (H_O) and expected (H_E) heterozygosities were calculated in Arlequin

3.0 as were tests for deviations from Hardy-Weinberg equilibrium (Excoffier et al., 2005)

and linkage equilibrium. FSTAT 2.9.3 (Goudet, 1995) was used to estimate allelic

richness (R_A) over all loci. R_A is a useful measure of allelic diversity, which takes sample size into account, unlike the total number of alleles at any given locus (El Mousadik and Petit, 1996).

To test for population structure in tui chubs two models were implemented in the population genetics program STRUCTURE 2.3.1 (Pritchard et al., 2000). Pritchard et al. (2000) found that microsatellite datasets with as few as five loci performed well when populations were discrete. However, when an admixture model was used the results were less consistent, even with 15 to 60 loci. Even if datasets contain either too few loci or individuals the authors' still suggest starting with the admixture model. If the population structure signal was weak Pritchard et al. (2000) suggest following the methods outlined by Hubisz et al. (2009), which allows for the use of sample location as an *a-priori* assumption. Firstly, using the methods of Pritchard et al. (2000) an admixture model, which allowed for admixed populations, was used to estimate *K*. Secondly, the methods of Hubsiz et al (2009) using the LOCPRIOIR model, which allowed for the assignment of population based on location, were followed.

The program STRUCTURE uses a Bayesian methodology to estimate the population of origin for an individual, given allele frequencies of all populations or "clusters" (*K*, which is user specified). This method basically permits the highest posterior probability to infer *K*. However, Evanno et al. (2005) have shown that STRUCTURE can lead to over estimations of *K*. These authors suggest using delta (Δ) *K*, which is a rate of change between *K* and *K*+1 clusters. To estimate ΔK, posterior probabilities for each estimate of *K* were obtained from STRUCTURE using both the

admixture and LOCPRIOR models. Individuals were grouped based on capture location and model iterations were as follows: burn-in 100,000 replications, 100,000 MCMC replicates, with 10 iterations for $K = 1$-10. Using the methods outlined in Evanno et al. (2005), results from STRUCTURE iterations were imported in STRUCTURE HARVESTER (http://taylor()biology.ucla.edu/struct_harvest/).

To evaluate the amount of hybridization in tui chubs from the Summer Basin and surrounding basins, 70 individuals that had both mtDNA cyt *b* and microsatellite data available were used. Microsatellite data for these individuals were grouped into one of two mtDNA cyt *b* clades, either *S. bicolor* or *S. obesa* and imported into STRUCTURE. Parameters for estimates of K were similar to those outlined above, with the exception of the number of clusters analyzed. The number of clusters investigated were one through five and estimates of K, from STRUCTURE, were imported into STRUCTURE HARVESTER to determine ΔK.

Morphometrics

Larvae were collected during late spring and summer from 2005-2007 with the exception of *S. boraxobius* which was collected during the month of November 2005 due to a large fall spawning population (Perkins et al., 1996). Gears used included larval seines, larval trawls, minnow traps and hand held dip nets. Specimens were fixed in a 5% formalin solution and later transferred to 50% isopropyl for permanent storage, and vouchers deposited in the Oregon State University Ichthyological Collection.

Individuals were staged based on caudal development (Kendall et al., 1984) with juveniles defined as those postflexion specimens with loss of finfold and presence of

adult fin ray counts. A total of 279 specimens were measured using a Zeiss dissecting scope and recorded to the nearest 0.01 mm. Only specimens which were in good physical condition were used and measurements were taken on the left side of the fish.

Measurements follow those of Remple and Markle (2005) with the following exceptions: Body Length (BL) is measured from the tip of the snout to the tip of the notochord (preflexion and flexion) and to the posterior edge of the upper hypural (postflexion, juveniles and adults), measurements are listed in Table 2.

Morphometrics Analysis

Specimens were grouped by stage and all measurements were standardized by dividing the measurement by either body length or head length. All morphological measurements are reported herein as either mean percent body or head length.

Meristics

A total of 305 larvae were cleared and stained following the methods of Pottoff (1984) and 276 adults were radiographed. Both methods allowed for collection of fin ray, vertebral and other osteological counts. Fin ray counts include all visible rays, except the last dorsal and anal rays which have two rays originating from one pterygiphore and are counted as one. Procurrents are counted as separate from the fin rays. Adult dorsal and anal fin ray counts in cyprinids typically exclude procurrents and only score principle rays (PR), thus some of the earlier postflexion specimens may include counts of the fins which are two higher than their later stage postflexion conspecifics. Osteological counts were made from both cleared and stained larvae and radiographed adults and are available in Table 3.

Meristics Analsysis

Adult and larval meristic datasets were examined separately using Principle Component (PC) analysis. Furthermore, mtDNA cyt *b* haplotypes were mapped onto scatterplots of PC scores to examine whether meristic characters were correlated with haplotype assignment.

Pigmentation

Pigmentation information was collected from each specimen used in morphometrics and photographs of developmental series were obtained. Descriptions of larvae were based on the overall sample population and developmental series were represented by those specimens which best characterize the description.

RESULTS

Mitochondrial DNA

There were 176 variable sites, 105 of which were parsimony informative, among the 117 cyt *b* sequences. A neighbor-joining (NJ) dendogram (Fig. 2) recovered a basal polytomy containing an eastern Alvord Basin clade and a western Sycan Marsh clade. Sequence divergence between the Alvord clade and the rest of the *S. bicolor* complex ranged from 9.0 % to 11.1 % but there was only 0.37% divergence between the two species in the Alvord clade, *S. boraxobius* and *S. alvordensis*. The "Sycan Marsh" clade had 6 autapomorphies at positions 79, 157, 229, 232, 373, and 496, and diverged 10.5 % from the Alvord clade and 7.7 % from the rest of the *S. bicolor* complex.

There were two monophyletic clades in the *S. bicolor* complex that diverged by 1.75 %. The *S. obesa* clade was diagnosable with two third position synapomorphies at positions 520 and 571. Within the clade, the monophyletic *S. oregonensis* lineage (54% bootstrap support) was restricted to Abert and Alkali Basins and was diagnosed by an autapomorphy (position 172). Sequence divergence between the basins was 0.0 - 0.37 %. Two individuals from Alkali Basin were autapomorphic at nucleotide position 37, but most individuals (14 of 19) from Alkali Basin were identical to individuals from Abert Basin. Divergence from *S. obesa* (Nevada) was 0.62 - 0.88 %, from the "Summer Basin" clade was 0.88 – 01.1% and from *S. bicolor* was 1.2 - 2.9%. The *S. oregonensis* clade was sister to a ploytomous *S. obesa* (Nevada) which was sister to a monophyletic "Summer Basin" clade. The "Summer Basin" clade (71% bootstrap support) had autapomorphies at positions 226, 325 and 370. Sequence divergence of the "Summer Basin" clade was 1.1 - 1.7% from *S. obesa* (Nevada), and 2.2 - 3.7% from *S. bicolor*. There were three lineages found within Summer Basin. In addition to the "Summer Basin" clade, we found *S. oregonensis* and *S. thalassinus* (Fig. 2).

The *S. bicolor* clade had autapomorphies at nucleotide positions 304, 586, 673, 655, and 787. The *S. bicolor* clade contained a monpohyletic *S. thalassinus* clade and a polytomous *S. bicolor* lineage. The *S. thalassinus* lineage occurred in Warner Basin, Goose Lake Basin and Pit River System while the *S. bicolor* lineage occurred in Malheur Basin, Catlow Basin, Guano Basin, Fort Rock Basin, Klamath Basin, and eastern Washington. The *S. thalassinus* clade (79% bootstrap support) had synapomorphies at positions 148, 202 and 409. Within *S. thalassinus* sequence divergence was 0.25 %

between Warner Basin and Goose Lake/Pit River, Sequence divergence of *S. thalassinus* was 2.6 - 3.0% from the *S. obesa* clade and 1.1 - 3.2% from the *S. bicolor* polytomy. One fish from the Warner Basin was a member of the "Summer Basin" clade and two fish from the Pit River and one from the Warner Basin were members of the *S. oregonensis* lineage. All? Individuals from Upper Klamath Lake and Thompson Reservoir (Fort Rock Basin) shared a transition at position 684. Most Fish from Sycan Marsh, located north of Upper Klamath Lake, were in the *S. bicolor* polytomy, except for two with the "Sycan Marsh"haplotype.

Microsatellites

Allele frequencies, number of alleles per locus for all populations, allele richness, observed (H_O) and expected (H_E) heterozygosities and deviations from Hardy-Weinberg equilibrium (HWE) are presented in Table 4. Observed (H_O) and expected (H_E) heterozygosities varied by populations with a total of eight significant deviations ($p \leq 0.01$) from HWE, four of which were from Gbi-G3, thus we excluded Gbi-G3 from further analysis.

Genetic diversity varied by location and locus. Fish from 20 Mile Slought in Warner Basin exhibited the greatest number of alleles with 18 at loci Gbi-G87 and Upper Klamath Basin had the highest allelic richness at 9.8 also at Gbi-G87. In contrast, fish from the Big Sage Reservoir, CA. had the lowest number of alleles and allelic richness at Gbi-G13with one and 1.0, respectively.

All sample locations and basins, where more than one sample was collected, had F_{st} values which indicated relatively little divergence (0.03 – 0.05) and were grouped

accordingly, with the exception of the Pit River System. Samples were collected from

two locations in the Pit River System; Big Sage Reservoir, CA. and the Pit River, CA.

The F_{st} value between these two locations was 0.21, which suggested genetic

differentiation and therefore were analyzed as separate entities (Table 5). The greatest F_{st}

values were shared between Malheur Basin and Summer Basin (0.31) and Malher Basin

and the Pit River (0.31) The lowest values were shared between Klamath Basin and the

following locations: Warner Basin and Abert Basin (0.03 and 0.06), respectively.

Results from Bayesian clustering of all individuals using both the admixture and

LOCPRIOR models indicated population structure. However, the LOCPRIOR model

performed better with the low number of loci available (n=3) and are the results report

herein. A gradual increase in the $\log_e P(X \mid K)$ from $K = 1 - 8$ was observed, which then

slightly decreased from $K = 9 - 10$ (Fig. 3). The steepest increase in the $\log_e P(X \mid K)$

identified by the ΔK statistic, was for $K = 2$ (Fig 3), with next greatest increase at $K = 8$

and a slight jump at $K = 5$.

Bar plots of proportional assignments for individuals at $K = 2$, 5 and 8 revealed

population structure (Fig 4). At $K = 2$ fish from Ana Reservoir and County Rd. 417 (both

from Summer Basin) formed a distinct cluster, while fish from all other locations grouped

into another cluster. At $K = 5$ and $K = 8$ a finer scale of resolution was observed. At $K =$

5, Hutton Spring (Alkali Basin), Crooked Creek (Abert Basin), Silver Creek (Malheur

Basin), County Road 417 (Summer Basin) and the Pit River System formed relatively

distinct clusters, while all other locations indicated varying degrees of admixture. At $K =$

8, clusters were closely linked to sample location. Individuals from Big Sage Reservoir

clustered separately from the Pit River, however all individual from the Pit River had

some membership probability to the Big Sage Reservoir. All individual from Upper

Klamath Lake had membership probabilities to the Pit River, Warner Basin and to a

lesser extent the Malheur Basin. All individuals from Ana Reservoir had membership

probability to the Big Sage Reservoir.

Using the criteria of cyt *b* haplotype to cluster individuals, both the mean

probability of K and ΔK indicated the steepest increase in the $\log_e P(X \mid K)$ was at $K = 3$

(Fig. 5a; 5b). At $K = 2$, two distinct clusters were present (Fig 6). Fish from Hutton

Spring (AlkB) formed one cluster, while all other fish formed another. However, two

individuals from Hutton Spring had some membership probability to the other cluster. At

$K = 3$, Ana Reservoir and County Road 417 (Summer Basin) formed a distinct cluster,

with one *S. obesa* cyt b haplotype individual having greater than 80 % membership

probability with the *S. bicolor* cyt *b* cluster. All individuals from both the Pit River and

Warner Basin, which were identified as having the *S. obesa* cyt *b* haplotype had greater

than a 50 % membership probability to the *S bicolor* cyt *b* haplotype. In contrast, Fish

from Summer Basin and County Road 417 identified with *S. bicolor* cyt *b* had few

individuals with greater than a 50 % membership probability to the *S. obesa* cyt *b*

haplotypes.

