Final Report

Title: **Genetic Purity and Subspecific Status of the Mohave Tui Chub**

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Abstract

The Mohave tui chub (*Gila bicolor mohavensis*) is listed by both the state of California and the federal government as an endangered species. It exists in only a few refugial populations. Concern has been raised about (1) possible past introgression with genes from the arroyo chub (*G. orcutti*) and (2) the subspecific status of the Mohave tui chub relative to the Owens tui chub (*G. b. snyderi*) and the Lahontan tui chubs (*G. b. obesa and G. b. pectinifer*). We examined and quantified variation for 34 allozyme loci and 51 amplified fragment length polymorphisms (AFLPs) in the above taxa as well as in the blue chub (*G. coerulea*) and the Klamath tui chub (*G. b. bicolor*). We analyzed the allozyme and AFLP data sets separately. The arroyo and Mohave tui chubs were fixed for alternate alleles at 10 allozyme loci and the arroyo chubs displayed 15 AFLP bands that were not present in any of the Mohave tui chubs examined. The lack of any of the arroyo specific nuclear alleles in the Mohave tui chub supports the view that the remaining populations of Mohave tui chub are pure, uncontaminated by arroyo chub genes. Secondly, we found one Mohave specific allozyme marker (Gam) that was not found in any of the other chub taxa, justifying the distinctness of the Mohave tui chub subspecies. All populations clustered with their respective co-taxonomic members when examined for genetic distance. The Mohave tui chub clustered most closely to the Lahontan tui chub for both allozyme and AFLP data sets, but at a similar genetic distance to the clustering of all tui chub subspecies (Mohave, Owens, Lahontan, and Klamath). The blue and the arroyo chubs clustered first with each other and then with the tui chub complex. These data support the view that the Mohave tui chub is a distinct evolutionary lineage and should be regarded as a separate subspecies from Klamath, Owens, and Lahontan tui chub subspecies. The Mohave tui chub should continue to receive protection as an endangered evolutionarily significant unit (ESU). Additional genetic work should be done on the other tui

chub subspecies, especially those whose taxonomic status is uncertain (*e.g.* the lake and creek forms of the Lahontan tui chub) or where possible introgression is presumed (*e.g.*, between the Owens and Lahontan tui chubs). Such a study will be crucial for the protection of any remaining natural populations of the endangered Owens tui chub.

Introduction

The Mohave tui chub is native to the Mojave River basin (Mohave Tui Chub Advisory Committee 1988). In the 1930's, the arroyo chub was introduced to the Mojave River by fishermen and has been known to hybridize with the Mohave tui chub. Hybridization with the Arroyo chub resulted in the loss of genetically pure Mohave tui chub throughout its native habitat. By 1970, the only population of what is believed to be genetically pure Mohave tui chub existed in a refugial site at Soda Springs, CA. This population is believed to have been established in this site, known as MC Spring, as the result of flooding by the Mojave River that filled Soda Lake in 1916 and 1938. However, the possibility also exists that the population may have been derived from introduced stock. A recovery plan was implemented in 1971 establishing refugia for the Mohave chub. Records indicate that the Mohave tui chub in MC Spring have been used to establish other refugial populations over the years. The extant refugial Mohave tui chub populations are located at Soda Springs (Lake Tuendae and MC Springs), the Naval Air Weapons Station at China Lake (introduced in 1971), Camp Cady Wildlife Area (2 ponds), and Barstow Desert Information Center. Regardless of whether the Mohave tui chub found in MC Spring were established as a result of a flood event or were human introduced, questions exist about whether the remaining Mohave tui chub are genetically pure or have been introgressed with genes from the arroyo chub.

The Mohave tui chub has gone through several nomenclatural changes since it was originally collected. The most recent change reclassified the Mohave tui chub to subspecific status, *Gila bicolor mohavensis*, because no morphological characters could be found to specifically separate it from all populations of *G. bicolor* in the Lahontan Basin (Miller 1973). Morphologically, the Mohave tui chub is similar to the Owens tui chub and the Lahontan tui chub. The Mohave tui chub, Owens tui chub, and Lahontan tui chub have been considered separate subspecies based on small morphological differences. Evidence exists that the Lahontan and Mojave basin drainages may have been connected during the Pleistocene era. The possibility that the drainages were connected in the past in addition to the morphological similarities of the Mohave tui chub, Owens tui chub and Lahontan tui chub raises the question of whether or not they are separate subspecies. Molecular genetic characters of the Mohave tui chub have never been compared with those of the Owens tui chub and the Lahontan tui chub to verify that they are separate subspecies.

Currently, the Mohave tui chub and the Owens tui chub are State and Federally listed as endangered, requiring special management, while the Lahontan tui chub is not listed. The arroyo chub is an introduced species that does not require special management. If the refugial populations of the Mohave tui chub are not genetically pure, then the Mohave tui chub may not require management as an endangered species. Alternately, if the Mohave tui chub is found to be genetically identical to the Owens or Lahontan tui chub, changes to the regulatory requirements for the Mohave tui chub might have to be considered.

We conducted an examination of allozyme variation and amplified fragment length polymorphisms (AFLPs) to determine (1) the genetic purity of the refugial populations of the Mohave tui chub and (2) its distinctness from Owens and Lahontan tui chubs.

Materials and Methods

- ***Permits***

Permits to take the endangered Mohave and Owens tui chubs were acquired from the US Fish and Wildlife Service (PRT-829201), the California Department of Fish and Game (8011-01-04), and the National Park Service (NRSP-0021-97).

- ***Sampling***

The populations sampled (see Table 1) included: refugial populations of the Owens tui chub at Little Hot Spring Pond (part of this sample was unfortunately taken from just below the pool before we knew Lahontan tui chub might be present in Little Hot Creek), Mule Spring, and Owens Gorge; Mohave tui chub at China Lake, Lake Tuendae, and Camp Cady Wildlife Area; Lahontan tui chub from Independence Lake and the E. Walker creek (tributary to Mono Lake); arroyo chub from three locations in the San Louis Rey and San Margarita River drainages, putative hybridized Mohave X arroyo chub from the Mojave River drainage and putative hybridized Owens X Lahontan tui chub from Hot Creek (to be examined in a later study). Additional samples of Klamath tui chub and blue chub were collected for comparative purposes. Whole fish were sacrificed, placed on dry ice and returned to the laboratory for allozyme and DNA analyses. Fin samples alone were taken from additional fish and dried or placed in 95% ethanol for examinations of DNA variation.

- ***Allozymes***

Muscle, liver, heart, and eye tissue samples were analyzed for allozyme variability for 45 enzymes with enzyme/tissue/gel buffer combinations chosen based on work in other fish species

(May 1992). Horizontal starch gel and histochemical methods followed those outlined in May (1992). During the initial phase we examined three fish from each Mohave, Owens, Lahontan, blue, and Klamath population; four fish from Little Hot Spring Pond; six fish from the putative hybridized Mohave x arroyo; and one fish from each of the arroyo populations for a total of 40 fish. Subsequently, an additional three fish from each of the three Mohave, the three Owens, and the two Lahontan populations were analyzed for Gam and Ck-1 variation.

- ***AFLPs***

The second method used in this study to evaluate the amount of differentiation between populations was Amplified Fragment Length Polymorphism (AFLP; Vos *et al.* 1995). The AFLP process uses a restriction endonuclease to cut the nuclear genome at specific recognition sequences. An adapter sequence (~15 bp) containing part of the restriction site is ligated onto each of the cut sequences. Finally, specific primers composed of the adapter sequence plus one to four bp are used to amplify this digested/ligated mix of genomic DNA. We used the *Pst* I restriction endonuclease (restriction recognition sequence is 5'–CTGCAG-3') and primers with four base extensions.

➢ Genomic DNA Extraction

Nuclear DNA was extracted from gill filaments of the whole fishes frozen for allozyme analysis using the CTAB phenol/chloroform protocol of Saghai-Maroof *et al.* (1984) and Doyle and Doyle (1987) as modified by Grewe *et al.* (1993). A 0.5cm length of gill filament was sterilely cut into strips and placed in a 1.5ml microfuge tube containing 200µl of CTAB buffer (50mM Tris, 10mM EDTA, 0.7M NaCl, 1% CTAB, 0.1% β-mercaptoethanol, pH 8.0). The tissue was ground with a sterile pestle and rinsed twice with 250µl of hot CTAB buffer into the tube.

