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Walker Lake Tui Chub Genetics Report

Project Title: Genetic analysis of tui chub in Walker Lake, Nevada

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Executive Summary of Work

This report details the research and findings of the Genomic Variation Laboratory (GVL) at the University of California, Davis, for Lahontan tui chubs (*Siphateles bicolor pectinifer*; *Siphateles bicolor obesa*) in Walker Lake, Nevada. Tui chubs in Walker Lake have experienced declines in recruitment as total dissolved salts (TDS) have increased over the last century. This work investigates how genetically differentiated the population in Walker Lake is relative to other populations of Lahontan tui chubs throughout the Walker, Carson and Truckee river basins.

To assess the genetic diversity and the genetic distinctiveness of Lahontan tui chubs in Walker Lake, nine microsatellite loci were used to genotype tui chubs from Topaz Lake, NV, Little Soda Lake, NV, Pyramid Lake, NV, Spooner Lake, NV, Twin Lakes, CA, Tahoe Keys, CA, East Fork Walker River, CA and Stillwater National Wildlife Refuge, NV. Three additional populations from Nevada were analyzed for comparison: South Fork Reservoir, Independence Valley, and Dixie Valley.

Funding in the amount of $40,000.00 was allocated by U.S. Fish and Wildlife Service-Lahontan National Fish Hatchery Complex to complete two objectives: 1) use genetic data to determine if the population of tui chubs in Walker Lake is genetically distinct and 2) identify a suitable source population for tui chub if it is necessary to create refugial populations.

We found that the population of Lahontan tui chubs in Walker Lake is genetically differentiated and has robust genetic diversity, and suggest that, rather than founding

refugial populations, to select fish from Pyramid Lake if restocking Walker Lake is deemed necessary.

Introduction

Walker Lake, a terminal saline lake fed by the Walker River in Walker River Basin, Nevada, was once part of the ancient Pleistocene Lake Lahontan. Lake Lahontan covered much of Northwestern Nevada and began to recede from its last highstand, or peak 12,500ya, leaving Walker Lake and Pyramid Lake today as the main remnants today. At this highstand, the Truckee, Carson, Walker, Humboldt, Susan and Quinn rivers drained into Lake Lahontan (Benson, 1988). At that time, fish populations in these rivers were probably connected, allowing gene flow. Between 12,500ya and the present, Walker Lake may have dried completely on two occasions (~4,700ya and ~2,600ya) either as a result of climate changes, or more likely, due to Walker River rerouting into the Carson River in the Carson Sink (Benson, 1988).

In 1882, Russell completed the first survey of Walker Lake hydrology and measured the total dissolved salts (TDS) at 2560 mg/L (Russell, 1885). Between 1882 and 2008, mostly as a result of water mining, the lake level dropped more than 150ft and TDS has increased to 17,000mg/L (Lopes & Allender, 2009). This increased TDS has been associated with the decline of Lahontan tui chubs (*Siphateles bicolor pectinifer* and *Siphateles b. obesa*) native to Walker Lake; Stockwell (1994) demonstrated a correlation between high TDS and lowered tui chub survival, suggesting that tui chubs will continue to decline if salinity is not reduced with increased inflows.

The Lahontan tui chub is a minnow (Cyprinidae) historically found throughout the Walker, Carson, Truckee and Humboldt River systems of the Lahontan basin (La

Rivers, 1962). The decline of tui chubs in Walker Lake is of conservation concern because they are the most abundant fish species in the lake and serve as an important prey item for the endangered Lahontan cutthroat trout and migratory birds. This genetic study to determine how distinct this population is relative to other Lahontan tui chubs throughout the Walker, Carson and Truckee River watersheds will assist in conservation efforts by determining which populations are suitable sources if it is necessary to restock Walker Lake or create refugial populations.

Tui chubs in Walker Lake have only been isolated from upstream locations since the construction of dams in the 1930s, so their degree of genetic distinction is uncertain. In addition, tui chubs from Walker Lake have been moved to other locations, such as Stillwater National Wildlife Refuge and Spooner Lake. If Walker Lake tui chubs are genetically distinct, management and conservation measures may be implemented.

The objectives of this genetic study are to 1) use genetic data to determine if the population of tui chubs in Walker Lake is genetically distinct and 2) identify a suitable source population for tui chub if it is necessary to create refugial populations.

Methods

Sample Collection

Between 10-50 individual Lahontan tui chub samples were collected from each of nine locations: Topaz Lake, NV (TPZ), Spooner Lake, NV (SPL), Little Soda Lake, NV (LSL), Stillwater National Wildlife Refuge, NV (STW), Tahoe Keys, CA (TKS), Pyramid Lake, NV (PYR), East Fork Walker River, CA (EWR), South Fork Reservoir, NV (Humboldt River; SFH), Twin Lakes, CA (TWN), and Walker Lake, NV (WLK) (Table 1; see Figure 1 for map). These locations are in the Walker River drainage,

adjacent to it, or were stocked with fish from Walker Lake. Two additional populations were sampled for comparison: Dixie Valley tui chub (*S. b. ssp*) from Casey Pond, Dixie Valley, NV (DXV), and Independence Valley tui chub (*S. b. isolata*) from Independence Valley, NV (IND). Each sample consists of a single $1mm^2$ pelvic fin clip placed in a coin envelope and dried for storage. Whole genomic DNA was extracted using the Promega Wizard SV 96 Genomic DNA Purification System.

Microsatellite Genotyping

Samples were genotyped at nine microsatellite loci from Meredith & May (2002; Gbi-G13, Gbi-G38, Gbi-G39, Gbi-G79 and Gbi-G87) and Baerwald & May (2004; Cyp-G3, Cyp-G41, Cyp-G47 and Cyp-G48). PCR reactions were conducted under conditions from Chen (2006) and electrophoresed on an ABI 3730XL capillary electrophoresis instrument (Applied Biosystems) after a 1:5 dilution with water and were scored using GeneMapper software (Applied Biosystems).

Heterozygosity and pairwise F_{ST} *values*

The software *MICRO-CHECKER* 2.2.3 (Van Oosterhout *et al*., 2004) was used to detect and correct any unusual values in the data set and to look for significant homozygote excess that might indicate the presence of null alleles.

The scoring of private alleles, calculations of allelic frequencies, observed heterozygosity (H_o), expected heterozygosity (H_e), and deviations from Hardy Weinberg equilibrium (HWE) were all performed using the software *GDA* (Lewis & Zaykin, 2001). Linkage disequilibrium (LD) values were calculated using Genepop ver. 4.0 (Raymond & Rousset, 1995). A Bonferonni correction was used in determining the significance of multiple tests in LD and HWE calculations.

Pairwise F_{ST} values, measuring the proportions of genetic diversity due to allele frequency differences among populations, were calculated with the software package *Arlequin* version 3.5 (Excoffier & Lischer, 2010), using the option of exact tests of population differentiation. F_{ST} values are expressed from 0 to 1, with 0 being panmixia, where populations interbreed freely, and 1 being complete separation of populations. Significance of each pairwise F_{ST} value was calculated in *Arlequin* using 1000 bootstrap permutations.