 Development

Larval Morphology

In general, all larvae of *Siphateles* experience positive growth in body proportions

during development, with the following exceptions; eye diameter and the distance from

the tip of the snout to the tip of urogenital pore (Table 6). All populations, except for Hutton Springs, exhibit a decrease in mean eye diameter during development. *Siphateles alvordensis* had the smallest mean diameter at 28.6% during flexion decreasing to 25.7% during postflexion and 24.8% during the juvenile stage. Larvae from Hutton Springs, however exhibited the largest mean eye diameter at 33.0 and 33.9 percent during flexion and postflexion, respectively. The other body proportion that decreased with growth was the distance from the tip of the snout to the lower edge of the urogenital pore, which ranged from 68.5% - 75.6% during flexion in larvae from Ana Reservoir and Co-Rd 417 (both Summer Basin), respectively and 63.7% - 70.0% during the juvenile stage in *S. alvordensis* and larvae from Sycan Marsh (Klamath Basin), respectively.

Of those body proportions which exhibited positive growth, two displayed the greatest amount of variation between populations. During flexion, mean head length ranged from 22.0% in larvae from Skull Creek to 27.6% in Co-Rd 417. However, postflexion larvae of *S. boraxobius* and *S. alvordensis* had the smallest mean head length at 25.4% and 24.4%, respectively; while larvae from Co-Rd 417 had the largest mean head length at 29.9%. During the juvenile stage specimens of *S. alvordensis* had the smallest mean head length at 25.8% while specimens from Upper Klamath Lake had the largest at 31.5%.

In flexion, mean snout length ranges from 9.4% - 20.2% in larvae from Hutton Spring and *S. boraxobius*, respectively. During postflexion, Hutton Spring larvae present with the smallest mean snout length at 13.6% while *S. boraxobius* has the largest mean snout length at 21.3%. During the juvenile stage larvae from Sycan Marsh (Klamath

Basin) have the smallest mean snout length at 18.0% while all other juveniles ranged

from 18.7% (Skull Creek) to 20.6% (*S. alvordensis*).

The first fin rays to develop are the caudal fin rays on the lower developing

hypural. By the end of flexion all larvae have a complete caudal fin ray count of 10+9

(Table 7). Dorsal fin rays are the next rays to develop, followed closely by anal rays.

Pelvic fin rays develop next while pectoral fin rays are the last rays to fully form. Primary

rays in the medial and paired fins develop from front to back, however dorsal, anal and

pelvic procurrents are the last to form and may not be fully developed until sometime

during late juvenile - early adult stages. Larvae of *S. boraxobius* have an adult

complement of all fin rays by roughly 15.5 mm, while specimens from Skull Creek

exhibit their adult complement by 22.0 mm.

Meristics

Differences between larval and adult meristic counts were due larval

development. Many osteological structures which were easily recognizable in adults were

either not present or so under developed in larvae that distinguishing and counting some

structures was difficult. Therefore, larval and adult meristic datasets were analyzed

separately.

During flexion, the developing hypurals, cleithrum and jaws are the first

structures to absorb alizarin red, which indicates ossification. In late stage flexion the

anterior vertebra and those posterior vertebrae associated with the hypural plates begin to

ossify. By early postflexion the Webberian Apparatus is formed and ossified along with

most of the cranium. By mid-postflexion all centra of the vertebral column have absorbed

alizarin red along with corresponding neural and hemal spines. Further, pterygiphores which have developing rays are ossified. During either late-postflexion or early juvenile stages all structures have completed ossification.

Pre-dorsal bones (PDB) were not visible on the radiographs of adults and therefore only observed in larvae. The first PDB forms just behind the Webberian Apparatus and occasionally behind the fifth neural spine. The number of PDB's continues to increase throughout development. Usually, only one to three PDB have ossified by the juvenile stage in all populations.

Principle component analysis for the 11 adult meristic characters indicated that PC score 1 explained 56.3% of the total variance (Table 7). Variable loading indicated PC 1 is related with pre-caudal vertebrae, anal fin insertion over vertebra number, anal pterygiophore and associated hemal spine and last anal fin pterygiophore and associated hemal spine (Table 7). Scatterplots of PC scores versus basins (Fig 7) showed considerable overlap in counts between individuals within and between basins. Further, mapping of cyt *b* haplotypes onto individual meristic PC scores did not reveal a phylogenetic signal within the meristic data.

Pigmentation.

Larvae of Oregon *Siphateles* are diagnosable based on the presence of an occipital heart-shaped patch of melanophores over the midbrain. From this occipital heart a row of either singly space or "bunched" melanophores extends down the dorsal surface from the nape to the origin of the dorsal fin. In early flexion, this single row does not extend to the

origin of the dorsal fin membrane. It typically ends either a quarter or half way down the dorsal surface.

In many of these locations the most commonly found cyprinid, in conjunction with *Siphateles* is *Rhinichthys osculus* (speckeled dace). *Rhinichthys* is easily identifiable, they too have the occipital heart over the midbrain, but lack the row of melanophores that extend along the dorsal surface (Feeney and Swift 2008; personal observation).

In general, pigment patterns amongst *Siphateles* populations from Oregon develop in a similar manner (Figs. 8 – 17). Typically, during flexion larvae exhibit melanophores on the anterior snout and a patch on the dorsal surface of the snout. On the upper operculum melanophores are either non-existent or slightly scattered (Figs. 10a-b and 17a-b) Melanophore concentration increases throughout development and into the juvenile stages for these areas (Figs. 8b-c, 9b-c, 14a-b, 10c-d – 17c-d).

Pigment on the lower jaw develops during flexion and is either non-existent or light (one-five melanophores). However, pigment increases with development and by postflexion larvae may have roughly 30 melanophores present on the lower jaw. All larvae exhibit heavy pigment over the cardiac region, typically in the shape of a "V", which begins during mid-late flexion and continues through postflexion.

In flexion, the solid line of pigment that extends from the nape to the origin of the dorsal fin is not full developed and will either extend a quarter of or half way to the origin of the dorsal fin membrane (Figs. 10a-b – 17a-b). As the fish develops this line of pigment continues to extend until it reaches the origin of the dorsal fin. Further, pigmentation extends from the insertion of the dorsal fin membrane up to the leading

edge of the upper caudal membrane (Figs 8c, 9c, 14b, 10d – 17d). In postflexion, pigment along the dorsal surface increases in numbers and are of various size. . Some fish develop a dark patch of melaophres at the base of the last two pterigphores, at the insertion of the dorsal fin (Figs. 9b, 11c, 13c, 16c). In late flexion, as the anal fin develops, a dashed line appears over the base or insertion of the anal fin rays (Fig. 9a) This pigment increases throughout development and in some cases becomes a solid line of pigment (Figs. 8b, 11c, 12c, 13c, 14c, 15c). On the ventral surface two lines of melanophores develop just posterior to the opening of the urogenital pore and run parallel to each other terminating, in most fish, at the leading edge of the lower hypural. This character is visible from a lateral view and starts during flexion (Figs. 9a – 13a, 15a – 17a). It is still visible in many postflexion fish; however it is not as prominent.

In flexion, pigment along the lateral myoseptum (anatomical structure that will develop into the lateral line) is either absent (Fig. 8a), confined to one to four melanophores posterior to the insertion of the dorsal fin membrane (Fig. 17a) , or extend to the origin of the dorsal fin membrane (Fig.9a – 13a, 15a-16a) During postflexion, a marked increase in melanophore numbers along the lateral myoseptum occurs and can extend from either mid-dorsal or to the posterior edge of the cleithrum (Figs. 8b – 17c; 11c- 12c, respectively). However, in larger juveniles melanophores, along what is now the developing lateral line, are harder to see for two reasons: 1) pigment is becoming embedded in the lateral line as tissue develops and 2) pigment form the dorsal surface is increasing in numbers and migrating ventrally, obscuring the previous patterns (Figs. 9b – 11c, 13c, 16c). In some fish this vertically migrating pigment extends past the lateral

myoseptum almost reaching the ventral surface (Figs. 9b – 11c, 13c, 16c). In other, pigment many only extend slightly past the lateral myoseptum with the greatest concentration of melanophores occurring along the anterior lateral myoseptum (Figs. 8b, 15c, 17c).

In the caudal region, pigmentation is present on both hypurals, with a heavier concentration on the upper hypural and only a few sporadically occuring melanophores on the lower hypural. Pigmentation in this area increases in number and size throughout development. Typically, a large dark patch of melanophores is present on the lower hypural and becomes embedded under the developing tissue (Figs. 9a – 13a and 15a – 17a). In most late stage postflexion and nearly all juveniles this patch is no longer visible.

In most specimens, when the caudal fin rays begin to develop during flexion, melanophores outline the rays, starting at the base and extending out towards the tips (Figs. 11a – 12a). This pattern is repeated in both dorsal and anal fins as they develop, the timing and amount of pigment differs by population. In the pectoral fin it is uncommon to have pigment present in the fin membrane during flexion and early postflexion. However, some fish will present with one to two melanophores during these stages (Fig. 11b). In postflexion, melanophores appear along the developing pectoral fin rays and increase in number as the fish develops (Figs. 9b; 10c – 16c), however it is not uncommon for pigment to be absent from this fin (Fig. 17c). In many fish pigmentation in the developing pelvic fin ray is absent until late postflexion or juvenile stages, if present at all (Figs.9b and 11c, 8b – 17c).

The amount and presence of pigmentation in an anatomical region differs based on the population, however larvae could be assigned to one of two types of pigment patterns; medium or heavy. Examples of each pigment pattern with corresponding larvae are as follows:

Medium pigment pattern (Figs.81-d, 9a-d, 13a-d, 14a-d, 15a-d) – In flexion larvae are relatively lightly pigmented on both the dorsal and lateral surfaces. The number of melanophores present on the lower jaw are either nonexistent or few (one to six). Pigment is present in the developing caudal fin rays, but either nonexistent or very light in the developing dorsal and anal fins. During postflexion and juvenile stages pigment increased in concentration both dorsally and laterally, however on the lateral surface pigmentation only extended slightly below the developing lateral line and just posterior to the cleithrum. Melanophore concentration is heavier in the medial fins, but is either absent from both the pelvic and pectoral fins or very light. Few melanophores were observed in the pectoral fins of later stage larvae and juveniles examined, larvae included in this pattern were: *S. alvordensis* (AB), *S. boraxobius* (AB), Upper Klamath Lake (UKL), Thompson Reservoir (FRB), Ana Reservoir (SB), and Dog Creek (GB).

Heavy pigment pattern (Figs.10a-d, 11a-d, 12a-d 16a-d) – In flexion, pigment along the lateral myoseptum extends either to the mid-point of the body or just anterior of the developing dorsal fin membrane. Pigment is present in the caudal fin and in the dorsal fin membrane of some populations. During postflexion and juvenile stages melanophores present of the lateral dorsal surface have migrated ventrally and extend below the developing lateral line on both the anterior and posterior body. Pigment is present in all

median and paired fins. Larvae included in the pattern were: Skull Creek (CB), Hutton Spring (ALKB), Co-Rd. 417 (SB), and Sycan Marsh (KB).

Hutton Spring tui chub (*S. oregonensis*) were the most unique larvae recovered from this study (Figs10a-d). These larvae had the heaviest pigmentation of any population. Further, at some point between 12 mm and 13 mm larvae from Hutton Spring lose the occipital heart shaped pigment. Melanophores appear to dissipate while the number increases, losing the characteristic heart shape. This may be an autapomorphic character found in fish from Hutton Springs, OR. The only larval specimen collected from neighboring 3/8 Mile Spring did not have the loss of the heart shaped crown at 15.6 mm.

DISCUSSION

Results from this study provide insight into the taxonomic relationship of tui chubs from Oregon's Great Basin. Both molecular and morphometric results for *S. alvordensis* and *S. boraxobius* provide corroborating evidence for differentiation of fish from the Alvord Basin. Pairwise percent sequence distances suggest a difference of 10.0 – 11.0 % between the Alvord Basin chubs and the rest of the *S. bicolor* complex. Assusing a one percent sequence divergence per one million years (Smith et al., 2002),the separation of *S. boraxobius* and *S. alvordensis* from the *S. bicolor* complex was 10 to 11 million years ago (mya). The separation of the Alvord chubs from other tui chubs coincides with the uplift of Steen Mountain Range 10 to 15 mya (Bishop, 2006). Morphological differences between *S. alvordensis* and *S. boraxobius* include a longer

head length, longer snout length and larger eye diameter in *S. boraxobius* (Hubbs and Miller, 1972; Williams and Bond 1980). I found these differences were also present in the early life stages and could be used to differentiate between the two taxa as larvae. For the *S. bicolor* complex developmental characters and adult meristics indicated overlap in morphology and meristics between basins. However, larval pigmentation and eye diameter were different in *S. oregonensis*.