Thirty µl of proteinase K (10µg/ml) was added, and the tubes were inverted to mix followed by incubation overnight at 70°C. An equal volume of PCI (Phenol: Chloroform: Isoamyl alcohol; 25:24:1) was added to the microfuge tube. The tubes were agitated for two minutes, then spun at max. speed for 10 minutes. The aqueous phase was decanted to a new microfuge tube avoiding contamination with any PCI or inter-phasic precipitate. An equal volume of CI (Chloroform: Isoamyl alcohol; 24:1) was added to the decanted aqueous phase. The tube was agitated for 2 min., and then spun at max. speed for 10min. The aqueous layer was decanted to a new tube, then the DNA was precipitated by adding 0.1 times the volume of 3M sodium acetate pH 8.0 followed by 2 times the volume of cold 20% isopropanol. The DNA was incubated at -80°C for 1hr., then spun at max. speed for 15 min. The supernatant was poured off and the pellet washed with 200µl of 70% ethanol. The tubes were spun for 5min., the ethanol was decanted, and the pellet was allowed to dry. The DNA pellet was resuspended in 100µl of 10mM Tris-Cl pH 8.0.

- **Digest-Ligation**

Two µg of genomic DNA were digested for 4 hr. at 37°C in a 30µl reaction containing 5U *Pst* I , 5 U T4 DNA ligase, 1X NEBuffer 3 (50mM Tris-Cl, 10mM $MgCl_2$, 100mM NaCl, 1mM DTT, pH 7.9), 1mM ATP, 100pmol *Pst* I adapter, then incubated overnight at room temperature. The digested-ligated DNA was diluted 1:5 with TE (10mM Tris-Cl; 0.1mM EDTA; pH 8.0) and stored at -20°C

- **AFLP amplification**

Five µl of diluted digest-ligation product was used as a template for a 25µl PCR reaction containing 1.5mM $MgCl_2$, 200µM of each dNTP, 1nmol of primer, 1X Gibco PCR Buffer (20mM Tris-Cl pH 8.0, 50mM KCl), and 0.3U Gibco Taq polymerase. DNA was amplified in an MJ Research PTC-100 thermocycler using an initial 1.5 min. denaturing step at 94°C followed by 12

cycles of 30 sec. at 94°C, 30 sec. at 65°C (0.7°C lower each cycle), and 2.5 min. at 72°C. The final 24 cycles were: 30 sec. at 90°C, 30 sec. at 56°C, and 2.5 min. at 72°C.

➢ Electrophoresis

Twenty-five µl of loading buffer (98% formamide, 10mM EDTA, 0.1% each: xylene cyanol and bromophenol blue) were added to the PCR product, which was then denatured for 5 min. at 95°C followed by immediately cooling on ice. Six µl of sample was loaded on a 3.5% polyacrylamide-7.5M Urea gel, and run at 45W until the xylene cyanol band was 2/3 of the way down the gel. The plates were separated and stained with Vistra Green DNA stain (Amersham). The gels were scanned on a Molecular Dynamics Fluorimager 595, and visualized with MD's FragmeNT analysis software.

Initial tests with three and four base extensions on the *Pst* I primers showed more clarity of banding for the four base extensions. We tried *Pst* I -TGAG, *Pst* I -CTGG, *Pst* I -CTCA, and *Pst* I -TGAT for 22 individuals. *Pst* I -TGAT was excluded from further analysis because very few bands showed on the gel. Forty-nine individuals were tested for the first three primers (see Table 5).

- ***Data Analysis***

Variability for both allozymes and AFLPs was scored for each of the taxa listed in Table 1 with the exception of the samples from Hot Creek (which was assumed to be a hybridized population of Owens and Lahontan tui chub) and Little Hot Creek Pond (because it was inadvertently sampled below the pond as well where hybridized tui chub might occur). Allozyme banding patterns were scored by attributing the variation to single Mendelian loci. AFLP gels were scored as presence or absence of particular bands. Each band was assumed to be a discrete

locus and assigned the nomenclature "*Pst* I -TGAG-670", which stands for "restriction enzyme-base extension-approximate size in bp"; individuals with the band were scored as homozygous "11" and individuals without the band as "22". If there was any doubt, the individual was not scored at that locus. Presumably some individuals with a band that were scored as 11 might be 12s. Allozyme and AFLP data sets were analyzed separately with "Genes in Populations", a computer program designed by B. May and C.C. Krueger and written in C by W. Eng and E. Paul. The UPGMA cluster algorithm was used to diagram the genetic relationships of the samples based on Nei genetic distance values.

Results

- ***Allozymes***

Variation was scored at 34 presumptive gene loci (see Table 2), 13 of which were monomorphic (Adh, Ald, Fum, Idh-1, Ldh-2, Mdh-2,3, Pp-1,2, Pgd, Pro-1,2, and Tpi). The results for the 21 polymorphic loci appear in Table 3. Mohave tui chub were fixed for allele 2 at Gam, while all other samples were fixed for allele 1. Arroyo chubs and Mojave tui chubs were fixed for alternate alleles at Gpi-2, Gam, Ldh-1, Aat-1, Aat-2, Ac-1, Idh-2, Sod, Sdh, and Pep-lgg-2. The three Mohave populations and the Owens population at Mule Springs had neglible variation ((Hs) = 0.000 to 0.008). All other populations had estimated heterozygosities of 0.031 to 078.

The allele frequency data were used to obtain Nei and Rogers genetic distance values (Table 3) and the Nei distances were combined with the UPGMA clustering algorithm to arrive at the dendrogram that appears in Fig. 4. In Fig. 4, all populations of a particular taxa cluster first, *e.g.*, all of the Owens, Mohave, or Lahontan samples. Note also that the arroyo from the coastal populations (here pooled as a single population) cluster tightly with the unknown sample from the

Mojave River These latter fish are clearly arroyo chub based on their allozyme genotypes. Subsequently, the Mohave combine with the Lahontan and then this cluster merges with the Owens and Klamath. The blue and arroyo chubs combine and then merge with the entire tui chub complex. These data are very consistent with the status of the Mohave as a subspecies of tui chub.

- ***AFLPs***

Primers *Pst* I-TGAG (Fig. 1), *Pst* I-CTCG (Fig. 2), and *Pst* I -CTCA (Fig. 3) were analyzed for variation among 49 individuals. The results appear in Table 5 where an allele designated 1 indicates presence of band and 2 indicates the absence of the band. Most taxa were fixed for displaying a band or not displaying a band, although several of the taxa were variable for expression of particular bands. Since limited data have been gathered on the inheritance of AFLP bands we are reluctant to speculate on the levels of this apparent intraspecfic variation. The arroyo chubs had the following bands that were not found in any of the Mohave samples: *Pst* I–CTCG-740, 675, 637, 592, 570, 532, and 510; CTCA-483 and 437; and TGAG-700, 646, 631, 617, 524, and 476. Further the Mohave had *Pst* I-CTCG-370 that was not found in any Lahontan or Owens samples.

Nei and Rogers genetic distance values appear in Table 6. The Nei values were combined with the UPGMA clustering algorithm to arrive at the dendrogram in Fig. 5. Overall the genetic distance values are about twice as high for the AFLP data than for the allozyme data. However, the same relationships among the taxa are shown in the AFLP dendrogram as found with the allozyme data set. All of the populations of a particular taxa cluster together. Again the Mohave populations are most similar to the Lahontan. The Owens, Klamath, and Mohave/Lahontan

groups cluster at similar levels. Then the blue and arroyo chubs cluster and finally this cluster merges with all of the tui chubs.

An interesting result is that the overall F_{ST} value for AFLPs was 0.862 and for allozymes was 0.837. This finding illustrates the comparable nature of these two data sets in assessing the partitioning of variation among the various taxa.