Allelic richness and private allelic richness

HP-Rare (Kalinowski, 2005) was used to estimate allelic richness (*A*), an estimate of the number of alleles across loci, and private allelic richness (*P*), an estimate of the number of unique alleles across loci. Both measures use rarefaction to correct for sample size bias, because the likelihood of detecting rare alleles increases with increased sample size (Kalinowski, 2004). Allelic richness is considered a measure of genetic diversity, and has been used in conservation settings (e.g. Kalinowski, 2004), while *P* is a measure of genetic distinctiveness. Petit *et al*. (1998) has suggested that *A* can provide information about which populations may need special management and which are best used as sources for restocking.

Genetic distance and Neighbor-joining tree

A neighbor joining (N-J) tree was used to visualize genetic distances. Note that N-J trees do not necessarily portray evolutionary relationships, just differences in frequencies of alleles. To construct the N-J tree, the *SEQBOOT* application in the software package *PHYLIP* version 3.69 (Felsenstein, 1995) was used to simulate 1000 data sets before calculating Cavalli-Sforza and Edwards cord distances (1967; D_{CE}) for

comparisons between all pairs of sites in *GENDIST* (Felsenstein, 1995). The main assumption when calculating D_{CE} is that differences in allele frequencies arise due to genetic drift only. D_{CE} was chosen because it does not assume that population sizes have remained constant or equal over time (Felsenstein, 1995), and Takezaki & Nei, (1996) found that D_{CE} is more likely to recover true tree topology than other genetic distance estimates. Unrooted N-J trees were constructed with the D_{CE} matrices calculated in *GENDIST* using the *NEIGHBOR* application in *PHYLIP* (Felsenstein, 1995). Finally, a consensus tree of all simulated N-J trees was built using *CONSENSE* in *PHYLIP* (Felsenstein, 1995).

FCA analysis

To visually depict the genetic relationships between tui chub individuals and populations, the software program *GENETIX* (Belkhir *et al*., 2003) was used to perform a factorial correspondence analysis (FCA). The FCA is calculated based on allele counts per individual in multiple dimensions.

Population structure

STRUCTURE 2.3.3 (Pritchard *et al.*, 2000) was used to determine the optimal number of genetic clusters (*K*) and to assign individuals to specific clusters. This program uses a Bayesian model-based clustering algorithm to group individuals into populations based on allele frequency patterns. 10 independent runs of *K* =1-10 were performed with a burn-in period of 100,000 and 1,000,000 Monte Carlo Markov Chain (MCMC) repetitions using no prior information and assuming admixture and correlated allele frequencies. We used the L(*K*) method of determining *K*, where the maximum value of the mean (over the 10 independent runs) of LnP(D) for each *K* is chosen. The 10

STRUCTURE outputs for each *K* were compiled with the software *CLUMPP* (Jakobsson & Rosenberg, 2007) using the Greedy K algorithm (described in Jakobsson & Rosenberg, 2007). *CLUMPP* aligns multiple replicate analyses of the same data set and creates an infile for the software *DISTRUCT* (Rosenberg, 2004), which creates a graphical representation of the mean *STRUCTURE* outputs for a chosen *K*. For the first analysis we included all 12 populations. For further analysis, Independence Valley and Dixie Valley are not included in the *STRUCTURE* analyses, as they are known to be divergent from Lahontan tui chubs.

Results

Heterozygosity and pairwise F_{ST} values

Seven out of 100 tests show significant deviation ($p<0.05$) from Hardy Weinberg equilibrium (HWE). One test in *MICRO-CHECKER* detected possible null alleles ($p<0.01$) at Gbi-G87 in Twin Lakes. Out of 432 tests for HWE, 12 are significant after a Bonferroni correction ($p<0.05$). No loci were dropped because there is no consistent pattern across populations and loci for significant HWE. For individual populations, after a Bonferroni correction there are 26 significant tests out of 550, but there is no significant linkage disequilibrium across all loci and all populations. See Table 5 for allele frequencies in each sampling location.

The observed heterozygosity values range from 0.61 (Dixie Valley) to 0.83 (Walker Lake). Expected heterozygosity values range from 0.62 (Dixie Valley) to 0.83 (Walker Lake). Due to missing data, loci Gbi-G39 and Gbi-G87 were not included in computing pairwise F_{ST} values, leaving a total of seven loci for these calculations (Table 3). Computed pairwise F_{ST} values range from 0.02- 0.36 and are all statistically

significant ($p<<0.05$). The lowest pairwise F_{ST} value is 0.02 (Pyramid Lake-Tahoe Keys), followed by 0.03 (Walker Lake-Topaz Lake). Generally, the highest pairwise F_{ST} values are between Independence Valley and all other populations, ranging from 0.17 (Independence Valley-Pyramid lake) to 0.36, (Independence Valley-Dixie Valley). The second highest pairwise F_{ST} values are generally between Dixie Valley and all other populations, ranging from 0.10 (Dixie Valley-Topaz Lake; Dixie Valley-Walker Lake) to 0.22 (Dixie Valley-Tahoe Keys).

Allelic richness and private allelic richness

The average number of alleles per locus (N_A) ranges from 5.44 (Dixie Valley) to 17.11 (Walker Lake, Table 3). Walker Lake has the most private alleles (N=16), while several populations have only one private allele (all populations with small sample sizes). Tahoe Keys and Twin Lakes were dropped from allelic richness (*A*) and private allelic richness (*P*) analyses due to small sample sizes. After correcting for sample sizes in the remaining locations, *A* varies from 5.44 (Dixie Valley) to 12.25 (Walker Lake). Walker Lake has the highest private allelic richness (*P*=1.06), and East Walker River has the lowest private allelic richness (*P*=0.13). *A* and *P* were calculated with a minimum number of genes N=34, because that is the smallest sample size included in the analysis (Dixie Valley).

FCA analysis

The FCA analysis that includes Independence Valley (Figure 2) shows a distinct Independence Valley cluster, a distinct Spooner Lake cluster, and a third cluster that includes all other locations. In this third cluster, Little Soda Lake, Topaz Lake and Dixie Valley each form somewhat distinct groups on the margins of a larger group that includes

Tahoe Keys, Twin Lakes, Stillwater National Wildlife Refuge, East Walker River, South Fork Reservoir, Walker Lake, and Pyramid Lake. Walker Lake, Pyramid Lake, and Stillwater span much of this third cluster because they have higher genetic diversity.

When Independence Valley is removed from the FCA analysis (Figure 3), Spooner Lake and Little Soda Lake each form distinct clusters, Dixie Valley forms a tight third cluster between Little Soda Lake and a fourth diffuse cluster composed of Walker Lake, Tahoe Keys, Twin Lakes, Pyramid Lake, Topaz Lake, Stillwater National Wildlife Refuge, East Walker River and South Fork Reservoir. Again, Walker Lake, Pyramid Lake and Stillwater National Wildlife Refuge form diffuse clusters that span much of this fourth cluster.