Harris (2000) found evidence for six nominal species from Oregon's Great Basin, ie., *Siphateles bicolor*, *Siphateles sp.*, *S. obesa*, *S. thalassinus*, *S. eurysomas*, and *S. columbianus*, all of which were geographically discrete. However, the shallow genetic structuring found outside of the Alvord Basin does not coincide with the timing of basin formation in Oregon or postulated ancient river connections. For example, it has been suggested that the disjunct distribution of the *S. obesa* clade from the Lahontan and Oregon Lakes region was once widespread and eventually bisected by the S. bicolor clade when the Snake River flowed west to Pacific through southeastern Oregon and northern California, however similarities in Pliocene fish fauna from the Snake, northern Califonia and southeastern Oregon point to a pre-Pliocene association (Smith et al., 2003). The average cyt *b* sequence divergence between tui chubs from the Klamath Basin (western Oregon) and Catlow Basin (eastern Oregon) is 1.04 % (Harris, 2000), which is younger than last hypothesized connection between the two basins. .Hershler and Lui (2004) found similar shallow genetic structure in the snail subgenus *Pyrgulopsis* from the Columbia-Snake River and Oregon Lakes region using the *COI* gene. In contrast, Arden et al., (2009) using cyt *b* from the cyprinid genus *Rhinichthys* from Goose Lake and the

Warner Basin found deep genetic structure, which was consistent with the uplift of the Hart Mountain range.

The cyt *b* analysis indicated the presence of more than one lineage within basins. Harris (2000) concluded that *S. thalassinus* from Goose Lake or one its tributaries was introduced into Summer Basin. Summer Basin mostly contained individuals of the *S. obesa* clade. But I also found individuals with *S. thalassinus* cyt *b* within the Summer Basin supporting the idea of introductions from one of the surrounding basins Members of *S. thalassinus* were otherwise restricted to Goose Lake Basin, Pit River system, Warner Basin and Cowhead Lake, which drains into the Warner Basin. I also found individuals with *S. obesa* cyt *b* DNA in the Warner Basin and Pit River system, possibly indicating reciprocal introductions with Summer Basin, Warner Basin and the Pit River system. Two individuals from the Pit River were identical to an *S. bicolor* haplotype from Silver Basin. These results suggest movement of fish between basins, which are no longer hydrologically connected.

Chen (2006) predicted Goose Lake as the source of the *bicolor/thalassinus* introductions into surrounding basins because of overlap in microsatellites, its proximity to the town of Lakeview, OR, and the popularity of tui chubs as bait fish. The STRUCTURE analysis at $K = 8$ indicated fish from both Ana Reservoir and the Pit River had some membership probability to Big Sage Reservoir, CA. The Big Sage Reservoir was constructed in 1921 as part of a Bureau of Reclamation irrigation project (State of California: Water Rights Board, 1964). To determine if *S. thalasinnus* from Goose Lake was the population of origin for this haplotype in the Big Sage Reservoir, the Pit River

and Summer Basin, individuals from Goose Lake will need to be included in further microsatellite analyses.

My data are consistent with movement of tui chubs involving multiple basins, whether these introductions are all recent or pre or post-European settlement will be hard to determine. Many Native American groups were active in the Great Basin during much of the late Pleistocene and early Holocene. The large pluvial lakes supported large human settlements. As the climate transitioned to a warmer, dryer period the larger lakes receded and left behind smaller, isolated bodies of water that were no longer able to support large settlements (Livingston 2002). Tui chubs appeared to have been a valuable food source due to their abundance, high catch per unit effort (CPUE), high tolerance to fluctuating environmental conditions, and high protein-caloric content (Raymond and Sobel 1990; Butler 1996). At one midden site 110 of 114 pharyngeal teeth belonged to tui chub (Butler 1996). Whether native Americans actually moved tui chubs is unknown.

European settlers did move fish. Standford ichthyologist, W. B. Evermann made a collecting trip to southeastern Oregon in 1897. In his field notes (Archives California Academy of Sciences) he noted in two places (pp. 65 and 73) of being informed of tui chub transplants. On August 2, 1897 (p. 65) referring to Abert Lake, he wrote, "No one has ever seen fish in the lake. At the north-end of the lake is a large spring 3 mi from the lake in which chubs were placed by Alvin Randall several years ago". XL Spring, the type locality of *S. oregonensis*, is 3.56 mi north of the lake. Evermann did not name the source population for this introduction. Evermann also mentions introductions of salmonids in the Oregon Lakes region in his 1897 field journal. Bills (1977) writes

"although bass and trout were at one time planted in XL Spring, I observed and collected only tui chubs" he further mentions "the XL Spring tui chub does not exhibit many of the characters associated with spring dwelling fishes". In my cyt *b* phylogeny and that of Harris (2000), fish from XL Spring formed a monophyletic group with fish from Hutton Spring, and were sister to a polytomy of fish from Nevada. Railroad Valley, NV, one of the closer populations is over 350 mi from XL Spring but there are closer Lahontan tui chub populations. The closest Oregon population was in McDermitt Creek about 150 mi from XL Spring, but that population was poisoned in August 2009. Harris (2000) explained this disjunct pattern as a result of Miocene viacariance. The small sequence differences ranged between (0.62 - 0.88 %), making a Miocene explanation unlikely and tend to corroborate Evermann's account suggesting the ESA listed Hutton Spring tui chub is an exotic.

Recent recorded introductions of tui chubs include: Walker Lake to the Stillwater National Wildlife Refuge, Spooner Lake, and the Owens River, (Finger and May 2010; Chen 2006) Diamond Lake, OR. (source population unknown; Eilers et al. 2011), and Paulina Lakes, OR. (likely Upper Klamath Lake; Bird 1975). Moyle (1982) in his inventory of fishes of the Pit River System found tui chubs present in great abundance in all reservoirs that contained sports fisheries. Sada and Vineyard (2002) reported the known translocation of 24 fish species endemic to the Great Basin, within and outside of the species original range, mostly to establish refuge populations.

When the STRUCTURE analysis was performed using cyt *b* haplotypes as *a priori* clusters there was a suggestion of hybridization among tui chubs in Summer Basin,

Warner Basin and the Pit River system. Fish from Summer Basin with the *S. obesa* cyt *b* haplotype had a high membership probability with the microsatellite cluster identified with *S. obesa* while those with *S. bicolor* cyt *b* haplotypes were more likely to have mixed microsatellite genotypes. This pattern suggested that, although both mitochondrial lineages remain, the *S. obesa* nuclear lineage was more dominant. In the Warner Basin and Pit River system, individuals with *S. obesa* cyt *b* haplotypes shared the nuclear genotypes of sympatric *S. bicolor* cyt *b* haplotype individuals. Hutton Spring (Alkali Basin) and Upper Klamath Lake (Klamath Basin) remained relatively homogeneous.

Hybridization in fish can either contribute to the diversification of a species (DeMarais et al. 1992; Gerber et al. 2001) or be detrimental to the survival due to reduced genetic diversity (Allendorf and Leary 1988; Allendorf et al. 2001). Gerber et al. (2001) noted the decoupling of morphological and molecular characters in hybridized populations of *Gila* from the Colorado River. This decoupling of morphological and molecular characters is similar to what has been observed in *Siphateles* and suggests local environmental adaptations play a strong role in shaping the morphology of these fish. In those areas where introductions and hybridization occurred the lack of diagnosable morphological and meristic characters suggest that evolution of these characters to match the surrounding environment is rapid.

CONCLUSIONS

I draw two general conclusions from this study. First, congruence between molecular and morphometric characters supported the recognition of *S. alvordensis* and

S. boraxobius. Outside of the Alvord Basin, congruence between molecular and morphometric characters was less clear. Second, the absence of congruence of mitochondrial haplotypes and basin geography and the congruence of microsatellite clusters and location rather than cyt b haplotypes was consistent with introductions and introgression of tui chubs. Both introduction and subsequent hybridization may explain the incongruence between datasets.

These findings could be problematic for conservation and management of *Siphateles.* The 1985 listing of Hutton Spring tui chub as threatened by USFWS was based solely on the isolation of Hutton Spring and its small population size. If these fish were introduced from the Lahontan Basin, their conservation status should come into question. However, if they do represent an introduction from McDermitt Creek then protection of Hutton Spring tui chubs would be justified. Although the current data are consistent with an introduction, further work is warranted.

The choice of populations for genetic and morphological studies must be made with care. For example, Big Sage, Ana and Thompson Reservoirs are all man-made and support sport fisheries, making these areas subject to bait fish releases. However, these locations are often convenient for biologists who may assume the samples represent native biota. Introduction of tui chubs as bait fish will be a continued problem and should be addressed when studying relationships in the genus *Siphateles.* Further, the continued introductions and subsequent hybridization will prove challenging for conservation management of threatened and endangered tui chubs.

MATERIALS EXAMINED

Taxon, locality information, size, and catalog number for specimens examined in this study. OS refers to Oregon State University fish collection specimens, DFM numbers are collectors field number, A0 are Upper Klamath Lake larval field numbers. Numbers in parentheses following catalog numbers are sample size used in morphmetrics, meristics and molecular analyses, respectively. Superscripted letters following sample size denote use; ^{L} = larvae, ^{A} = adult and ^{G} = genetics. Those locations were cyt *b* were obtained from Genbank begin with AF370. The ^{H} following taxon indicate those taxonomic designations of Harris (2000).

Siphateles boraxobius. Alvord Basin. Borax Lake, Harney Co., OR: OS17841 (11, 19)^L; OS17942 (2, 8) ^{L}; OS18037 (6)^A; OS18053 (2)^A; OS18304 (1)^A; AF37042.1.

Siphateles alvordensis. Alvord Basin. Janas Pond, Harney Co., OR: OS18036 (6) ^{A}; OS18039 (9)^A; Dufferena Ponds, Elko Co., NV: OS06926 (6)^L; OS03725 (7, 5)^L; OS06924 (2)^L; OS0627 (2)^L; AF37041.1.

Siphateles mohavensis^H. Mohave Desert, CA. AF37043.1

Siphateles newarkensis^H. Fish Creek. Fish Creek Valley, Eureka Co., NV. AF37087.1.

Siphateles isolatus^H. Warm Springs Ranch. Elko Co., NV. AF37084.1

Siphateles eurysomas^H. Catlow Basin. Skull Creek, Harney Co., OR: OS17775 (3, 20)^L; OS16770 (8)^A; OS17921 (19)^L; OS17838 (19)^L; OS17922 (16)$^{L;}$ OS03418 (4)^A; OS05775 (2)^A; AF370991.1; AF37097.1; AF37095.1.

Siphateles columbianus[H]. <u>Malheur Basin</u>. Silver Creek, Malheur Co., OR: OS15577 (13, 27)[A, G]; AF37101.1.

Siphateles thalasinnus[H]. <u>Warner Basin</u>. 20 Mile Slough, Lake Co., OR: OS17847 (31)[A] OS17848 (15, 21)[A, G]; OS17849 (2, 2)[A, G]. Hart Lake, Lake Co., OR: OS05159 (5) [A].; AF37107.1; AF370108.1; <u>Goose Lake Basin</u>. Thomas Creek, Lake Co., 15430 (3, 5)[A, G]; Dog Creek DFMDC01 (3)[L]; AF37114.1; AF112.1. <u>Pit River System</u>. Pit River, Modoc Co., CA. 17852 (16, 16)[A, G]. Big Sage Reservoir, Modoc Co., CA. 17853 (15, 15)[A, G].

Siphateles obesa. <u>Summer Basin</u>. Ana Reservoir, Lake Co., OR: OS17935 (21, 13)[L]; OS17839 (4, 5)[L]; OS17938 (13, 9)[L]; OS17936 (6)[L]; OS17937 (2)[L]; OS15440 (18, 18)[A, G]. County Road 417, Lake Co., OR: OS18000 (13, 6)[L]; OS15437 (25, 62)[A, G]; AF37076.1; AF37077.1; AF37079.1; AF37081.1; AF37082.1; AF37110.1

Siphateles oregonensis. <u>Abert Basin</u>. XL Spring, Lake Co., OR: OS05315 (15)[A]. Crooked Creek, Lake Co., OR: OS15082 (10, 10)[A, G]; OS17854 (4)[A]; OS17856 (19, 28)[A, G]; AF37066.1; AF37069.1; AF37073.1; <u>Alkali Basin</u>. Hutton Spring, Lake Co., OR: OS17918 (8, 5)[L]; OS17924 (4)[L]; OS17925 (3)[L]; OS17943 (4)[L]; OS05136 (13)[A]; ODFW07 (34)[G]. 3/8 Mile Spring, Lake Co., OR: OS05316 (10)[A]

Siphateles bicolor. <u>Klamath Basin</u>. Upper Klamath Lake, Klamath Co., OR: A06648 (1)[A]; A08469 (4, 33)[A, G]; A08845 (2)[A]; A96194 (6)[L]; A09336 (1)[L]; A09286 (2)[L]; A09212 (1)[L]; A09332 (1)[L]; A96137 (1)[L]; A02195 (1)[L]; A01157 (1)[L]; A03186 (3)[L]; A99210 (2)[L]; A02199 (1)[L]; A01170 (2)[L]; A00210 (2)[L]; A96194 (1)[L]; A09198 (1)[L]; A96195 (3)[L]; A02267 (6)[L]; AF37105.1; AF37106.1 <u>Sycan Marsh</u>, Klamath Co., OR:

OS17933 (14)[L]; OS17932 (2, 1)[L]; OS17840 (5)[L]; OS17926 (5, 20)[L]; OS17928 (5)[L];

OS17927 (4,4)[L].