Discussion

- ***Genetic Purity of Mohave Tui Chub***

Both allozymes and AFLPs have answered the two questions that motivated this project. Have the remaining populations of Mohave tui chub been compromised by introgression with the arroyo chub? Since none of the arroyo specific nuclear markers were shown in any of the Mohave refugial samples, these Mohave populations should be considered genetically pure. As an aside, the samples of chub we took from the Mojave River near Victorville appear to be "pure" arroyo chub. Whether any Mohave chub exist elsewhere in the Mojave River remains unknown.

Is the Mohave tui chub a distinct taxon from the Owens or Lahontan tui chubs? Both the allozyme and AFLP data sets clearly show differentiation of the Mohave tui chubs from the Owens and the Lahontan. The closest association is between the Mohave and the Lahontan groups. Overall the genetic distances suggest that the Mohave tui chub are a distinct lineage and should be considered a subspecies of tui chub. The allozyme data suggest that the Mohave tui chub possess limited intraspecific variation. This finding may be a result of the derivation of all remaining Mohave individuals from a single, small population at Soda Springs. At this point we are reluctant to attach meaning to the variability in expression of AFLP bands as a reflection of level of variation within the Mohave tui chubs.

- ***Allozymes vs. AFLPs***

Allozymes have a rich history of use to assess genetic variation within and among populations and higher level taxa (Murphy *et al.* 1996). We routinely assign genotypes to individual banding patterns, attributing the variation to single discrete loci even when multiple loci code for the same enzyme. With about twice the effort we could have gained an approximately 50% increase in the number of loci, with a concordant increase in data resolving ability. Unfortunately, that would be nearly the maximum amount of information that could be gained from an allozyme analysis. This limit is coupled with the need to sacrifice individuals to gain these data.

The AFLPs used in this study have shown themselves to be a robust method of detecting nuclear genomic variability. With AFLPs each band is treated as a single locus and genotypes are assigned as homozygous for the band or alternately homozygous for not having the band. Many loci are represented on a single gel. While the assumption of homology of bands of similar mobility is probably reasonably accurate, some homology (the same locus) between bands of different mobility is lost. The net effect will be to somewhat inflate the degree of difference between two individuals (taxa). Further, we score a band as being present no matter how bright it is. A band may be very bright in one taxa and not nearly so bright in another, yet both taxa would be scored as possessing this band. Clearly some population variability exists in the AFLP data set. Some individuals will show a band and others will not. As mentioned above, we are reluctant to attach meaning to the intraspecific variation in expression of AFLP bands observed for Mohave samples. The two biggest advantages of AFLPs over allozymes are that (1) the organism to be examined does not have to be sacrificed for AFLPs and (2) there is a much greater number of loci that can be examined. With the same *Pst* I four base primer approach we used, we could have

examined sixty times as many bands as we did. Given a longer period of time for a study, one could do more preliminary screening to choose the primers to be used. We used a single restriction enzyme (*Pst* I) and single 4 base extension primers for this project that generated usable bands in the 300-1000 bp range. We currently are moving to a two restriction enzyme methodology to produce banding patterns in the range of 50 to 500 bp range spread out over a wider gel area. We advocate the use of this latter technology to enhance the discriminatory power of this approach.

- ***Future Work on Tui Chubs***

While we have shown the Mohave tui chub to be a distinct subspecies with little genetic variation, such clarity is not true for other tui chubs. Much genetic work remains to be done on the tui chub of California, as well as the few populations in Oregon and Nevada. As pointed out by Moyle *et al.* (1995), there are currently 10 subspecies of tui chub of which three do not have formal taxonomic descriptions (Eagle Lake, High Rock Spring, and Pit River tui chubs). Most of the 10 subspecies are found in isolated lakes or river drainages, with the exception of the two putative forms of the Lahontan tui chub, the lake form (*G. b. pectinifer*) and the creek form (*G. b. obesus*). Questions exist regarding the purity of populations of the Owens tui chub in the Owens River system. Hybridization with Lahontan tui chubs has been proposed (S. Parmenter, personal communication). Suspicions about the distinctness of the Cowhead tui chub from the Goose Lake tui chub have been raised (R. Miller, personal communication). We recommend that an extensive examination of all tui chub be undertaken using AFLP markers which have the capacity to quantify degrees of genetic variability within and among populations, to detect hybridization, and to be sampled non-destructively.

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Glossary

AFLP	amplified fragment length polymorphism. A procedure for examining many different random genetic sequences on a single gel. Genomic DNA is digested with one (or two) restriction enzymes and then a short (~15 bp) adapter is ligated to the end of the restriction site. The adapter is constructed so that it does not fully restore the restriction site so that restriction and ligation can be done at the same time to prevent religation of genomic fragments. Primers are chosen that include the adapter, part of the restriction site, and 1-4 bp extensions. The number of random base pair extensions determines the number of bands that will be shown on the gel.
agarose	the neutral gelling fraction of agar commonly used in gel electrophoresis.
allele	one of a series of possible alternative forms of a given gene differing in DNA sequence and affecting the structure and/or function of a single product (RNA and/or protein).
allozyme	alternative (allelic) forms of proteins (enzymes) differentiated by net charge, and therefore detectable by electrophoretic separation and histochemical staining.
bp	base pair, a pair of hydrogen-bonded nucleotides that join the two strands of a DNA double helix. In a double-stranded DNA molecule, adenine (A) forms a base pair with thymine (T), and guanine (G) pairs with cytosine (C).
dendrogram	any branching tree-like diagram.
DNA ligase	enzyme that joins two double-stranded DNAs together, end to end, by catalyzing 3'OH and 5'P termini bond formation.
electrophoresis	the separation of macromolecules in the presence of an electric current. Electrophoresis is routinely used to separate both proteins and DNA fragments; allozymes are separated based on differences in net charge, whereas DNA fragments are separated based on differences in size.
F-statistics	a set of coefficients that describe how genetic variation is partitioned within and among populations and individuals (see coancestry coefficient and inbreeding coefficient).
genetic distance	a measure of the number of allelic substitutions per gene that have occurred during the separate evolution of two populations or species.
genetic marker	mutant gene usually recognizable by a restriction enzyme that is useful in genetic mapping studies for locating sites of other genes.
heterozygosity	the condition of having a pair of dissimilar alleles at a locus.
homozygous	the presence of identical alleles at a corresponding homologous chromosome loci.
inbreeding	reproduction between related individuals.

kb an abbreviation for 1,000 nucleotide base-pairs of DNA or RNA.

ligation enzymatically catalyzed formation of a phosphodiester bond that links two DNA molecules.

locus a specific position on a chromosome.

nuclear genome the portion of the genome contained in the nucleus of eukaryotes, i.e., the chromosomes.

polymerase chain reaction (PCR) a series of thermal cycles of denaturation, annealing of primers, and primer extension catalyzed by a thermostable DNA polymerase, in which a target DNA fragment is amplified exponentially; primers that have nucleotide sequences complementary to the DNA that flanks the target region are added to sample DNA along with a heat-stable DNA polymerase. The DNA is heated to separate the complementary strands and then cooled to let the primers bind to the flanking sequences. The polymerase initiates synthesis of complementary DNA. The reaction is allowed to proceed for a series of replication cycles. Twenty cycles will yield a millionfold amplification; thirty cycles will yield an amplification factor of one billion.

polymorphism intraspecific variation. On the DNA level, this refers to differences in base pair sequence between two individuals.

primers short pieces of single stranded DNA (10-30 bp) annealed to the 5' end of a DNA template used to initiate synthesis of the complementary strand of the template piece of DNA. Primers can be designed so that they will bind only to a very specific region of the DNA, and will thus initiate synthesis of a targeted sequence (as in PCR or DNA sequencing).

proteinase K a hydrolytic enzyme used in the digestion of proteins to amino acids.

restriction enzyme (endonuclease) an enzyme that cleaves double-stranded DNA. Type I restriction endonucleases are not sequence-specific; type II restriction endonucleases cleave DNA at particular recognition sequences (typically 4-6 bp palindromes).The enzymes are named by an acronym that indicates the bacterial species from which they were isolated, followed by a Roman numeral that gives the chronological order of discovery when more than one enzyme came from the same source. DNA fragments produced by certain enzymes, such as EcoRI, can anneal with any other fragment produced by that enzyme. This property allows splicing of foreign genes into E. coli plasmids or bacteriophage vectors.

similarity a generic measure of the resemblance between two objects, usually on a scale from 1 to 0.