Population structure

When all 12 sample locations are included, the optimal *K*-value for the structure analysis is *K*=9 (Figure 4). Here seven locations form independent clusters: Independence Valley, Dixie Valley, Spooner Lake, Stillwater National Wildlife Refuge, South Fork Reservoir, Little Soda Lake and East Fork Walker River. Pyramid Lake, Tahoe Keys and Walker Lake form a cluster. Twin Lakes is grouped as an intermediate between the Pyramid-Walker cluster and East Fork Walker River. See Figure 7 for a map of the sample locations with corresponding *STRUCTURE* cluster colors.

The optimal *K*-value for the *STRUCTURE* analysis when Dixie Valley and Independence Valley are removed is *K* =7 (Figure 5). Where *K* = 7, Spooner Lake, Little Soda Lake, Stillwater National Wildlife Refuge, East Fork Walker River and South Fork Reservoir are distinct genetic clusters. Tahoe Keys, Pyramid Lake, Twin Lakes, Walker

Lake and Topaz Lake form a poorly resolved cluster, indicating that *STRUCTURE* cannot distinguish between these groups.

Genetic distance and Neighbor-joining tree

The consensus N-J tree (Figure 6) supports a close relationship between Walker Lake and Pyramid Lake (60% bootstrap support). The only other well supported relationships are between Twin Lakes and East Fork Walker River (52% bootstrap support) and South Fork Reservoir and Topaz Lake (54% bootstrap support). All other branches had bootstrap support values under 50%. Independence Valley is clearly distinct from all other locations.

Discussion

Genetic diversity

Our findings suggest that Walker Lake is a genetically differentiated population of Lahontan tui chubs with ample genetic diversity. Though *STRUCTURE* and the FCA analysis could not distinguish Walker Lake from Pyramid Lake, the very high number of private alleles (16) in the Walker Lake sample is a strong indicator of genetic differentiation.

The robust genetic diversity in Walker Lake is likely due to four non-exclusive factors, explored in more detail below: 1) the historical connectivity of Walker Lake to Lake Lahontan; 2) the better conditions in Walker Lake and its ability to support a very high number of tui chubs since its last desiccation event; 3) the terminal location of Walker Lake for the Walker River watershed; and 4) the long lives (20-30 years) and high fecundity of Lahontan tui chubs.

Lake Lahontan supported a very large population of Lahontan tui chubs, and large populations that remain large over many generations have more genetic diversity than small populations or populations that fluctuate in size. Smaller populations and populations that undergo genetic bottlenecks are more susceptible to genetic drift, which removes genetic diversity from a population. Since the last highstand of Lake Lahontan, the population of tui chubs in Walker Lake was still probably connected, even during desiccation events, to the Walker River and potentially the Carson River. This connection to Walker River allowed a larger population size and ample gene flow that would support retention of genetic diversity. Aside from Pyramid Lake, among the locations included in this study, Walker Lake has the largest volume and area and could support many fish, allowing Walker Lake and Pyramid Lake to retain more genetic diversity over time than smaller populations that are susceptible to bottlenecks and genetic drift.

If Walker Lake completely desiccated ~2600ya, a genetically robust population of fish must have recolonized the Lake when inflows returned, or the fish in Walker Lake were able to escape into the Walker River and perhaps into the Carson River drainage. Since the rewatering of the Lake, records indicate higher volume, and better conditions (lower TDS) of Walker Lake up until very recently. These conditions would support a larger population size and the maintenance of high genetic diversity.

Walker Lake is the downstream terminal location for the entire Walker River watershed, and this may allow one-way gene flow downstream from populations on both the West Fork Walker River (Topaz Reservoir since 1922) and the East Fork Walker River (East Fork Walker River, Twin Lakes), contributing to increased genetic diversity in Walker Lake.

Finally, the relatively long lives and high fecundity of individual tui chubs provide a fourth explanation of high genetic diversity in Walker Lake. Adults as old as 35 years are recorded and young of the year fish have been observed spawning (Steve Parmenter, CDFG, personal communication). Longer-lived fish such as tui chubs may maintain more genetic diversity because multiple generations of fish in Walker Lake act as a reservoir of diversity, much like a multi-year seed bank in plants. Populations of imperiled Colorado River fishes with similar life histories to Lahontan tui chubs have a similar pattern of high genetic diversity despite recent declines. For example, the endangered razorback sucker has declined steeply over the last few decades. There is no evidence of successful recruitment, so most of the surviving razorback suckers are large, old adults. However the remaining adults maintain surprisingly high genetic variation (Garrigan *et al*., 2002). The author suggests that this is due to the long generation time and the historically large and geographically wide range of the razorback sucker. As recently as the mid-20th century, there was probably a large population of razorback suckers in the Lower Colorado River (Hedrick, 2004). Therefore the steep decline in census number has not yet overwhelmed the genetic diversity of the remaining population. Walker Lake tui chub, like the razorback sucker, still have substantial genetic variation despite recent declines in population size. This has two conservation ramifications. First, their high fecundity coupled with their high existing diversity makes their recovery potential very high if conditions in Walker Lake improve. Second, the high diversity in the existing population does not reflect the current conditions in the lake, and much of this diversity will be lost as the existing fish die out.

Genetic relationships

The relationships between Walker Lake and the other sample locations were somewhat surprising. Hydrologic connections suggested that the populations most closely related to the Walker Lake Population would be the other Walker River watershed locations: Topaz Lake, East Fork Walker River and Twin Lakes. However the data support a surprisingly close relationship between Walker Lake and Pyramid Lake, based on the FCA and *STRUCTURE* analysis, low pair wise F_{ST} value, and the N-J tree. This close relationship exists despite the Lakes' location in different watersheds.

This close relationship may be explained by the same factors that result in high genetic diversity in Walker Lake. Pyramid Lake is the second most genetically diverse population, and it and Walker Lake are the only remnants of Pleistocene Lake Lahontan. Walker Lake and Pyramid Lake are known to have been connected ~12,500ya and may have been connected more recently; it is probable that these populations retained a larger proportion of genetic diversity than any other location due to their large size and more stable hydrology. After Pyramid Lake, Walker Lake is most closely related to Topaz Lake, Twin Lakes, East Fork Walker River, Tahoe Keys and South Fork Reservoir.

Topaz Lake is closely related to East Fork Walker River, but it is different. Topaz Lake was created in 1922 on the site of Alkali Lake, a historical lake isolated from the Walker River. Upon its creation, Topaz Lake was connected via a diversion to the West Fork Walker River. This complicated history muddies the implications of the relationships detected between Walker Lake and Topaz Lake; it may reflect a combination of a possible residual genetic signature of tui chubs that may have inhabited historic Alkali Lake, or fish that may have migrated into Topaz Lake after the diversion was constructed. There is no known stocking of Topaz Lake.