Siphateles sp[H]. <u>Fort Rock Basin</u>. Thompson Reservoir, Lake Co., OR: OS17919

(4, 7)[L]; OS17920 (12, 9)[L]. Silver Creek, Lake Co., OR: 05120 (6)[A].

Siphateles obesa <u>(Nevada)</u> AF37043.1; AF37045.1; AF37047.1; AF37049.1;

AF37051.1; AF37053.1; AF37059.1; AF37061.1; AF37063.1; AF37065.1; AF37067.1;

AF37069.1; AF37071.1; AF37073.1; AF37075.1.

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Table 1 Material collected and taxa information for *Sipahteles*. Numbers following location correspond to those in Fig. 1. [H] indicates the current taxonomic designations from Harris (2000).

Basin (abrev.)	Current Taxon Designation	Proposed Taxon Designation[H]	Location (code)
Alvord Basin (AL)	*Siphateles alvordensis*	*Siphateles alvordensis*	Dufferena Ponds, Elko Co., NV.[2]
			Janas Pond, Harney Co., OR.[3]
	Siphateles boraxobius	*Siphateles boraxobius*	Borax Lake, Harney Co., OR.[1]
Catlow Basin (CB)	*S. b. eurysomas*	*S. eurysomas*	Skull Creek, Harney Co., OR.[4]
Malheur Basin (MB)	*S. b. columbianus*	*S. columbianus*	Silver River, Malheur Co., OR.[5]
Warner Basin (WB)	*S. b. ssp*	*S. thalassinus*	Twenty mile Slough, Lake Co., OR.[11]
Goose Lake Basin (GB)	*S. b. thalassinus*	*S. thalassinus*	Thomas Creek, Lake Co., OR.[12]
			Dog Creek, Lake., OR.[12]
Pit River System (PR)	*S. b ssp*	*S. thalassinus*	Big Sage Reservoir, Modoc Co., CA.(PR[1])[13]
			Pit River, Modoc Co., CA. (PR[2])[14]
Summer Basin (SB)	*S. b. ssp*	*S. obesa*	Ana Reservoir, Lake Co., OR.(SB[1])[9]
			County Road 417, Lake Co., OR.(SB[2])[10]
Abert Basin (AB)	*S. b. oregonensis* (XL Spring and Chewacan River), *S. b. ssp*	*S. oregonensis*	XL Spring, Lake Co., OR.[7]
			Crooked Creek, Lake Co., OR.[8]
Alkali Basin (AlkB)	*S. b. ssp.*	*S. obesa*	Hutton Springs, Lake Co., OR.[6]
			3/8 Mile Spring, Lake Co., OR.[6]
Fort Rock Basin (FRB)	*S. b. ssp*	*S. sp.*	Thompson Reservoir, Lake Co., OR.[15]
Klamath Basin (KB)	*S. bicolor*	*S. bicolor*	Upper Klamath Lake, Klamath Co., OR.[17]
			Sycan Marsh, Klamath Co., OR.[16]

Table 2. Morphometric characters with descriptions of measurement taken.

Measurement	Description
Body Length (BL)	Tip of snout to end of notochord (preflexion/flexion) or edge upper hypural
Snout Length (SntL)	Tip of snout to anterior edge of the eye
Eye Diameter (ED)	Linear measurement from the anterior to the posterior edges of the eye
Head Length (HL)	Tip of the snout to the anterior edge of the cleithrum
Tip of the snout to edge of anus	Tip of the snout to the urogenital opening, posterior most edge
Body depth at Cleithrum	Depth from dorsal surface to ventral surface just behind the cleithrum
Body depth at Anus	Depth from dorsal surface to ventral surface just behind the anus
Body depth at Caudal	Least Depth from dorsal surface to ventral surface on the caudal peduncle
Anus to the Hypurals	Horizontal measurement from the urogenital opening to the posterior edge of the upper hypural

Table 3. Meristic characters, abbreviations and brief description of character.

Character	Description
Precaudal Vertebra (PCV)	Vertebra before the caudal peduncle
Caudal Vertebra (CV)	Vertebra in the caudal peduncle
Total Vertebra (TV)	Both caudal and precaudal vertebra
Dorsal Fin Origin over Vertebrae (DO)	Origin of dorsal fin and its alignment of the corresponding vertebra
Dorsal Fin Insertion over Vertebrae (DI)	Insertion of dorsal fin and its alignment of the corresponding vertebra
First Dorsal Fin Pterygiophore in front of vertebrae neural spine (FDFP)	The neural spine and corresponding vertebrae in which the first dorsal pterygiphore sit directly in front off.
Last Dorsal Fin Pterygiophore in front of vertebrae neural spine (LDFP)	The neural spine and corresponding vertebrae in which the last dorsal pterygiphore sit directly in front off.
Anal Fin Origin over Vertebrae (AO)	Origin of anal fin and its alignment of the corresponding vertebra
Anal Fin Insertion over Vertebrae (AI)	Insertion of anal fin and its alignment of the corresponding vertebra
First Anal Fin Pterygiophore in front of vertebrae hemal spine (FAFP)	The hemal spine and corresponding vertebrae in which the first dorsal pterygiphore sit directly in front off.
Last Anal Fin Pterygiophore in front of vertebrae hemal spine (LAFP)	The hemal spine and corresponding vertebrae in which the last dorsal pterygiphore sit directly in front off.
Predorsal bones (PDB)	Free floating bones that are posterior to the cleithrum and anterior of dorsal fin origin

Table 4. Allele frequencies for three nDNA loci by basin. KB=Upper Klamath Lake, PR[1]=Pit River Big Sage Reservoir, PR[2]= Pit River, WB= Warner Basin, SB[1]= Summer Basin Ana reservoir, SB[2]= Summer Basin Thousand Springs, AB=Abert Basin, AlkB= Alkali Basin, and MB=Malheur Basin. H_O= observed and H_E=expected heterozygosities, HWE= deviations from Hardy-Weinberg equalibrium R_A= allelic richness, N_A=number of alleles present, and N_S=number of individuals sampled.

Gbi-G13	KB	PR[1]	PR[2]	WB	SB[1]	SB[2]	AB	AlkB	MB
204	0.64	1	0.27	0.7	0.5	0.16	0.7	0.41	0.98
208	0.05	-	-	0.1	0.12	0.15	-	-	-
212	0.05	-	-	-	-	-	0.03	-	0.02
218	0.16	-	0.67	-	-	-	0.08	-	-
220	-	-	-	0.14	0.3	0.61	-	0.11	-
222	0.03	-	0.07	-	-	-	-	-	-
224	-	-	-	-	0.02	-	-	0.22	-
226	-	-	-	0.02	-	-	-	-	-
228	-	-	-	-	0.02	-	0.03	0.2	-
230	0.03	-	-	-	-	-	0.03	-	-
234	0.02	-	-	0.02	-	-	-	-	-
236	-	-	-	-	0.2	-	0.02	-	-
252	-	-	-	0.01	-	-	0.08	0.06	-
254	-	-	-	-	-	-	-	-	-
258	-	-	-	-	-	-	-	-	-
260	-	-	-	0.01	-	-	-	-	-
264	-	-	-	-	0.07	0.08	0.05	-	-
274	0.02	-	-	-	-	-	-	-	-
H_O	0.28	-	0.35	0.22	0.65	0.45	0.56	0.48	0.08
H_E	0.58	-	0.51	0.49	0.71	0.6	0.34	0.74	0.08
HWE	0	-	0.3	0	0.46	0.02	0.58	0.01	1
R_A	4.72	1	4.63	3.8	4.83	4.53	4.63	3.6	1.3
N_A	8	1	3	7	7	5	3	5	2
N_S	29	11	15	46	21	44	33	27	24

Table 4. Contiuned

Gbi-G79	KB	PR[1]	PR[2]	WB	SB[1]	SB[2]	AB	AlkB	MB
200	0.02	-	-	-	-	-	-	-	-
202	-	-	-	-	-	0.01	-	-	-
203	-	-	0.06	0.03	-	-	-	-	-
204	0.3	0.13	-	0.34	0.12	0.14	0.02	0.03	0.6
207	-	-	0.06	-	0.13	0.29	0.1	0.01	-
208	0.1	0.54	0.06	0.01	-	-	-	-	-
211	-	-	-	-	0.02	0.02	-	-	-
212	0.2	-	-	0.01	0.02	0.01	0.19	0.21	-
215	-	-	-	0.13	-	0.03	-	0.11	0.04
216	0.1	-	-	-	-	0.04	-	-	0.08
219	-	-	0.13	0.03	-	-	-	0.48	-
220	0.13	0.08	0.31	0.02	0.25	0.13	-	0.03	0.06
223	-	-	-	0.02	-	-	-	0.06	0.02
224	0.03	0.08	0.13	0.14	-	-	0.05	-	-
227	-	-	-	-	-	-	0.02	-	-
228	0.08	-	-	0.04	0.06	-	0.25	0.03	0.06
231	-	-	-	0.02	0.02	-	0.03	-	-
232	0.03	0.08	0.06	0.13	0.13	-	-	0.01	0.12
235	-	-	-	-	0.08	0.17	0.11	-	0.02
236	0.03	-	-	0.05	-	-	0.05	-	0.02
239	-	-	-	0.01	0.11	0.14	-	-	-
240	0.02	-	-	0.01	0.04	-	0.14	-	-
243	-	0.08	0.13	-	0.02	-	-	-	-
244	-	-	-	-	-	-	0.05	-	-
247	-	-	-	0.01	-	-	-	-	-
248	-	-	-	-	-	-	-	-	-
251	-	-	0.06	-	-	-	-	0.01	-
268	-	-	-	-	-	-	-	-	-
272	-	-	-	-	-	-	-	-	-
284	-	-	-	-	-	-	-	-	-
293	-	-	-	-	-	-	-	-	-
352	-	-	-	-	-	0.01	-	-	-
H_O	0.66	0.5	0.9	0.77	0.75	0.8	0.79	0.63	0.62
H_E	0.85	0.69	0.88	0.83	0.88	0.82	0.85	0.75	0.74
HWE	0.04	0.03	0.03	0.03	0.02	0.13	0.16	0	0.15
R_A	7.26	5.5	9	7.3	7.89	5.72	7.37	6.35	5.6
N_A	11	6	9	16	12	11	11	10	9
Ns	31	12	8	51	26	56	32	31	25

Table 4. Continued

Gbi-G87	KB	PR[1]	PR[2]	WB	SB[1]	SB[2]	AB	AlkB	MB
157	-	-	-	-	0.07	-	-	-	0.1
161	-	-	0.25	0.01	0.07	-	0.12	0.12	-
165	0.02	0.06	-	-	-	-	-	-	0.06
169	0.02	0.11	-	-	-	-	0.06	-	0.42
173	0.1	-	0.1	0.03	-	-	-	-	0.12
177	0.15	0.11	0.05	0.23	0.02	-	-	0.01	0.06
181	0.12	0.06	0.1	0.06	-	-	-	-	0.02
185	0.15	-	-	0.1	0.63	0.75	0.11	-	0.02
189	-	-	0.15	0.05	-	-	0.02	-	0.04
193	0.02	-	-	0.03	-	-	-	-	-
197	0.02	-	-	0.05	-	-	-	-	-
201	0.08	-	0.05	0.13	-	-	0.04	0.04	-
205	0.06	-	-	0.1	-	0.01	-	-	0.04
209	0.08	-	-	0.08	0.04	-	-	0.04	-
213	-	0.11	0.05	0.09	0.02	-	0.13	-	0.02
217	0.02	-	-	0.01	-	0.04	-	0.03	-
221	0.06	0.06	-	0.01	0.02	0.06	0.06	0.15	0.04
225	0.02	0.17	0.05	0.01	0.11	0.09	0.02	-	0.02
229	0.02	-	0.05	-	0.02	0.02	0.31	-	-
233	0.04	-	-	-	-	-	0.07	-	0.02
237	0.04	-	-	0.01	-	-	-	-	-
241	-	-	0.15	0.02	-	-	-	0.01	0.02
245	-	-	-	0.01	-	0.04	-	0.57	-
249	-	-	-	-	-	-	-	0.01	-
255	-	0.28	-	-	-	-	-	-	-
259	-	0.06	-	-	-	-	-	-	-
285	-	-	-	-	-	-	-	-	-
H_O	0.92	0.77	0.91	0.87	0.64	0.45	0.5	0.51	0.74
H_E	0.93	0.88	0.86	0.89	0.59	0.4	0.8	0.63	0.8
HWE	0.36	0.18	0.44	0.44	0.39	0.98	0	0.2	0.12
R_A	9.8	8.5	8.9	8.8	5.12	5.07	6.9	3.8	7.72
N_A	17	9	10	18	9	7	10	9	14
N_S	26	9	10	52	27	54	27	34	25

Table 5. F_{st} values for pairwise comparisons between tui chub populations from eight basins within Oregon using 3 microsatellite loci.