***Taq* polymerase** a thermostable DNA polymerase from *Thermus aquaticus*, thermophilic bacterium. Used for amplification via the polymerase chain reaction.

UPGMA unweighed pair group method using arithmetic average. A cluster analysis technique.

References

Ayala, F.J., Hedgecock, D., Zumwalt, G.S., and Valentine, J.W. (1973). Evolution, 27, 177.

Cardy, B.J., Stuber, C.W. and Goodman, M.M. (1980). Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Dept. of Statistics Mimeo Series No. 1317. North Carolina State University, Raleigh, N.C.

Clayton, J.W. and Tretiak, D.N. (1972). J. Fish. Res. Bd. Can. 29, 1169.

Doyle, J.J., and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytol. Bull. 19: 11-15.

Grewe, P.M., C.C. Krueger, C.F. Aquadro, E. Bermingham, H.L. Kincaid, and B. May. 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario. Can. J. Fish. Aquat. Sci. 50: 2397-2403.

Markert, C.L. and Faulhaber, I. (1965). J. Exp. Zool. 159, 319.

May, B. 1992. Starch gel electrophoresis of allozymes. In: Molecular Genetic *Analysis of Populations: A Practical Approach*. A.R. Hoelzel, ed. Oxford Univ. Press. pp. 1-27 and 271-280.

Miller, R.R. 1973. Two new fishes, *Gila bicolor snyderi* and *Catastomus fumeiventris*, form the Owens River basin, California. Occ. Pap. Mus. Zool. Univ. Michigan 667:1-19.

Mohave Tui Chub Advisory Committee. 1988. Revised Recovery Plan for the Mohave Tui Chub, *Gila bicolor mohavensis*. U.S. Fish and Wildlife Service.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Pp. 145-153. *In: Studies in genetics*. VII. Ed., M.R. Wheeler. Univ. Texas Publ. 7213. 354 p.

Sneath, P.H.A. and R. R. Sokal. 1973. Numerical taxonomy. Freeman, San Franciso.

Moyle, P.B., R.M. Yoshiyama, J.E. Williams, and E.D. Wikramanayake. 1995. Fish Species of Special Concern in California. Final Report for Contract N. 2128IF to California Department of Fish and Game.

Murphy, R.W., Sites, J.W., Buth, D.G. and Haufler, C.H. (1996). In Molecular Systematics (ed. D.M. Hillis, C. Moritz, and B.K. Mable), pp. 51-120. Sinauer Associates, Sunderland, MA.

Ridgway, G.J., Sherburne, S.W. and Lewis, R.D. (1970). Trans. Am. Fish. Soc. 99, 147.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 81: 8014-8018.

Selander, R.K., Smith, M.H., Yang, S.Y., Johnson, W.E. and Gentry, J.B. (1971). Studies in Genetics 6, 49.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids. Res. 23:4407-4414.

Table 1. Samples obtained for this study.

Scientific name	Common name	Location	Code	Sacrifice	Fin clips
G. b. mohavensis	Mohave tui chub	Camp Cady	M-cc	6	20
"	"	China Lake	M-cl	6	20
"	"	Lake Tuendae	M-lt	6	20
G. b. snyderi	Owens tui chub	Little Hot Creek	O-lhc	6	20
"	"	Mule Springs	O-ms	6	13
"	"	Owens Gorge	O-og	6	7
G. b. spp. hybrids	arroyo/Mohave hybrids	Mojave River	H-axm	6	20
	Lahontan/Owens hybrids	Hot Creek	H-lxo	6	20
G. b. spp.	Lahontan tui chub	E. Walker Creek	L-wc	7	20
G. b. spp.	Lahontan tui chub	Independence Lake	L-il	41	0
G. coerulea	blue chub	U. Klamath Lake	B-kl	50	0
G. b. bicolor	Klamath tui chub	U. Klamath Lake	K-kl	38	0
G. orcutti	arroyo chub	Agua Caliente/San Luis Rey R.	A-ac	4	0
"	"	West Fork San Luis Rey R.	A-wf	4	0
"	"	Rainbow Cr./Santa Margarita R	A-rc	4	0

Table 2. Allozyme abbreviations, names, E.C. number, locus names, tissue, and buffer combinations.

Abbrev.	Enzyme name	E.C. #	Locus	Tissue	Buffer*
AC	Aconitase	4.2.1.3	Ac-1	muscle	4
			Ac-2	liver	4
ADH	Alcohol dehydrogenase	1.1.1.1	Adh	liver	C
ALD	Aldolase	4.1.2.13	Ald	eye	C
AAT	Aspartate aminotransferase	2.6.1.1	Aat-1	muscle	R
			Aat-2	muscle	R
			Aat-3	muscle	R
CK	Creatine kinase	2.7.3.2	Ck-1	muscle	R
EST	Esterase	----------	Est-1	muscle	R
FUM	Fumarase	4.2.1.2	Fum	muscle	4
GAM	Galactosaminidase	----------	Gam	muscle	R
GPI	Glucosephosphate isomerase	5.3.1.9	Gpi-1	muscle	R
			Gpi-2	muscle	R
IDH	Isocitrate dehydrogenase	1.1.1.42	Idh-1	muscle	4
			Idh-2	eye	4
LDH	Lactate dehydrogenase	1.1.1.27	Ldh-1,2	eye	C
MDH	Malate dehydrogenase	1.1.1.37	Mdh-1	eye	4
			Mdh-2	muscle	4
			Mdh-3	liver	C
MPI	Mannosephophate isomerase	5.3.1.8	Mpi	muscle	9
PEP	Peptidase: resolved with glycyl-leucine,	3.4.11-13	Pep-pap-1	muscle	4
	leucyl-alanine, leucyl-glycyl-glycine,		Pep-lgg-1	liver	R
	leucyl-leucyl-leucine, or phenyl-alanyl-proline		Pep-lgg-2	liver	R
PGM	Phosphoglucomutase	5.4.2.2	Pgm-1,2	eye	9
PGD	Phosphogluconate dehydrogenase	1.1.1.43	Pgd	muscle	C
PRO	General protein	----------	Pro-1,2	muscle	R
PP	Inorganic pyrophosphatase	----------	Pp-1	muscle	9
			Pp-2	eye	9
SDH	Sorbitol dehydrogenase	1.1.1.14	Sdh	liver	R
SOD	Superoxide dismutase	1.15.1.1	Sod	liver	R
TPI	Triosephosphate isomerase	5.3.1.1	Tpi	muscle	4

* As modified in May (1992) from the following references: A - (Ayala *et al.* 1973), C - (Clayton and Tretiak 1972), H - (Cardy *et al.* 1980), M - (Markert and Falulhaber 1965), R - (Ridgway *et al.* 1970), 9 and 4 - (Selander *et al.* 1971).

Table 3. Allozyme allele frequencies by population.

Locus	Allele		Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owen's Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub	Mojave River "hybrids"
Aat-1	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
	89		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
	111		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Aat-2	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
	87		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
		N	3	3	3	3	3	3	3	3	3	3	6
Aat-3	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	0.67	1.00
	67		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
	113		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Ac-1	100		1.00	1.00	1.00	1.00	1.00	0.83	0.00	0.00	0.00	0.00	0.00
	90		0.00	0.00	0.00	0.00	0.00	0.17	1.00	1.00	1.00	1.00	0.92
	70		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
		N	3	3	3	3	3	3	3	3	3	3	6
Ac-2	100		0.00	0.17	0.00	0.00	0.00	1.00	1.00	0.50	0.75	0.00	0.20
	115		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	1.00	0.70
	131		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
	83		1.00	0.83	1.00	1.00	1.00	0.00	0.00	0.50	0.00	0.00	0.00
		N	1	3	3	2	2	2	1	3	2	1	5
Ck-1	100		1.00	1.00	1.00	0.58	0.79	0.00	0.00	1.00	1.00	1.00	1.00
	89		0.00	0.00	0.00	0.42	0.21	1.00	1.00	0.00	0.00	0.00	0.00
		N	6	6	6	6	7	6	6	3	3	3	6