The relationships of Twin Lakes, East Fork Walker River, Tahoe Keys and South Fork Reservoir to each other and to other locations are also confusing, which could be due to low sample sizes. Twin Lakes and East Fork Walker River grouped together in most of the analyses, which is predicted given that they are geographically close and hydrologically connected. There is also a surprising relationship between South Fork Reservoir and other locations; perhaps this location was also connected to Lake Lahontan in the past.

Aside from Dixie Valley and Independence Valley (the outgroups), the locations least closely related to Walker Lake include Spooner Lake, Little Soda Lake, and Stillwater National Wildlife Refuge. Spooner Lake is surprisingly differentiated given that it was stocked with fish from Washoe Lake in 1993, which was in turn stocked with fish from Walker Lake. However Spooner Lake is a small area and tui chubs have always been abundant there (Kim Tisdale, NDOW, personal communication). There may be a genetic signature of the historic population of tui chubs in Spooner Lake, and this historic population may have undergone genetic drift due to small population size.

Little Soda Lake and the nearby Big Soda Lake are natural lakes that lie in volcanic craters which were formed after Lake Lahontan receded from the area. The Lakes are thought to have formed from between 10,000ya and 1500ya (Garside and Schilling, 1979). Little Soda Lake is relatively small, 300m across, and the population of tui chubs there is moderately divergent from the other locations in the study. Though the N-J tree pairs Dixie Valley with Little Soda Lake, pairwise F_{ST} values indicate a closer relationship with Walker Lake (0.08) than with Dixie Valley (0.12). There is no inflow or outflow to Little Soda Lake; it is thought that the water comes from subsoil seepage from

the Carson River. Little Soda Lake tui chubs may be genetically differentiated due to genetic drift since the initial (recorded) stocking event in 1931, the relatively small population size and isolation (Kim Tisdale, NDOW, personal communication).

The Carson River drains into Stillwater Marsh during high flow years, and the Humboldt River has been known to flow into the Carson River drainage (King, 2000). Fishing records for Stillwater National Wildlife Refuge stated that tui chubs were present and abundant in the 1950s, so they have been naturally present. However subsequent records show that Stillwater was stocked with 5500 tui chubs in 1994 from Sleeper Mine District, Humboldt County, NV, and later with 198 fish from Walker Lake in 1995. Genetic drift in an isolated historic population or human stocking events may explain the divergence of Stillwater National Wildlife Refuge from other Lahontan tui chub populations.

Refuge implications

The creation of refugial populations may be a desirable option to preserve the genetic composition of Lahontan tui chubs in Walker Lake if the population continues to decline. If this plan were to be adopted, it should include multiple refuges with all founders selected from Walker Lake. Use of founders from Walker Lake is important, because it is a genetically differentiated population with both substantial diversity and private alleles found nowhere else. This differentiation may indicate adaptation to the environment in Walker Lake and should be preserved.

However, if collecting tui chubs from Walker Lake for refugial populations is not a viable option, the next best option may be to select Lahontan tui chubs from Pyramid Lake for restocking Walker Lake if and when possible. The rationale behind this is the

high genetic diversity in Pyramid Lake, the close genetic relationship between Walker Lake and Pyramid Lake, and the similar ecology of these two locations. Walker Lake and Pyramid Lake were connected by Pleistocene Lake Lahontan, and are its main remnants. Lahontan tui chubs ranged throughout Lake Lahontan, so perhaps Walker Lake and Pyramid Lake are closest genetically to this ancestral population. To further understand these evolutionary relationships between and among Lahontan tui chub populations, we recommend further studies using mitochondrial and nuclear sequence data.

Caution is required when making the decision to establish refugial populations. Refugial populations are often small and subject to genetic drift, bottlenecks and inbreeding. In addition, creation and management of refugial populations requires intensive and expensive management and genetic analysis to ensure the populations are able to serve their purpose. Moreover, natural selection can cause adaptation of a refugial population to its refuge environment, making the population less adapted to its native habitat if it is ever reintroduced. At present, the population of tui chubs in Walker Lake is large and genetically diverse, which gives it a high potential for recovery. Therefore, from a conservation standpoint, preservation of a healthy population of Lahontan tui chubs in Walker Lake *in situ* is the best option to ensure the long-term survival of tui chubs in Walker Lake.

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Table 1. List of sample locations, location code, number of samples collected in each location (N), and known stocking history

Location	Code	N	Stocking history	Year Collected
Topaz Lake, NV	TPZ	49	Formerly Alkali Lake; connected to the West Fork Walker River through a diversion ditch in 1922.	2006
Little Soda Lake, NV	LSL	50	Originally stocked in 1931; source is unknown (Kim Tisdale, personal communication).	2006
Spooner Lake, NV	SPL	48	There may have been tui chub here historically; stocked from Washoe Lake in 1993. Washoe may have had fish naturally but was also stocked with fish from Walker Lake in the 1980s and 1990s.	2006; 2008
Tahoe Keys, CA	TKS	10	No recorded stocking.	2007
Pyramid Lake, NV	PYR	33	No recorded stocking.	2008
Twin Lakes, CA	TWN	15	No recorded stocking.	2008
Walker Lake, NV	WLK	50	No recorded stocking.	2007
Stillwater National Wildlife Refuge, NV	STW	32	Fisheries inventory records indicate fish were present and common; later stocked with fish from Sleep Mine Wetlands and Walker Lake.	2007
Dixie Valley (Casey Pond), NV	DXV	17	No recorded stocking.	1998
East Fork Walker River, CA	EWR	20	No recorded stocking.	1997
South Fork Reservoir, NV	SFH	24	No recorded stocking.	2007
Independence Valley, NV	IND	41	No recorded stocking.	2007