Basin	N	KB	PR[1]	PR[2]	WB	SB	AB	AlkB	MB
KB	31	0	0.10	0.16	0.03	0.18	0.06	0.16	0.10
PR[1]	12		0	0.21	0.11	0.29	0.14	0.26	0.18
PR[2]	15			0	0.17	0.21	0.16	0.21	0.31
WB	49				0	0.19	0.10	0.17	0.10
SB	64					0	0.21	0.26	0.31
AB	33						0	0.26	0.17
AlkB	30							0	0.30
MB	27								0

Table 6. Morphometrics of larvae from 10 populations of *Siphateles*, represented as a mean percentage of body length or head length ± standard deviation, with ranges in parentheses and superscript = sample size.

Measurement / Stage	Upper Klamath Lake (KB)	Sycan Marsh (KB)	Thompson Reservior (FRB)	Dog Creek (GB)	Ana Reservior (SB)
Body Length					
Preflexion	5.75±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	7.6±0.73 (6.6-8.5)[7]	8.6±1.2 (7.75-9.5)[2]	9.53±0.31 (9.1-9.9)[5]	n=0	9.52±0.11 (9.4-9.6)[2]
Postflexion	13.3±2.5 (9.1-18.5)[35]	14.1±2.2 (10.0-17.4)[28]	12.3±1.4 (25.2-28.0)[10]	11.14± .32(10.88-11.5)[3]	13.5±2.1 (9.88-17.75)[37]
Juvenile	20±0 (-)[1]	18.9±0.94 (18.0-20.4)[9]	n=0	n=0	18.5±0 (-)[1]
Head Length					
Preflexion	19.1±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	23.9±2.1 (20.9-26.8)[7]	23.9±2.1 (22.5-25.2)[2]	24.5±2.6 (21.9)[5]	n=0	24.5±0.34 (24.3-24.8)[2]
Postflexion	28.8±1.9 (25.0-32.1)[35]	28.7±1.9 (23.0-31.4)[28]	27.1±0.82 (25.2-28.0)[10]	29.1±0.7 (28.3-29.6)[3]	27.0±1.1 (24.5-29.5)[37]
Juvenile	31.5±0 (-)[1]	29.4±0.9 (27.8-30.5)[9]	n=0	n=0	28.3±0 (-)[1]
Snout Length					
Preflexion	11.8±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	11.9±3.0 (9.0-17.5)[7]	16.9±0.33 (16.6-17.1)[2]	14.5±1.7 (11.5-15.8)[5]	n=0	10.5±0.03 (10.5-10.6)[2]
Postflexion	16.7±1.5 (19.8)[35]	16.7±2.3 (14.6-21.7)[28]	15.9±1.2 (13.3-16.9)[10]	15.1±2.0 (12.9-17.0)[3]	16.0±1.9 (12.0-19.5)[37]
Juvenile	20±0 (-)[1]	18.0±1.3 (16.2-19.6)[9]	n=0	n=0	19.0±0 (-)[1]
Eye Diameter					
Preflexion	36.3±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	33.7±2.3 (31.5-37.5)[7]	33.8±0.67 (33.3-34.0)[2]	33.6±3.0 (30.7-37.5)[5]	n=0	34.3±3.7 (31.6-36.1)[2]
Postflexion	30.7±2.5 (24.2-34.0)[35]	31.2±1.6 (28.7-33.3)[28]	31.9±1.2 (30.5-33.5)[10]	28.9±1.9 (26.9-30.8)[3]	33.1±2.9 (29.4-34.7)[37]
Juvenile	31.7±0 (-)[1]	30.0±2.2 (27.2-34.0)[9]	n=0	n=0	18.5±0 (-)[1]

Table 6. Continued

Measurement / Stage	Upper Klamath Lake (KB)	Sycan Marsh (KB)	Thompson Reservior (FRB)	Dog Creek (GB)	Ana Reservior (SB)
Body depth at Caudal					
Preflexion	2.3±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	4.6±0.78 (3.8-5.9)[7]	6.38±0.1 (6.3-6.4)[2]	6.7±0.4 (6.3-7.2)[5]	n=0	6.51±0.73 (6.4-6.5)[2]
Postflexion	8.4±1.3 (6.3-10.8)[35]	9.7±1.5 (6.3-11.6)[28]	7.9±0.4 (7.2-8.5)	7.9±0.42 (7.5-8.3)[3]	7.8±1.0 (6.2-9.0)[37]
Juvenile	11.0±0 (-)[1]	10.3±0.46 (10.1-11.4)[9]	n=0	n=0	8.1±0 (-)[1]
Body Depth at Cleithrum					
Preflexion	10.9±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	15.8±1.1 (14.5-17.8)[7]	16.6±3.3 (14.1-18.9)[2]	17.6±1.5 (15.4-19.1)[5]	n=0	14.3±1.1 (14.3-14.4)[2]
Postflexion	20.6±1.5 (16.2-22.4)[35]	21.1±1.7 (19.6-23.3)[28]	19.4±0.93 (17.9-21.1)[10]	20.1±0.5 (19.5-20.5)[3]	19.6±1.2 (17.4-22.6)[37]
Juvenile	24.5±0 (-)[1]	21.4±0.75 (20.4-22.5)[9]	n=0	n=0	21.0±0 (-)[1]
Body Depth at Anus					
Preflexion	4.3±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	6.4±1.0 (5.5-8.2)[7]	8.6±1.2 (7.7-9.4)[2]	8.0±0.61 (7.0-8.5)[5]	n=0	7.75±8.4 (7.6-7.8)[2]
Postflexion	11.1±2.0 (8.0-15.0)[35]	12.8±2.4 (8.7-15.0)[28]	9.54±1.1 (8.1-10.5)[10]	10.4±0.47 (9.9-10.8)[3]	10.8±1.7 (7.8-13.5)[37]
Juvenile	15.5±0 (-)[1]	14.5±1.1 (12.6-15.9)[9]	n=0	n=0	13.5±0 (-)[1]
Snout to tip of Anus					
Preflexion	67.5±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	69.6±2.8 (65.0-74.5)[7]	72.2±3.6 (69.6-74.7)[2]	72.8±3.3 (69.2-76.9)[5]	n=0	68.5±2.9 (66.5-70.5)[2]
Postflexion	68.8±3.4 (64.0-73.0)[35]	71.1±1.9 (66.0-73.5)[28]	70.7±2.3 (67.8-75.4)[10]	72.0±3.0 (69.5-75.4)[3]	70.3±1.5 (68.2-73.4)[37]
Juvenile	69.0±0 (-)[1]	70.0-±1.5 (68.6-72.2)[9]	n=0	n=0	68.2±0 (-)[1]

Table 6. Continued

Measurement / Stage	Upper Klamath Lake (KB)	Sycan Marsh (KB)	Thompson Reservior (FRB)	Dog Creek (GB)	Ana Reservior (SB)
Anal Fin Origin to Edge Upper Hypural					
Preflexion	32.7±0 (-)[1]	n=0	n=0	n=0	n=0
Flexion	33.3±2.8 (29.4-37.1)[7]	30.1±6.7 (35.2-34.8)[2]	29.7±1.3 (28.7-31.8)[5]	n=0	32.0±0.35 (32.1-32.6)[2]
Postflexion	32.1±1.8 (27.4-35.2)[35]	30.1±1.6 (27.2-34.0)[28]	28.6±1.3 (27.1-31.5)[10]	31.5±0.86 (30.8-32.5)[3]	30.5±1.5 (27.7-34.5)[37]
Juvenile	32.5±0 (-)[1]	31.2±1.1 (29.5-33.3)[9]	n=0	n=0	34.5±0 (-)[1]

Measurement / Stage	Co-Rd 417 (SB)	Hutton Spring (AlkB)	Skull Creek (CB)	*S. alvordensis* (AB)	*S. boraxobious* (AB)
Standard or Notochord Length					
Preflexion	n=0	n=0	6.6±3.2 (6.25-6.9)[3]	n=0	n=0
Flexion	9.32±1.7 (9.2-9.44)[2]	8.4±1.3 (6.88-9.4)[4]	8.46±0.67 (7.4-9.4)[9]	7.25±0 (-)[1]	7.8±1.32 (6.88-8.75)[2]
Postflexion	12.03±1.5 (10.4-15.2)[8]	13.1±2.4 (11.0-17.75)[17]	13.7±2.3 (9.75-19.1)[29]	14.2±3.6 (8.75-17.75)[8]	12.2±1.5 (9.1-14.5)[10]
Juvenile	n=0	n=0	23.0±1.7 (21.9-25.0)[3]	20.1±1.5 (18.0-22.5)[7]	18.0±1.87 (15.4-21.25)[15]
Head Length					
Preflexion	n=0	n=0	15.8±1.4 (14.8-17.4)[3]	n=0	n=0
Flexion	27.6±0.7 (27.1-28.1)[2]	23.0±2.1 (20.0-23.9)[4]	22.0±1.4 (18.9-23.7)[9]	24.4±0 (-)[1]	25.4±2.4 (23.7-27.2)[2]
Postflexion	29.9±1.6 (26.9-31.3)[8]	26.9±1.1 (24.2-29.3)[17]	26.0±1.6 (21.5-27.9)[29]	25.9±2.4 (20.0-28.0)[8]	28.5±1.1 (27.2-30.7)[10]
Juvenile	n=0	n=0	27.8±0.9 (26.8-28.5)[3]	25.8±0.8 (24.6-27.2)[7]	29.7±1.1 (28.1-31.7)[15]
Snout Length					
Preflexion	n=0	n=0	12.6±1.6 (11.0-14.0)[3]	n=0	n=0
Flexion	14.3±1.5 (13.2-15.4)[2]	9.4±1.8 (7.0-11.1)[4]	10.1±1.6 (8.1-14.1)[9]	14.3±0 (-)[1]	20.2±4.8 (16.8-23.7)2
Postflexion	16.9±1.8 (14.4-19.3)[8]	13.6±2.4 (10.9-17.6)[17]	14.8±2.3 (10.4-19.5)[29]	17.3±2.6 (13.8-21.7)[8]	21.3±3.4 (18.1-25.2)[10]
Juvenile	n=0	n=0	18.7±2.1 (17.1-21.0)[3]	20.6±1.3 (17.9-22.0)[7]	20.4±1.4 (17.6-23.1)[15]