Locus	Allele		Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owen's Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub	Mojave River "hybrids"
Est-1	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
	121		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Gam	100		0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	171		1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		N	6	6	6	6	7	6	6	3	3	3	6
Gpi-1	100		1.00	1.00	1.00	0.67	1.00	1.00	1.00	0.83	0.67	1.00	1.00
	320		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00
	0		0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.33	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Gpi-2	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.00	0.00
	121		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
	113		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Idh-2	100		1.00	1.00	1.00	0.83	1.00	1.00	1.00	1.00	0.00	0.00	0.00
	111		0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	89		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
	80		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
		N	3	3	3	3	3	3	3	3	3	3	6
Ldh-1	100		1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	0.00	0.00	0.00
	0		0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
	60		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
		N	3	3	3	3	3	3	3	3	3	3	6

Locus	Allele		Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owen's Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub	Mojave River "hybrids"
Mdh-1	100		1.00	1.00	1.00	1.00	1.00	0.83	1.00	1.00	0.83	1.00	1.00
	50		0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.17	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Mpi	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	1.00	1.00
	73		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Pep-Lg	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00
	120		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Pep-Lg	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
	94		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
		N	3	3	3	3	3	3	3	3	3	1	6
Pep-Pa	100		1.00	1.00	1.00	0.83	1.00	0.67	1.00	0.00	0.50	1.00	1.00
	110		0.00	0.00	0.00	0.00	0.00	0.33	0.00	1.00	0.50	0.00	0.00
	90		0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		N	3	3	3	3	3	3	2	3	3	3	6
Pgm-1	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	1.00	0.33	0.50
	79		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00
	121		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33
	93		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
	111		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.08
		N	3	3	3	3	3	3	3	3	3	3	6

Locus	Allele		Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owen's Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub	Mojave River "hybrids"
Pgm-2	100		1.00	1.00	1.00	0.67	0.83	0.33	0.00	1.00	1.00	0.67	0.92
	94		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.08
	85		0.00	0.00	0.00	0.33	0.17	0.67	1.00	0.00	0.00	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	3	6
Sdh	100		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
	120		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
	-20		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
		N	3	3	3	3	3	3	3	3	3	1	6
Sod	100		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
	42		1.00	1.00	1.00	0.33	0.50	1.00	1.00	1.00	0.00	0.00	0.00
	-42		0.00	0.00	0.00	0.67	0.50	0.00	0.00	0.00	0.00	0.00	0.00
		N	3	3	3	3	3	3	3	3	3	1	6
Avg Hs			0	0.008	0	0.07	0.046	0.042	0	0.031	0.078	0.046	0.041
std err			0	0.006	0	0.02	0.017	0.015	0	0.013	0.021	0.019	0.017
Avg Ho			0	0.01	0	0.064	0.063	0.039	0	0.029	0.123	0.02	0.047
std err			0	0.007	0	0.022	0.026	0.017	0	0.012	0.035	0.014	0.019

Table 4. Genetic distances between populations from allozyme data

Nei's Distance above diagonal; Roger's below

	Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owen's Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub	Mojave
Mohave (China Lake)	*	0.005	0	0.056	0.042	0.113	0.159	0.102	0.478	0.425	0.395
Mohave (Lake Tuendae)	0	*	0	0.057	0.043	0.103	0.149	0.098	0.467	0.421	0.389
Mohave (Camp Cady)	0	0.005	*	0.056	0.042	0.113	0.159	0.102	0.478	0.425	0.395
Lahontan (Independence)	0.091	0.096	0.091	*	0.045	0.109	0.135	0.092	0.436	0.392	0.365
Lahontan (Walker Creek)	0.065	0.07	0.065	0.007	*	0.111	0.131	0.084	0.422	0.369	0.341
Owens (Owen's Gorge)	0.127	0.123	0.127	0.065	0.073	*	0.049	0.09	0.447	0.438	0.405
Owens (Mule Springs)	0.147	0.142	0.147	0.106	0.115	0.027	*	0.102	0.441	0.41	0.386
Klamath Tui Chub	0.113	0.108	0.113	0.131	0.119	0.123	0.113	*	0.335	0.372	0.344
Blue Chub	0.404	0.399	0.404	0.379	0.378	0.385	0.385	0.361	*	0.252	0.228
Arroyo Chub	0.36	0.358	0.36	0.352	0.328	0.377	0.347	0.329	0.264	*	0.006
Mohave River "hybrids"	0.334	0.331	0.334	0.332	0.305	0.356	0.33	0.304	0.244	0.034	*

Table 5. AFLP allele frequencies by population.

Allele "1" = band present; "2" = band absent

locus	allele	Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owens Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub
CTCG 800	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 790	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 765	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 750	1	0.33	0.33	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00
	2	0.67	0.67	1.00	1.00	0.00	0.00	0.00	0.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 740	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 706	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 675	1	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00
	N	6	6	6	2	6	6	6	3	5	3

locus	allele	Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owens Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub
CTCG 637	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 592	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 570	1	0.00	0.00	0.00	0.00	0.33	0.00	1.00	0.00	0.00	0.00
	2	1.00	1.00	1.00	1.00	0.67	1.00	0.00	1.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 532	1	1.00	1.00	1.00	1.00	0.50	1.00	1.00	1.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 510	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 475	1	0.83	0.83	1.00	0.50	0.50	1.00	1.00	0.33	1.00	1.00
	2	0.17	0.17	0.00	0.50	0.50	0.00	0.00	0.67	0.00	0.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 456	1	0.25	0.25	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00
	2	0.75	0.75	0.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00
	N	4	4	6	2	6	6	6	3	5	3
CTCG 439	1	0.50	1.00	1.00	0.00	0.17	0.33	0.00	0.67	0.00	0.00
	2	0.50	0.00	0.00	1.00	0.83	0.67	1.00	0.33	1.00	1.00
	N	2	2	6	2	6	6	6	3	5	3
CTCG 380	1	1.00	1.00	1.00	1.00	0.00	0.33	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	1.00	0.67	1.00	1.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3

locus	allele	Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owens Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub
CTCG 370	1	0.67	1.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.33	0.00	0.33	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCG 327	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
	N	6	6	6	2	6	6	6	3	5	3
CTCA 709	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCA 628	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCA 549	1	1.00	1.00	1.00	0.50	0.80	0.67	0.00	1.00	0.00	0.00
	2	0.00	0.00	0.00	0.50	0.20	0.33	1.00	0.00	1.00	1.00
	N	6	6	6	2	5	6	6	2	5	3
CTCA 510	1	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00	1.00	0.00
	2	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	1.00
	N	6	6	6	2	4	6	6	3	5	3
CTCA 483	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCA 479	1	0.17	0.40	0.67	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.83	0.60	0.33	0.00	1.00	1.00	1.00	1.00	1.00	1.00
	N	6	5	6	2	6	6	6	3	5	3
CTCA 472	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
	N	6	6	6	2	6	6	6	3	5	3

locus	allele	Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owens Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub
CTCA 437	1	0.00	0.00	0.00	1.00	0.50	0.17	0.00	0.00	0.00	0.00
	2	1.00	1.00	1.00	0.00	0.50	0.83	1.00	1.00	1.00	1.00
	N	6	4	6	2	4	6	6	3	5	3
CTCA 423	1	1.00	1.00	1.00	1.00	1.00	0.67	0.00	1.00	1.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.33	1.00	0.00	0.00	1.00
	N	6	6	6	2	6	6	6	3	5	3
CTCA 384	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.67
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.33
	N	6	4	6	2	3	6	4	3	5	3
CTCA 356	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.33
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67
	N	6	6	6	2	6	6	6	3	5	3
CTCA 351	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.33
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.67
	N	6	6	6	2	6	6	6	3	5	3
CTCA 327	1	1.00	1.00	1.00	0.00	0.60	0.50	1.00	1.00	1.00	0.00
	2	0.00	0.00	0.00	1.00	0.40	0.50	0.00	0.00	0.00	1.00
	N	6	6	6	2	5	6	6	3	5	3
CTCA 293	1	0.33	1.00	1.00	0.00	0.33	0.83	1.00	0.00	0.00	0.00
	2	0.67	0.00	0.00	1.00	0.67	0.17	0.00	1.00	1.00	1.00
	N	6	6	6	2	6	6	6	2	5	3
TGAG 897	1	0.83	1.00	1.00	1.00	0.00	1.00	1.00	0.00	0.00	0.00
	2	0.17	0.00	0.00	0.00	1.00	0.00	0.00	1.00	1.00	1.00
	N	6	6	6	2	5	5	6	3	5	3
TGAG 841	1	0.00	1.00	0.00	1.00	0.67	1.00	1.00	0.00	0.00	0.00
	2	1.00	0.00	1.00	0.00	0.33	0.00	0.00	1.00	1.00	1.00
	N	4	6	6	2	6	5	6	3	5	3