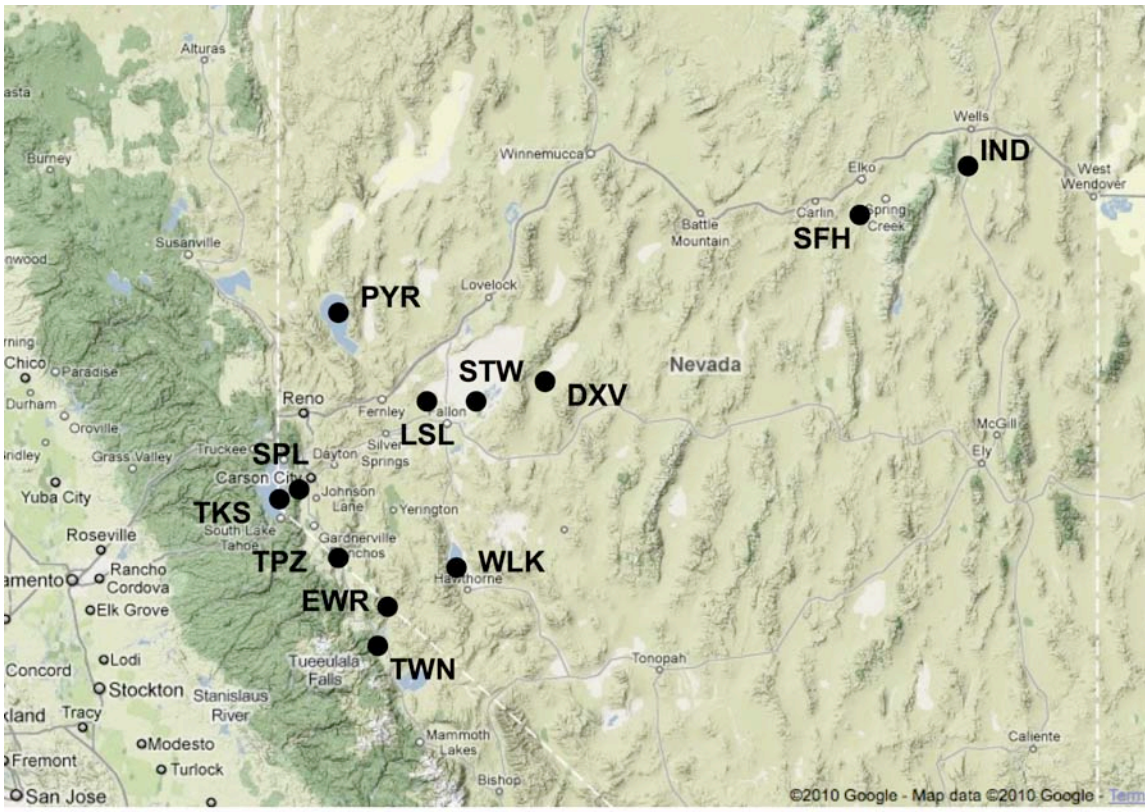

Figure 1. Sample locations for Walker Lake tui chub project. Location codes are listed in Table 1.

Table 2. Pairwise F_{ST} values calculated in *Arlequin* 3.5 with seven microsatellite loci. All values were significant (p<<0.05) after 1000 bootstrap replicates. Walker Lake data are highlighted.

Location	**TPZ**	**LSL**	**SPL**	**TKS**	**PYR**	**TWN**	**WLK**	**STW**	**DXV**	**EWR**	**SFH**	**IND**
TPZ	-											
LSL	0.08	-										
SPL	0.08	0.14	-									
TKS	0.15	0.19	0.06	-								
PYR	0.12	0.18	0.07	0.02	-							
TWN	0.07	0.13	0.08	0.07	0.06	-						
WLK	0.03	0.08	0.05	0.06	0.04	0.04	-					
STW	0.06	0.12	0.07	0.10	0.08	0.09	0.04	-				
DXV	0.10	0.12	0.15	0.22	0.20	0.17	0.10	0.16	-			
EWR	0.05	0.10	0.12	0.14	0.13	0.04	0.04	0.09	0.16	-		
SFH	0.06	0.14	0.06	0.08	0.06	0.07	0.04	0.05	0.15	0.09	-	
IND	0.27	0.35	0.21	0.21	0.17	0.23	0.22	0.24	0.36	0.31	0.20	-

Table 3. Number of sample genotyped, expected (H_e) and observed (H_o) heterozygosities, and average number of alleles across loci (N_A) and number of private alleles (N_P) were calculated in GDA (Lewis and Zaykin 2001). Allelic richness (*A*) and private allelic richness (*P*) values were calculated in HP-Rare (Kalinowski 2005) using a minimum of 34 genes. Tahoe Keys and Twin Lakes did not have enough samples to reliably provide *A* and *P* estimates, and were not included in that analysis.

Location	**N**	**H_e**	**H_o**	**N_A**	***A***	**N_P**	***P***
TPZ	49	0.78	0.80	13.89	10.5	9	0.54
LSL	50	0.67	0.62	7.33	6.28	2	0.18
SPL	48	0.77	0.76	8.78	7.19	5	0.65
TKS	10	0.76	0.72	6.78	-	1	-
PYR	33	0.82	0.72	13.33	11.35	4	0.47
TWN	15	0.77	0.70	8.44	-	1	-
WLK	50	0.83	0.83	17.11	12.25	16	1.06
STW	32	0.80	0.80	10.44	8.92	3	0.33
DXV	17	0.62	0.61	5.44	5.44	2	0.26
EWR	20	0.73	0.68	8.33	8.00	1	0.13
SFH	24	0.78	0.78	9.78	8.88	3	0.35
IND	41	0.53	0.50	6.56	5.73	12	1.26