Table 6. Contiued

Measurement / Stage	Co-Rd 417 (SB)	Hutton Spring (AlkB)	Skull Creek (CB)	*S. alvordensis* (AB)	*S. boraxobious* (AB)
Eye Diameter					
Preflexion	n=0	n=0	34.0±4.3 (31.5-39.0)[3]	n=0	n=0
Flexion	30.4±1.1 (29.6-31.2)[2]	33.0±5.2 (27.5-38.1)[4]	36.9±4.6 (30.0-43.0)[9]	28.6±0 (-)[1]	34.0±4.4 (30.7-37.0)[2]
Postflexion	29.5±1.6 (26.8-32.5)[8]	33.9±2.2 (31.2-38.0)[17]	31.1±3.1 (26.8-40.0)[29]	25.7±2.6 (23.3-31.4)[8]	31.2±2.1 (27.5-35.7)[10]
Juvenile	n=0	n=0	26.5± 0.5 (26.1-27.0)[3]	24.8±0.4 (24.3-25.5)[7]	27.3±2.2 (23.1-31.3)[15]
Body Depth at Caudal					
Preflexion	n=0	n=0	4.1±0.3 (3.9-4.3)[3]	n=0	n=0
Flexion	6.6±0.5 (6.3-6.9)[2]	5.7±1.4 (3.6-6.7)[4]	5.2±0.77 (3.9-6.1)[9]	8.7±0 (-)[1]	5.4±2.5 (3.6-7.2)[2]
Postflexion	8.0±0.7 (7.1-9.1)[8]	7.6±1.6 (6.3-9.6)[17]	8.4±1.1 (5.9-9.9)[29]	9.55±.81 (8.5-10.6)[8]	9.0±1.1 (7.3-11.2)[10]
Juvenile	n=0	n=0	10.1±0.9 (9.1-11.0)[3]	10.3±.27 (9.9-10.6)[7]	9.6±0.5 (8.8-10.2)[15]
Body Depth at Cleithrum					
Preflexion	n=0	n=0	14.7±1.2 (13.6-16.0)[3]	n=0	n=0
Flexion	17.1±0.16 (16.9-17.1)[2]	15.6±2.2 (12.7-18.2)[4]	16.1±0.78 (14.6-17.0)[9]	19.0±0 (-)[1]	18.0±1.0 (17.4-18.6)[2]
Postflexion	20.9±1.3 (18.5-22.6)[8]	18.9±1.2 (17.1-21.8)[17]	19.5±1.5 (16.2-22.0)[29]	19.9±0.74 (19.1-21.1)[8]	20.0±0.9 (19.1-21.6)[10]
Juvenile	n=0	n=0	22.9±1.5 (16.2-22.0)[3]	19.4±0.7 (18.5-20.8)[7]	22.1±1.3 (19.7-24.3)[15]
Body Depth at Anus					
Preflexion	n=0	n=0	6.1±0.3 (6.0-6.4)[3]	n=0	n=0
Flexion	8.5±0.4 (8.2-8.8)[2]	7.3±1.2 (5.5-7.9)[4]	6.5±9.8 (4.1-7.4)[9]	6.0±0 (-)[1]	7.0±2.2 (5.5-8.5)[2]
Postflexion	11.2±1.3 (8.8-12.8)[8]	10.5±1.3 (7.5-12.3)[17]	11.1±1.9 (6.5-13.7)[29]	10.6±2.5 (6.8-13.7)[8]	10.7±3.3 (8.2-11.0)[10]
Juvenile	n=0	n=0	15.7-±1.7 (13.7-16.9)[3]	13.0±0.3 (12.5-13.3)[7]	13.6±1.0 (11.5-15.8)[15]

Table 6. Continued

Measurement / Stage	Co-Rd 417 (SB)	Hutton Spring (AlkB)	Skull Creek (CB)	*S. alvordensis* (AB)	*S. boraxobious* (AB)
Flexion	75.6±1.0 (75.0-76.2)[2]	70.1±1.5 (66.8-71.8)[4]	69.1±2.5 (65.8-73.4)[9]	70.8±0 (-)[1]	69.6±0.6 (69.2-70.1)[2]
Postflexion	70.0±3.0 (64.0-77.1)[8]	69.3±1.8 (66.4-70.9)[17]	68.4±2.2 (61.6-74.6)[29]	66.4±3.8 (62.9-74.3)[8]	68.5±1.4 (65.5-69.8)[10]
Juvenile	n=0	n=0	68.6±0.2 (68.4-68.9)[3]	63.7±1.5 (61.6-66.3)[7]	67.2±1.9 (65.1-72.4)[15]
Anal Fin Origin to Edge Upper Hypural					
Preflexion	n=0	n=0	32.4±2.2 (30.4-35.0)[3]	n=0	n=0
Flexion	31.6±2.7 (29.6-31.5)[2]	30.5±1.3 (29.2-32.5)[4]	31.6±1.5 (28.6-33.7)[9]	32.3±0 (-)[1]	30.2±1.6 (29.1-31.4)[2]
Postflexion	33.0±1.9 (30.2-34.6)[8]	31.1±1.7 (28.5-34.8)[17]	31.2±1.5 (27.1-33.5)[29]	31.1±0.8 (30.1-32.5)[8]	31.6±1.1 (30.4-33.3)[10]
Juvenile	n=0	n=0	32.9±0.8 (32.4-33.9)[3]	32.9±1.1 (31.3-34.3)[7]	34.5±1.4 (31.0-36.5)[15]

Table 7. Variable loading for principle component one of meristic characters for *Siphateles* species groups.

Meristic Character	Principle Component 1
Pre-caudal vertebrae	0.354
Caudal vertebrae	0.110
Total vertebrae	0.333
Dorsal fin origin over vertebrae number	0.203
Dorsal fin insertion over vertebrae number	0.328
Anal fin origin over vertebrae number	0.308
Anal fin insertion over vertebrae number	0.354
Dorsal fin pterygiphore in-front of neural spine number	0.197
Last dorsal fin pterygiphore in front of neural spine number	0.307
Anal fin pterygiphore in front of hemal spine number	0.307
Last anal fin pterygiphore in front of hemal spine number	0.350
% Total Variance	58.6%
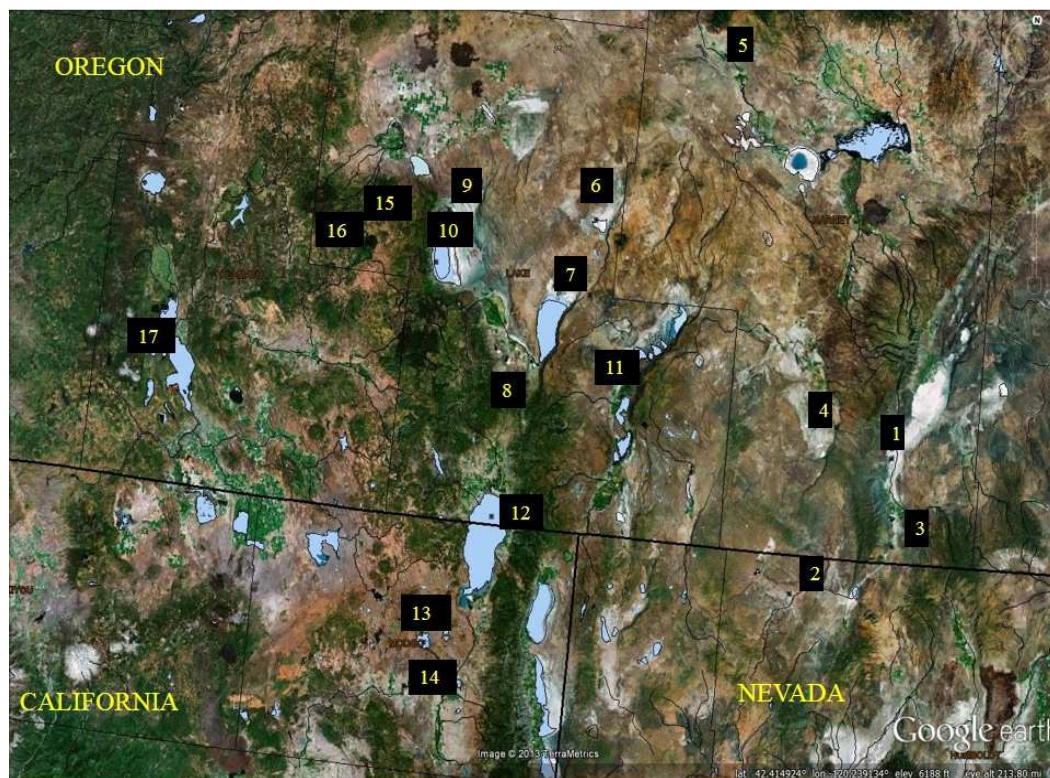
Figure 1. Map of sample location, see Table 1 for sample location code.
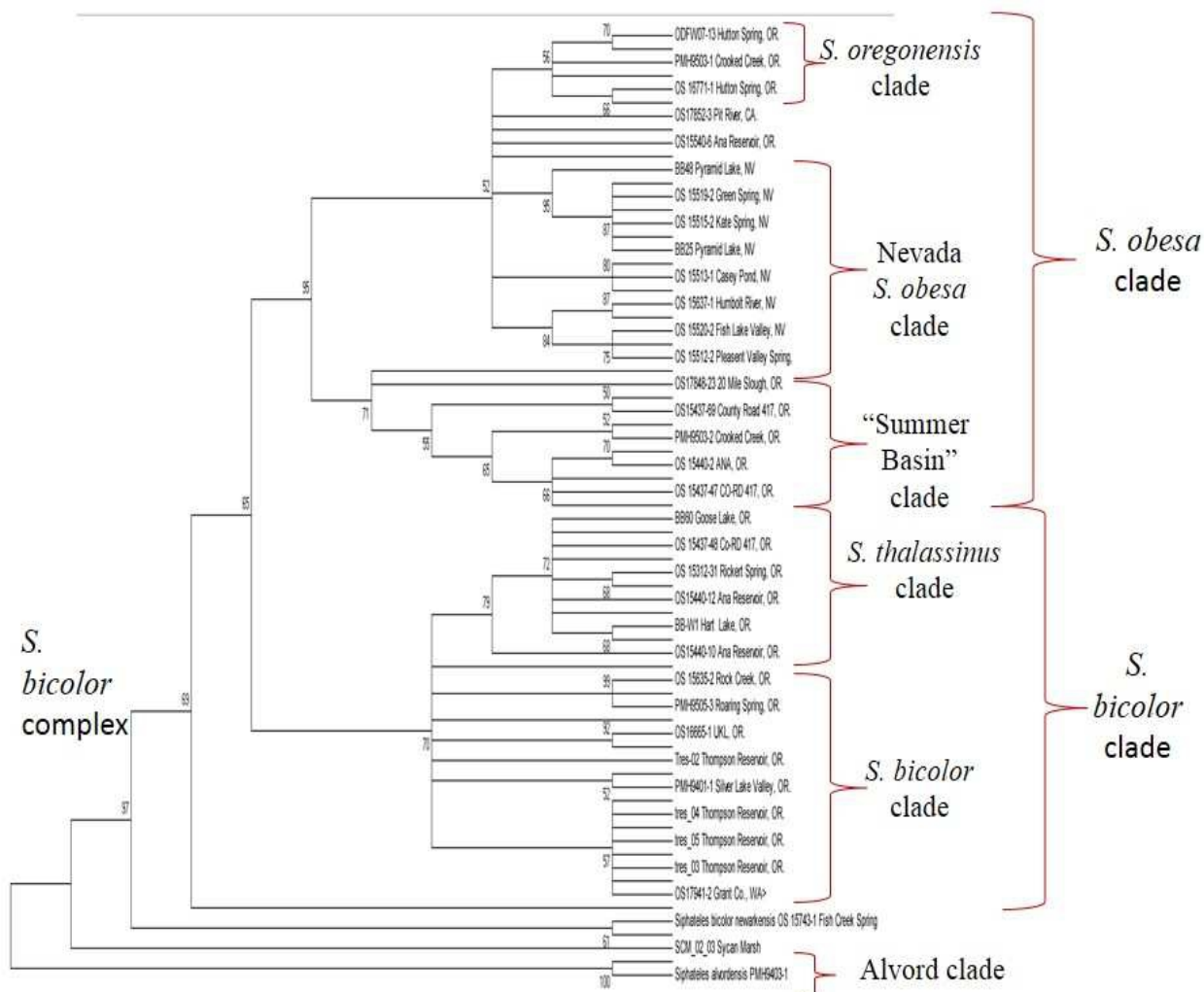
Figure 2. Neighbor-Joining dendogram based on mtDNA cytochrome *b* sequences for *Siphateles*. Numbers before nodes are bootstrap values that indicated 50 % or greater bootstrap support.
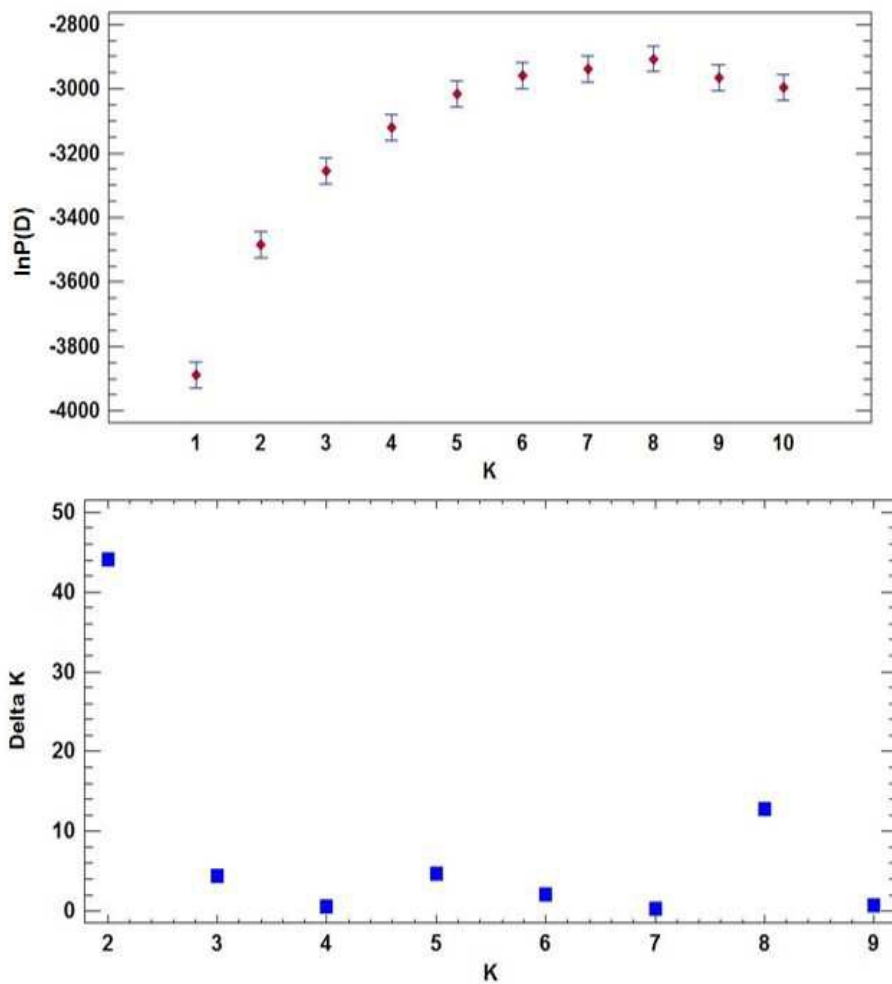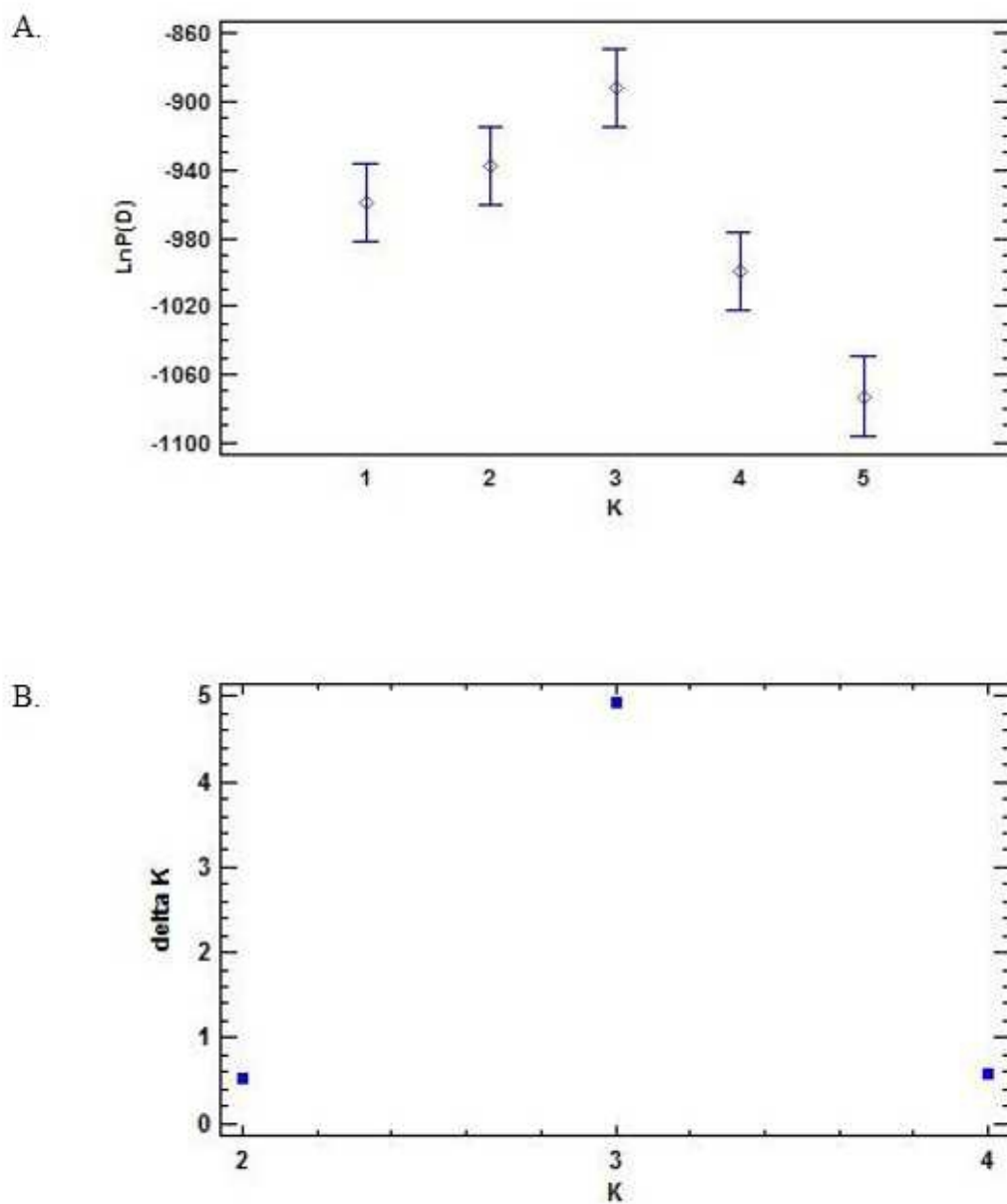
Figure 3. Estimated untransformed log likelihood probability (lnP(D)) of genetic groups at different runs of *K* (1 – 10). Each *K* was run at 10 iterations and each assigned a LnP(D) value and variance. B) Results of Δ*K* analysis, which give the rate of change between *K* and *K* + 1.
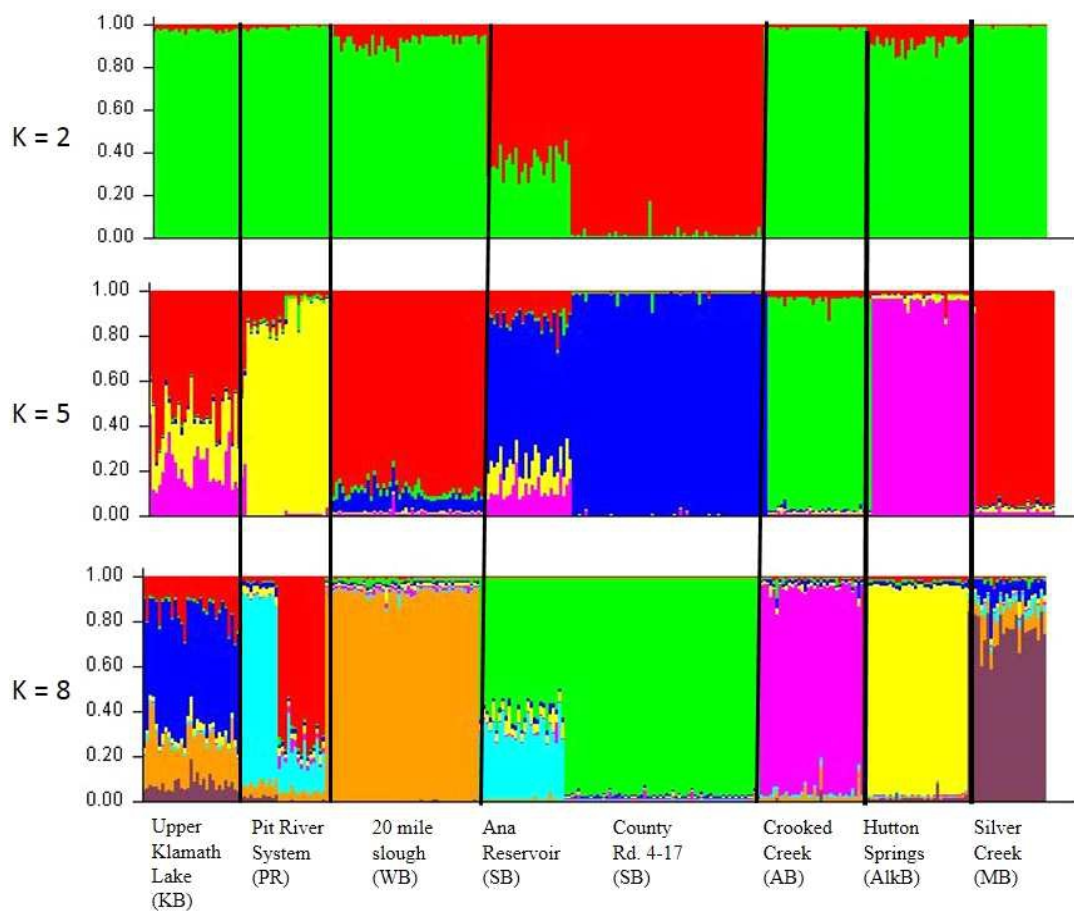
Figure 4. Proportion membership of tui chub populations based on Bayesian clustering of individuals at *K* and Δ*K* for 2, 5, and 8. Vertical bars indicate an individual's probability of membership to a population.