locus	allele	Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owens Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub
TGAG 818	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.33
	N	6	6	6	2	6	5	6	3	5	3
TGAG 800	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00
	N	6	6	6	2	6	5	6	3	5	3
TGAG 785	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	N	6	4	6	2	6	5	6	3	5	3
TGAG 748	1	0.00	0.00	0.17	0.00	0.00	1.00	1.00	1.00	1.00	0.00
	2	1.00	1.00	0.83	1.00	1.00	0.00	0.00	0.00	0.00	1.00
	N	4	4	6	2	6	4	6	3	5	3
TGAG 700	1	0.00	0.00	0.00	0.00	0.17	1.00	0.67	1.00	1.00	0.00
	2	1.00	1.00	1.00	1.00	0.83	0.00	0.33	0.00	0.00	1.00
	N	4	6	6	2	6	4	6	3	5	3
TGAG 680	1	0.00	1.00	0.17	1.00	0.60	0.20	1.00	0.00	0.00	0.00
	2	1.00	0.00	0.83	0.00	0.40	0.80	0.00	1.00	1.00	1.00
	N	4	3	6	2	5	5	6	3	5	3
TGAG 660	1	1.00	1.00	1.00	1.00	1.00	0.33	0.00	0.67	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.67	1.00	0.33	1.00	1.00
	N	6	6	6	2	6	3	6	3	5	3
TGAG 646	1	0.00	0.00	0.00	0.50	0.17	1.00	1.00	0.33	0.00	0.00
	2	1.00	1.00	1.00	0.50	0.83	0.00	0.00	0.67	1.00	1.00
	N	4	2	6	2	6	5	6	3	5	3
TGAG 631	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	1.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.20	0.00
	N	4	4	6	2	6	4	4	3	5	3

locus	allele	Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owens Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub
TGAG 617	1	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00
	N	4	2	6	2	3	4	6	3	5	3
TGAG 613	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	N	4	2	6	2	3	4	5	3	5	3
TGAG 606	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00
	N	4	2	6	2	3	4	6	3	5	3
TGAG 576	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	N	4	2	6	2	3	4	6	3	5	3
TGAG 524	1	0.00	0.00	0.00	0.00	0.00	0.75	0.60	1.00	0.00	0.00
	2	1.00	1.00	1.00	1.00	1.00	0.25	0.40	0.00	1.00	1.00
	N	4	2	6	2	6	4	5	3	5	3
TGAG 476	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00
	N	5	2	6	2	3	5	6	3	4	3
TGAG 469	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	N	6	6	6	2	6	4	6	3	5	3

Table 6. Genetic distance between populations from AFLP data

Nei's Distances above diagonal; Roger's below

	Mohave (China Lake)	Mohave (Lake Tuendae)	Mohave (Camp Cady)	Lahontan (Independence)	Lahontan (Walker Creek)	Owens (Owens Gorge)	Owens (Mule Springs)	Klamath Tui Chub	Blue Chub	Arroyo Chub
Mohave (China Lake)	*	0.058	0.033	0.146	0.099	0.163	0.32	0.208	0.699	0.657
Mohave (Lake Tuendae)	0.076	*	0.053	0.141	0.133	0.172	0.288	0.31	0.889	0.843
Mohave (Camp Cady)	0.067	0.075	*	0.15	0.176	0.203	0.36	0.287	0.741	0.777
Lahontan (Independence)	0.175	0.157	0.167	*	0.145	0.214	0.343	0.384	0.834	0.767
Lahontan (Walker Creek)	0.158	0.184	0.218	0.181	*	0.133	0.233	0.177	0.686	0.587
Owens (Owens Gorge)	0.203	0.207	0.225	0.233	0.182	*	0.112	0.166	0.718	0.754
Owens (Mule Springs)	0.304	0.27	0.319	0.309	0.257	0.146	*	0.276	0.772	0.855
Klamath Tui Chub	0.221	0.291	0.275	0.33	0.211	0.195	0.263	*	0.656	0.674
Blue Chub	0.521	0.597	0.532	0.575	0.526	0.534	0.544	0.493	*	0.621
Arroyo Chub	0.508	0.585	0.556	0.552	0.485	0.558	0.587	0.51	0.475	*