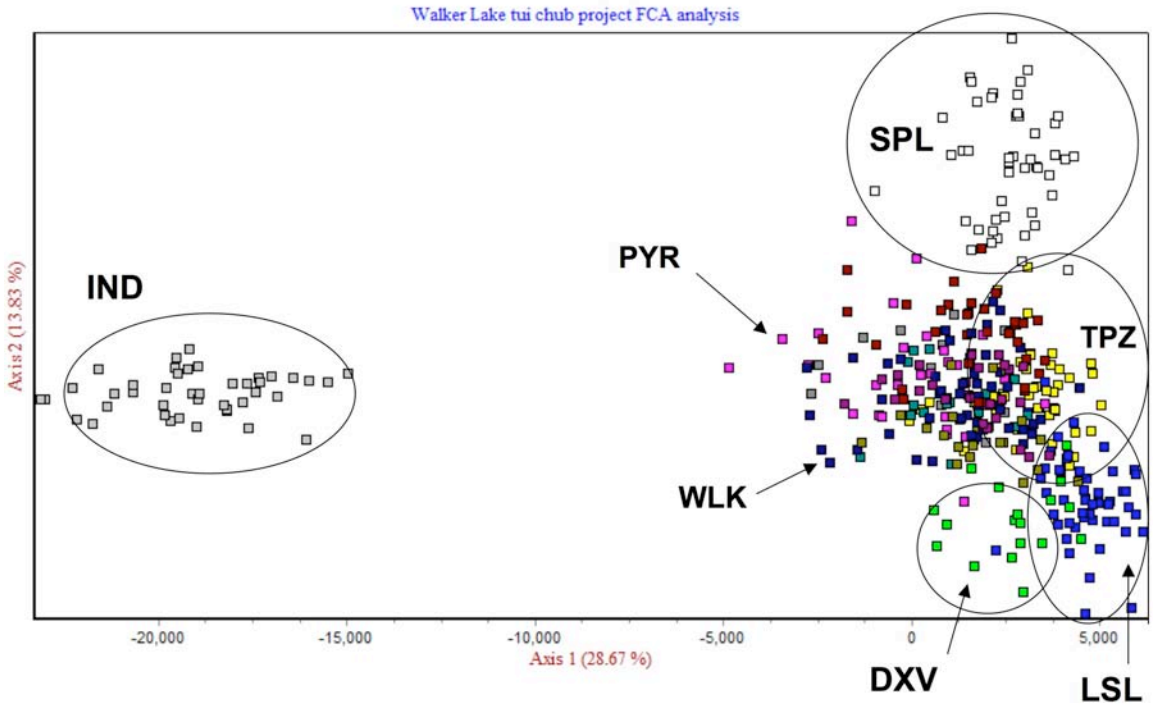

Figure 2. A graphical representation of the FCA analysis that includes all 12 locations sampled. Independence Valley (IND; gray) forms the most distinct cluster, followed by Spooner Lake (SPL; white). The remaining locations form a diffuse cluster with Dixie Valley (DXV; bright green), Topaz Lake (TPZ; yellow) and Little Soda Lake (LSL; blue) on the margins. Pyramid Lake (PYR; pink), Stillwater National Wildlife Refuge (STW; dark red) and Walker Lake (WLK; dark blue) cover the widest area, indicating higher genetic diversity. Twin Lakes (TWN; brown) and Tahoe Keys (TKS; green) fall within this third cluster.

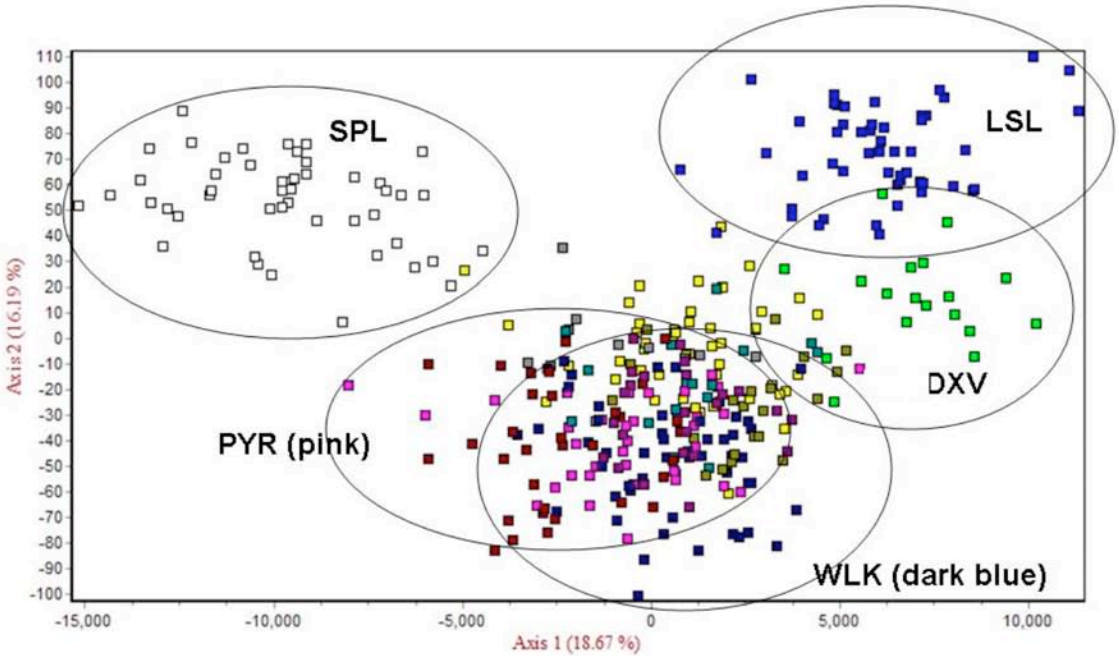

Figure 3. Graphical representation of the FCA analysis with 11 locations (Independence Valley is removed). Spooner Lake (SPL; white) forms an independent cluster. Little Soda Lake (LSL; blue) forms a distinct cluster with Dixie Valley (DXV; bright green) forming a cluster between it and a fourth cluster of Pyramid Lake (PYR; pink), Walker Lake (WLK; dark blue), Stillwater National Wildlife Refuge (STW; dark red), Topaz Lake (TPZ; yellow), Twin Lakes (TWN; brown), and Tahoe Keys (TKS; green).

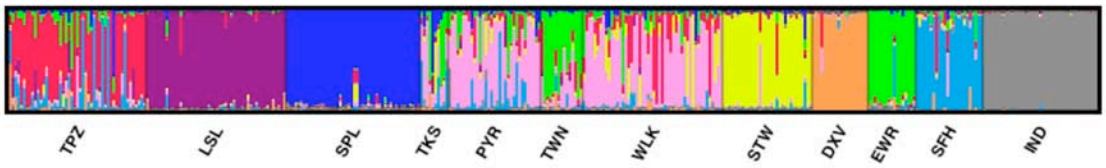

Figure 4. *STRUCTURE* output with *K* = 9 showing population substructure among 12 sample locations. Independence Valley and Dixie Valley form distinct independent clusters. Tahoe Keys, Pyramid Lake, Walker Lake, Twin Lakes and Topaz Lake are somewhat unresolved, though Topaz Lake is different from the former locations. East Fork Walker River appears to be somewhat similar to Twin Lakes, though Twin Lakes also groups in part with the Walker-Pyramid cluster. Little Soda Lake, Spooner Lake, Stillwater National Wildlife Refuge, and South Fork Reservoir each form their own independent cluster.

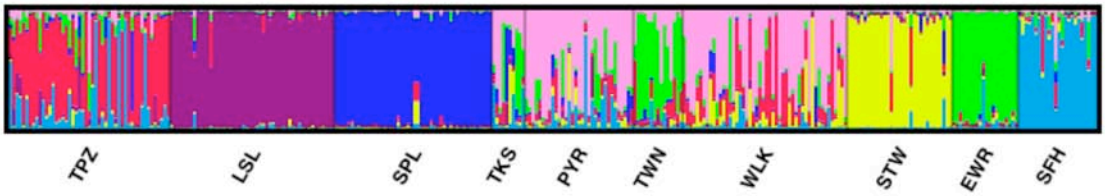

Figure 5. *STRUCTURE* output with *K* = 7 showing population substructure among 10 sample locations (excluding Dixie Valley and Independence Valley). Tahoe Keys, Pyramid Lake, Walker Lake, Twin Lakes and Topaz Lake are somewhat unresolved, though Topaz Lake is different from the former locations. Little Soda Lake, Spooner Lake, Stillwater National Wildlife Refuge, East Fork Walker River and South Fork Reservoir each form their own cluster.