Figure 5. Estimated untransformed log likelihood probability (lnP(D)) of genetic groups at different runs of K (1 – 5). Each K was run at 10 iterations and each assigned a LnP(D) value and variance. B) Results of ΔK analysis, which give the rate of change between K and $K + 1$.
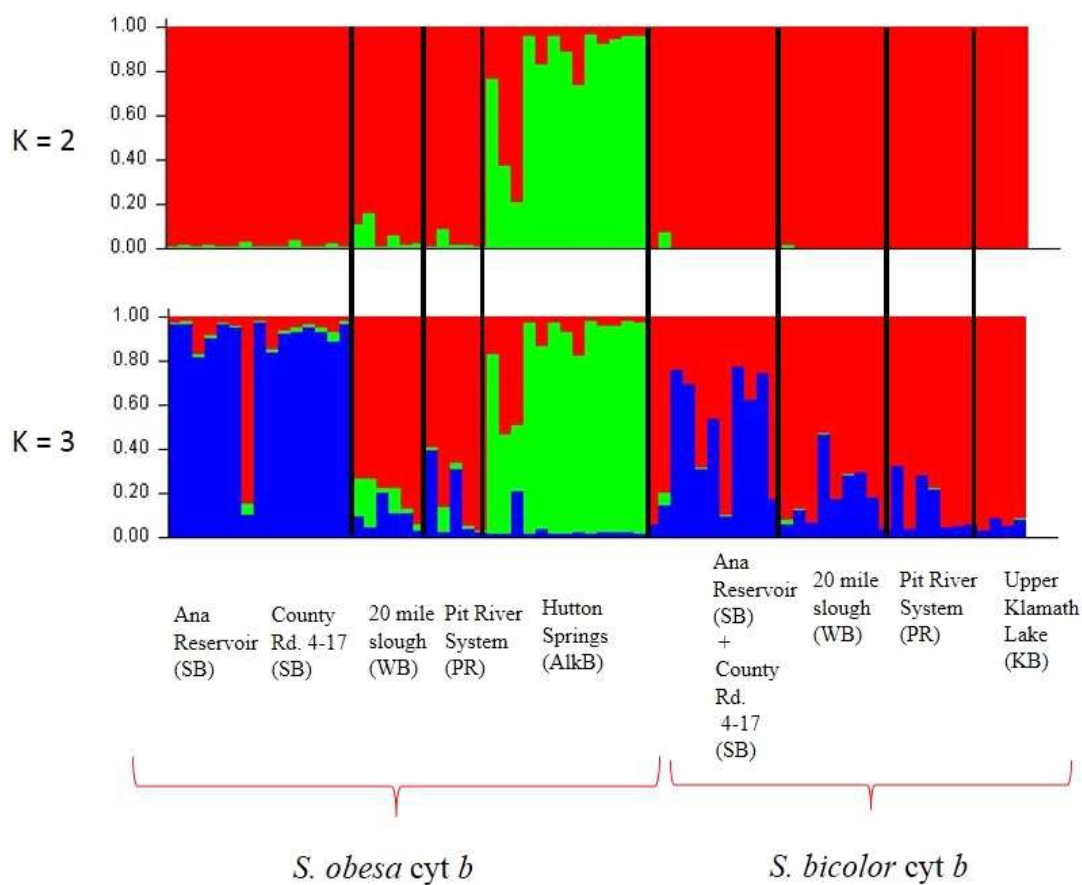
Figure 6. Bayesian clustering of tui chub based on an individual's cytochrome *b* haplotype for *K* and Δ*K* at 2 and 3. Vertical bars indicate an individual's probability of membership to a cytochrome *b* haplotype.
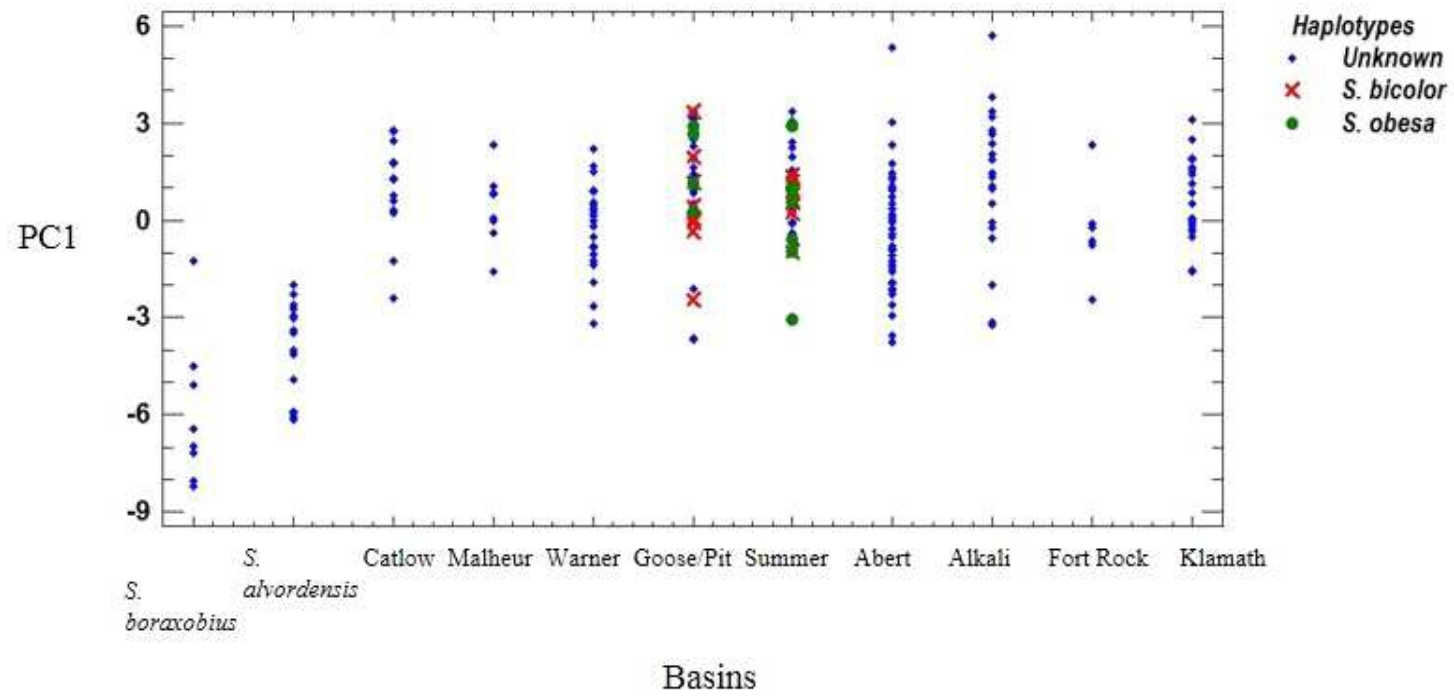
Figure 7. Principle component analysis of 11 meristic adult characters with individual cytochrome *b* haplotypes. See Table 8 for variable loading.