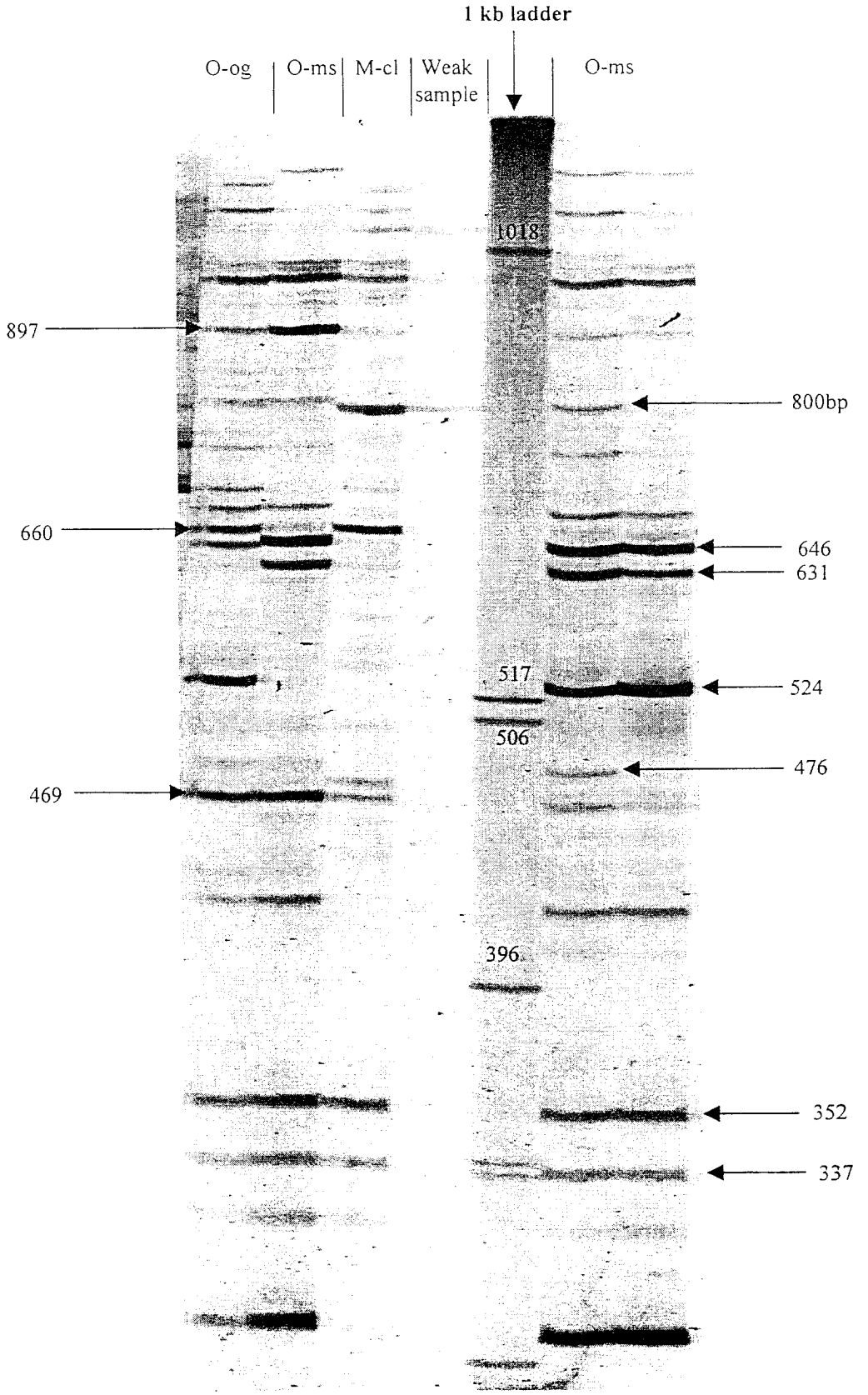

Figure 1. Representative AFLP *Pst*-TGAG variation in tui chub

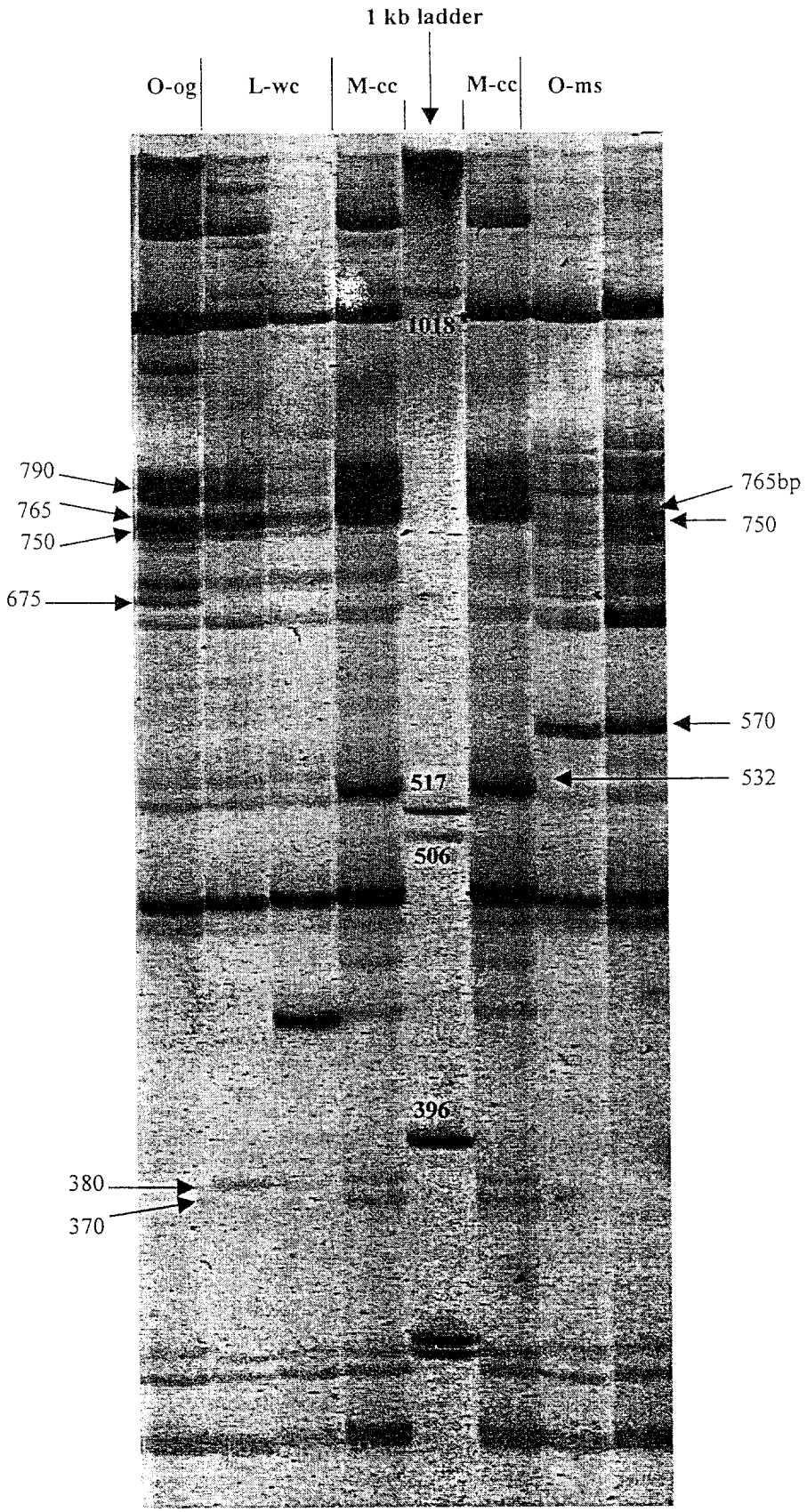

Figure 2. Representative AFLP *Pst*-CTCG variation in tui chub

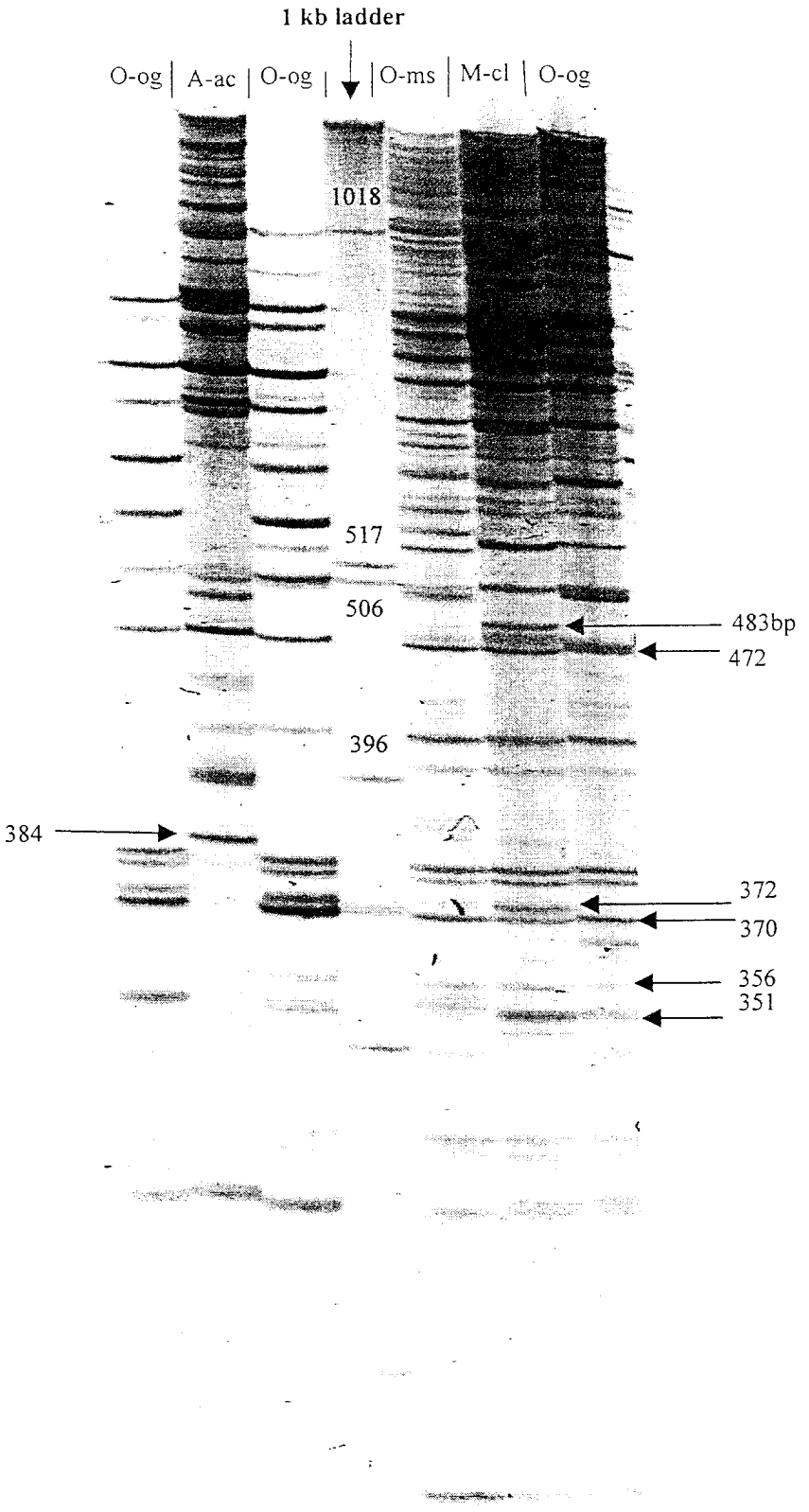

Figure 3. Representative AFLP *Pst*-CTCA variation in tui chub.