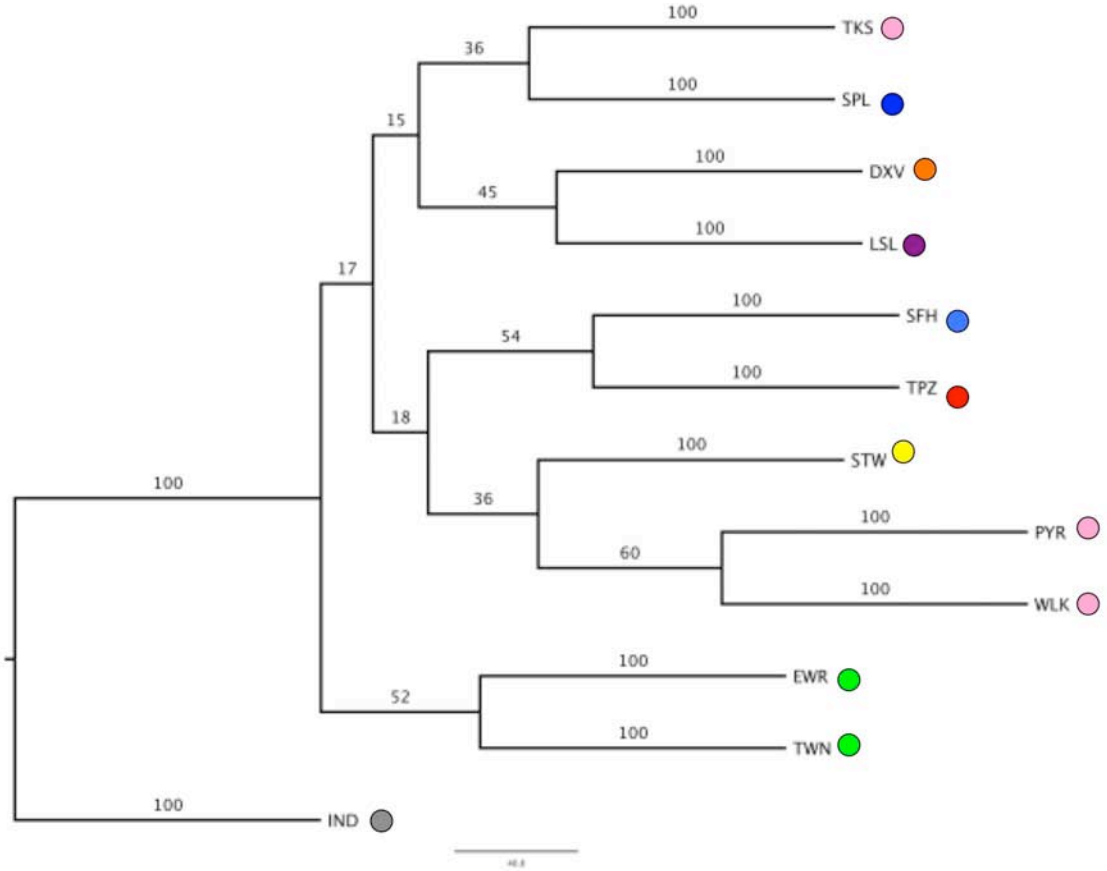

Figure 6. Unrooted Neighbor-Joining tree built with 9 microsatellite loci using Cavalli-Sforza cord distances (D_{CE}). Independence Valley is designated as outgroup. The numbers above branches are bootstrap values after 1000 replicates. Values under 60 cannot be considered reliable (e.g. only 36% of 1000 trees placed Stillwater as a sister taxon to Pyramid Lake and Walker Lake). Colored dots correspond to groupings in the *STRUCTURE* analysis.

Figure 7. Map showing locations analysed with *STRUCTURE* where *K*=9. Colored dots correspond to groupings in the respective *STRUCTURE* bar graph.

Table 4. Loci used in this study with allele sizes and frequency of each allele in each location, and over all locations, weighted for sample size.

Allele size	TPZ	LSL	SPL	TKS	PYR	TWN	WLK	STW	DXV	EWR	SFH	IND	All (weighted)
Gbi-G13													
202	-	-	-	-	-	-	-	-	-	-	-	0.84	0.09
206	-	-	-	-	0.04	-	0.01	-	-	-	-	0.16	0.02
210	0.10	0.27	0.13	0.38	0.28	0.63	0.23	0.07	0.16	0.55	0.19	-	0.20
214	0.08	-	-	0.06	0.11	0.07	0.04	0.13	-	-	0.17	-	0.05
218	0.08	0.21	0.17	0.13	0.15	-	0.09	0.15	0.13	0.05	0.06	-	0.11
222	0.09	0.15	0.27	0.31	0.11	0.03	0.27	0.47	-	0.20	0.21	-	0.18
226	0.20	0.21	0.12	-	0.13	0.23	0.14	0.08	-	0.18	0.06	-	0.13
230	0.04	-	-	-	0.04	-	0.07	-	0.13	-	0.13	-	0.03
234	0.09	-	-	-	0.11	0.03	0.06	0.05	-	-	0.04	-	0.04
238	0.19	-	0.32	-	0.02	-	0.02	0.07	0.13	0.03	0.15	-	0.09
242	0.09	-	-	-	-	-	0.02	-	-	-	-	-	0.02
246	0.01	0.12	-	0.06	0.02	-	0.03	-	-	-	-	-	0.02
250	0.01	0.02	-	-	-	-	0.01	-	0.47	-	-	-	0.03
254	-	0.02	-	0.06	-	-	-	-	-	-	-	-	<0.01
N	49	50	48	8	27	15	48	31	16	20	24	41	
Gbi-G38													
244	-	0.02	-	-	-	-	-	-	-	-	-	-	<0.01
248	-	0.13	-	-	0.02	-	-	-	-	-	-	-	0.02
252	-	-	-	-	0.04	-	0.01	-	-	0.08	-	-	0.01
256	-	-	0.05	-	0.02	-	0.03	0.13	-	-	-	-	0.02
260	0.05	-	-	0.06	0.02	0.10	0.10	-	0.06	0.05	-	-	0.03
262	0.01	-	-	-	0.02	-	-	-	-	-	-	-	<0.01
264	0.07	0.02	0.03	-	0.02	-	0.01	-	-	-	0.02	-	0.02
268	0.08	-	0.09	-	0.05	0.03	0.04	0.02	-	-	-	-	0.04
272	-	-	0.04	-	0.02	-	0.02	0.09	-	-	-	-	0.02
276	0.04	0.03	-	-	-	-	0.01	0.02	-	-	-	-	0.01
280	0.03	-	0.01	0.06	0.02	-	0.02	-	0.15	0.08	-	-	0.02
284	0.07	-	-	0.06	0.04	-	0.03	-	-	0.10	-	-	0.02
288	0.01	0.32	0.02	0.17	0.13	0.10	0.05	0.36	-	0.05	0.17	-	0.12
292	0.06	-	0.16	0.17	0.09	0.20	0.09	0.09	-	0.10	0.10	-	0.08
296	0.07	0.23	0.03	0.11	0.11	0.03	0.11	0.05	0.09	0.18	0.04	-	0.09
300	0.06	0.16	0.04	0.17	0.04	0.03	0.13	-	0.56	-	0.02	-	0.09
304	0.10	0.03	0.16	0.11	0.13	0.13	0.11	0.09	-	0.03	0.06	-	0.08
308	0.13	-	0.08	-	0.02	-	0.02	-	-	0.03	0.29	-	0.05
312	0.05	-	0.09	0.06	0.04	-	0.03	0.06	-	0.13	0.15	-	0.05
316	0.04	-	-	-	0.04	-	0.01	0.05	-	0.03	0.02	0.03	0.02
322	0.02	-	0.05	0.06	0.12	0.03	0.04	-	-	-	-	0.06	0.03
326	0.04	0.06	0.09	-	0.05	0.13	0.02	0.02	-	0.03	-	0.09	0.05
330	-	-	-	-	0.02	0.13	0.02	0.02	0.15	0.03	-	0.15	0.03
334	0.02	-	-	-	-	-	0.03	-	-	-	-	0.15	0.02
338	0.01	-	-	-	-	-	0.01	-	-	-	0.02	0.02	<0.01
342	0.01	-	0.01	-	-	-	0.01	0.02	-	0.05	0.10	0.11	0.02
346	-	-	0.03	-	-	0.07	-	-	-	0.08	-	0.08	0.02
350	-	-	-	-	-	-	0.02	-	-	-	-	-	<0.01
354	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01