Figure 8. Larvae of *Siphateles alvordensis* A. 7.2 mm BL (OS06924) B. 15.0 mm BL (OS06924) lateral view C. 15.0 mm BL (OS06924) dorsal view.

Figure 9. Larvae of *Siphateles boraxobius* A. 6.9 mm BL (OS17841) B. 18.9 mm BL (OS17841) lateral view C. 18.9 mm BL (OS17841) dorsal view.

Figure 10. Larvae of *Siphateles* from Skull Creek (CB) A. 8.7 mm BL (O17775) lateral view B. 8.7 mm (OS17775) dorsal view C.15.8 mm BL (O17838) lateral view d. 15.8 mm BL (O17838) dorsal view.
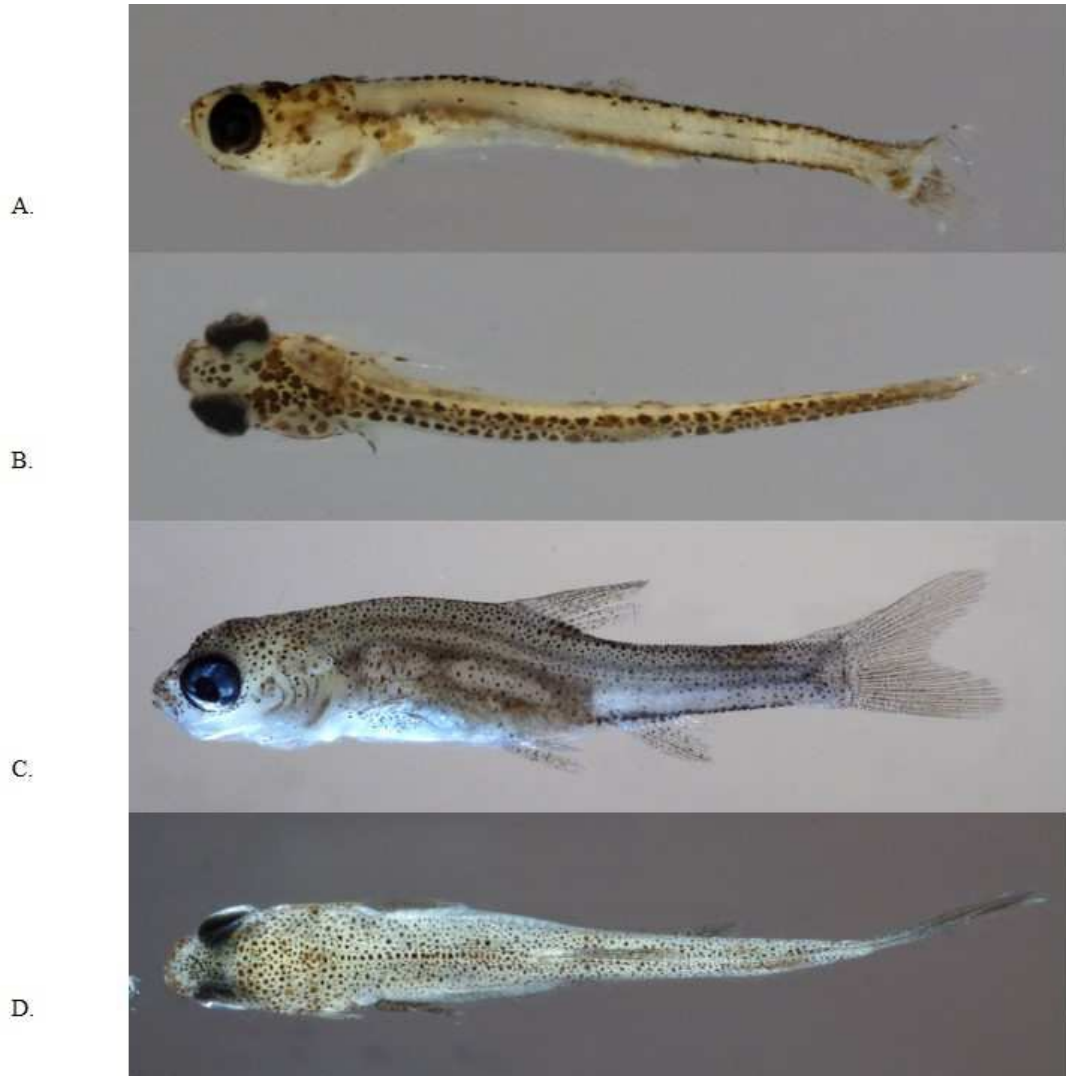
Figure 11. Larvae of *Siphateles* from Hutton Spring (AlkB) A. 8.1 mm BL (OS17924) lateral view B. 8.1 mm BL (OS17924) dorsal view C. 17.75 mm BL (OS17918) lateral view D. 17.75 mm BL (OS17918) dorsal view.

Figure 12. Larvae of *Siphateles* from County Road 417 (SB) A. 9.6 mm BL (OS18000) lateral view B. 9.6 mm BL (OS18000) dorsal view C. 12.5 mm BL (OS18000) lateral view D. 12.5 mm BL (OS18000) dorsal view.

Figure 13. Larvae of *Siphateles* from Ana Reservoir (SB) A. 8.5 mm BL (OS17935) lateral view B. 8.5 mm BL (OS17935) dorsal view C. 14.5 mm BL (OS17839) lateral view D. 14.5 mm BL (OS17839) dorsal view.

Figure 14. Larvae of *Siphateles* from Dog Creek (GL) A. 11.5 mm BL (DMFDC01) lateral view B. (DMFDC01) dorsal view.
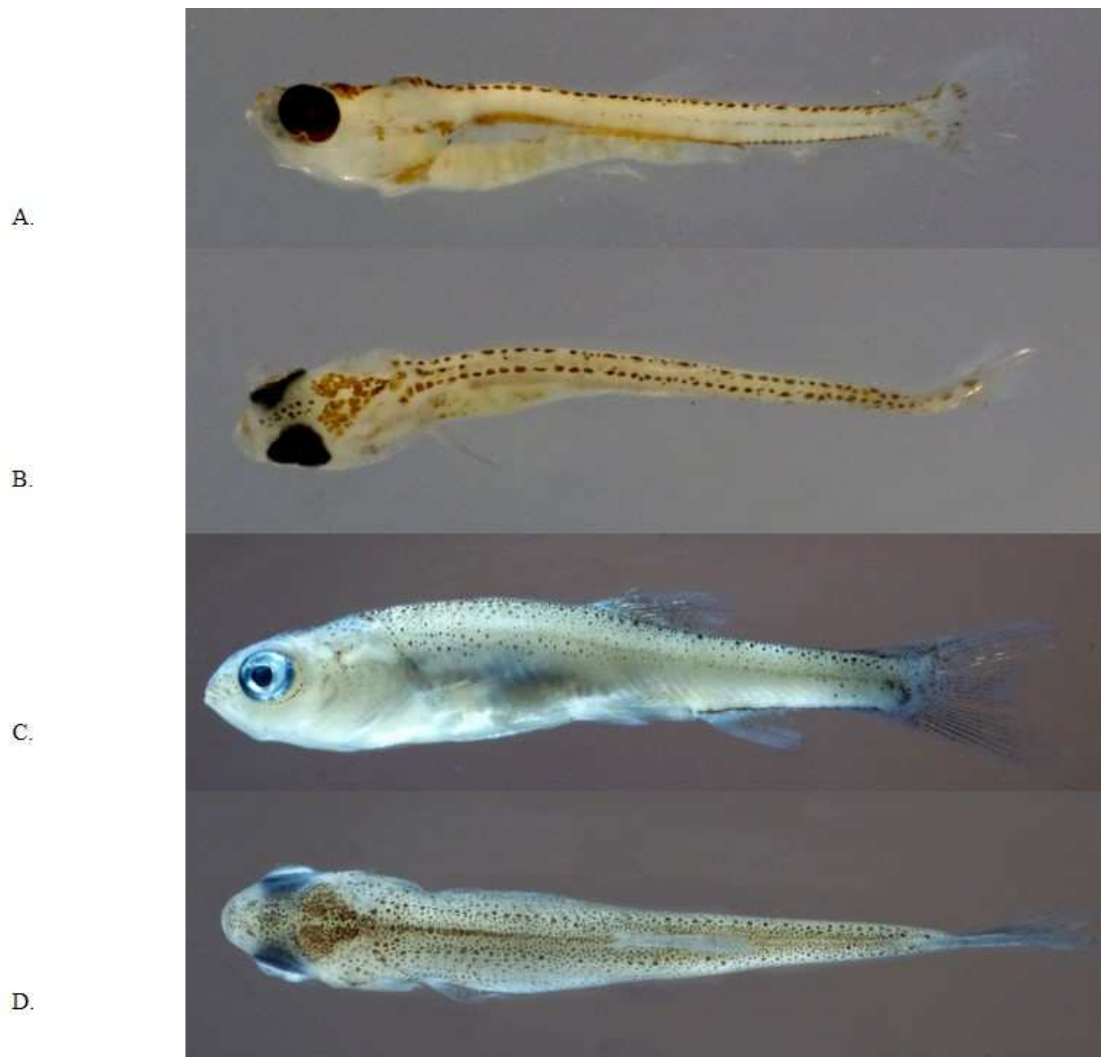
Figure 15. Larvae of *Siphateles* from Thompson Reservoir (FRB) A. 7.9 mm BL (OS17920) lateral view B. 7.9 mm BL (OS17920) dorsal view C. 15.2 mm BL (OS17919) lateral view D. 15.2 mm BL (OS17919) dorsal view.

Figure 16. Larvae of *Siphateles* from Sycan Marsh (KB) A. 7.9 mm BL (OS17840) lateral view B. 7.9 mm BL (OS17840) dorsal view C. 15.2 mm BL (OS17933) lateral view D. 15.2 mm BL (OS17933) dorsal view.

Figure 17. Larvae of *Siphateles bicolor* from Upper Klamath Basin (KB) A. 8.4 mm BL (A09286) lateral view B. 8.4 mm BL (A09286) dorsal view C. 14.0 mm BL (A09318) lateral view D. 14.0 mm BL (A09318) dorsal view.