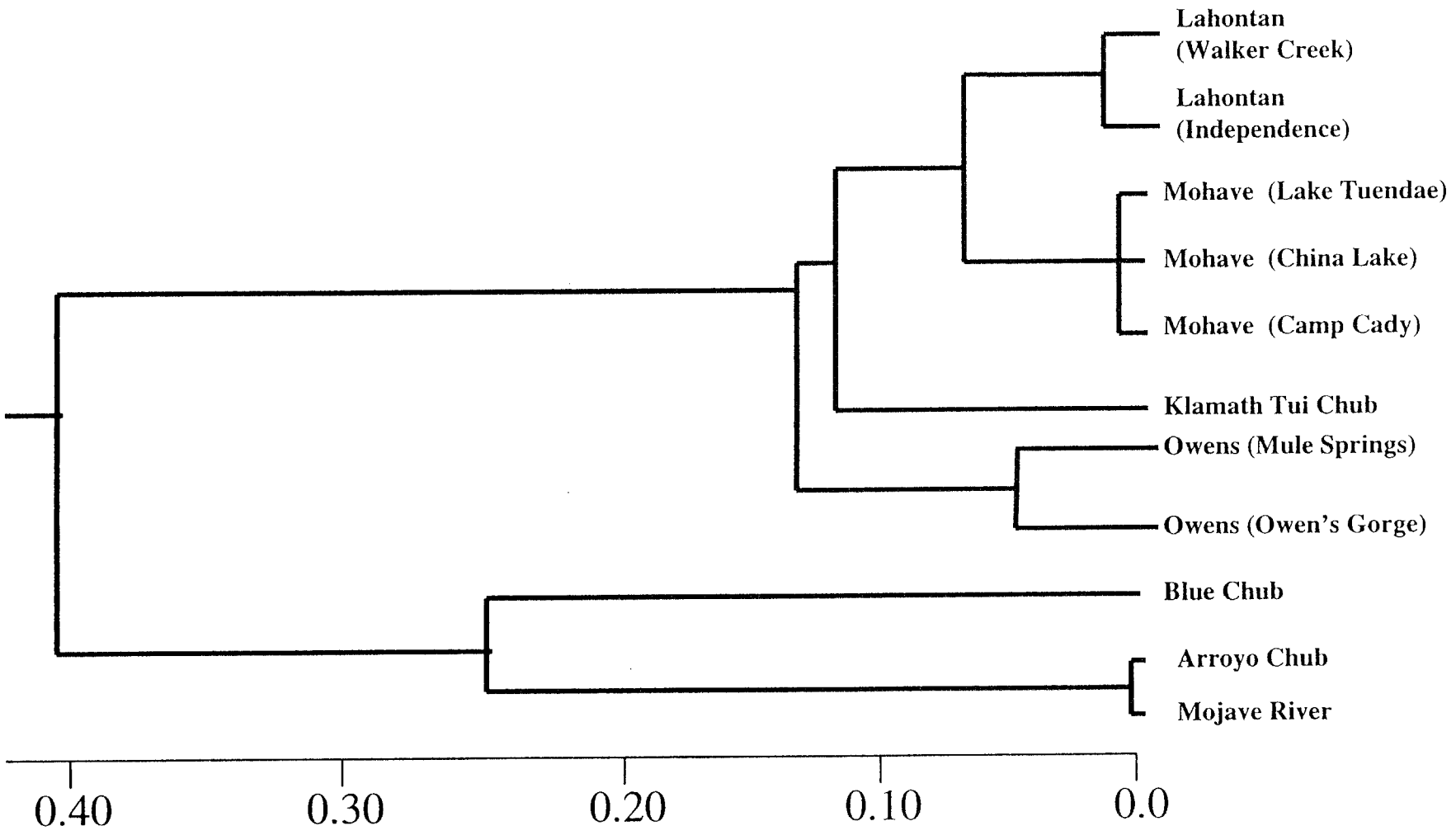

Figure 4. UPGMA of Nei's genetic distance from allozyme variation in populations of *Gila* spp.

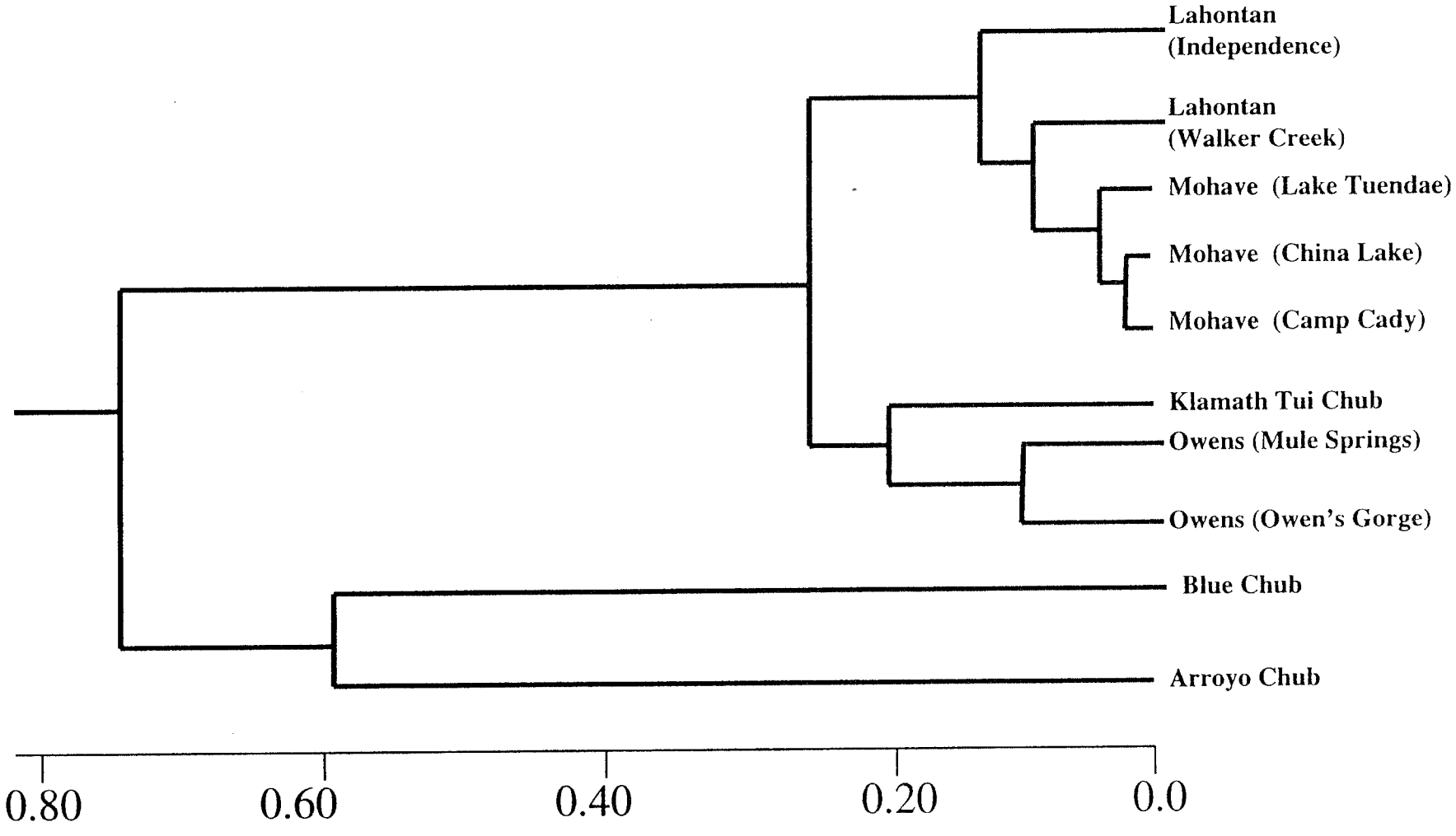

Figure 5. UPGMA of Nei's genetic distances from AFLP variation in populations of *Gila* spp.