358	-	-	-	-	-	-	-	-	-	-	-	0.03	<0.01
378	-	-	-	-	-	-	-	-	-	-	-	0.06	0.01
382	-	-	-	-	-	-	-	-	-	-	-	0.12	0.01
386	-	-	-	-	-	-	-	-	-	-	-	0.06	0.01
388	-	-	-	-	-	-	-	-	-	-	-	0.05	<0.01
N	48	50	48	9	28	15	49	32	17	20	24	33	

Gbi-G39													
186	-	-	-	-	-	-	0.04	-	-	-	-	-	0.01
190	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01
194	-	-	-	-	0.05	-	0.04	0.11	-	-	-	1.0	0.12
198	-	-	-	-	0.08	0.03	0.13	0.03	-	-	-	-	0.03
202	0.03	-	-	-	0.03	-	0.09	0.02	-	-	0.02	-	0.02
206	-	-	-	0.08	-	0.07	0.04	0.02	-	0.18	-	-	0.02
210	0.03	0.12	0.03	-	0.08	0.03	-	-	0.32	-	0.06	-	0.05
214	0.17	0.42	-	0.17	0.18	-	0.06	-	-	0.10	0.13	-	0.12
218	0.09	0.17	0.17	-	0.16	0.10	0.05	0.05	-	0.15	0.04	-	0.09
222	0.17	0.06	0.05	0.08	0.03	0.13	0.03	0.02	0.09	-	-	-	0.06
226	0.04	0.07	-	-	-	0.17	0.05	0.03	-	-	0.02	-	0.03
230	0.04	0.01	0.06	-	0.08	0.23	0.05	0.14	0.09	0.10	0.04	-	0.06
234	0.11	0.07	0.04	0.08	0.03	0.07	0.05	0.08	0.41	0.18	0.10	-	0.08
238	0.09	0.04	0.05	0.08	0.08	-	0.05	0.05	-	-	0.17	-	0.05
242	0.07	0.04	-	-	0.11	0.03	0.05	0.17	-	0.23	0.31	-	0.08
246	0.07	-	0.01	0.17	-	0.10	0.08	0.08	-	0.05	0.10	-	0.05
250	0.07	-	-	-	0.03	-	0.08	0.11	-	0.03	-	-	0.03
254	-	-	0.21	-	0.05	-	0.03	0.11	-	-	-	-	0.05
258	-	-	0.11	-	-	-	0.01	-	-	-	-	-	0.02
262	-	-	0.18	0.08	-	-	0.04	-	-	-	-	-	0.03
270	-	-	0.06	0.08	0.03	-	-	-	-	-	-	-	0.01
274	-	-	0.01	-	-	-	-	-	-	-	-	-	<0.01
278	-	-	-	0.08	-	0.03	-	-	-	-	-	-	<0.01
282	-	-	-	0.08	-	-	-	-	0.05	-	-	-	<0.01
286	-	-	-	-	-	-	-	-	0.05	-	-	-	<0.01
N	49	50	47	6	19	15	49	32	11	20	24	37	

Gbi-G79													
226	-	-	-	-	0.06	-	-	-	-	-	-	-	0.01
228	0.01	0.04	0.10	0.10	-	0.17	0.01	0.08	-	0.20	-	-	0.05
230	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01
232	0.16	0.10	0.23	-	0.02	0.20	0.06	-	-	-	0.04	0.05	0.09
234	-	-	-	-	-	-	-	0.02	-	-	-	-	<0.01
236	0.11	-	0.06	-	0.02	0.13	0.04	0.16	0.06	0.10	-	0.05	0.06
240	0.08	-	-	0.10	0.23	-	0.13	0.03	0.09	0.08	0.04	0.31	0.09
244	0.06	0.12	0.18	0.50	0.09	0.30	0.14	0.02	0.15	0.28	0.07	0.30	0.15
248	0.10	0.10	0.29	0.25	0.11	0.03	0.15	0.19	0.21	-	0.44	0.21	0.17
252	0.16	0.03	0.12	-	0.06	0.07	0.10	0.20	0.18	0.18	0.15	0.10	0.11
256	0.17	0.06	-	-	0.12	-	0.08	0.11	-	0.15	0.20	-	0.08
260	0.07	0.55	-	0.05	0.09	0.07	0.07	0.08	0.29	0.03	-	-	0.12
264	0.05	-	-	-	0.05	0.03	0.14	0.06	-	-	0.04	-	0.04
268	0.01	-	-	-	-	-	0.01	0.03	0.03	-	-	-	0.01
272	-	-	-	-	0.06	-	0.03	-	-	-	0.02	-	0.01
276	-	-	0.02	-	0.02	-	0.01	-	-	-	-	-	0.01
280	-	-	-	-	0.02	-	0.01	0.03	-	-	-	-	0.01
284	-	-	-	-	0.03	-	-	-	-	-	-	-	<0.01
290	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01

292	-	-	-	-	0.03	-	-	-	-	-	-	-	<0.01
298	-	-	-	-	0.02	-	-	-	-	-	-	-	<0.01
N	49	50	47	10	33	15	48	32	17	20	23	41	

Gbi-G87													
181	0.13	-	-	-	-	-	-	-	-	0.05	0.04	-	0.02
185	0.04	0.11	-	-	-	-	-	-	-	-	0.02	-	0.02
193	0.10	0.09	0.18	-	0.02	-	-	0.07	-	-	0.10	-	0.06
197	0.13	0.06	0.09	0.30	0.04	0.03	0.01	0.03	0.07	0.45	0.42	-	0.11
201	0.07	-	0.16	0.10	0.04	-	0.01	0.02	0.07	0.08	0.02	-	0.05
205	0.02	-	-	-	0.02	0.30	0.03	-	0.49	0.03	0.19	-	0.05
207	-	-	-	-	0.02	-	-	0.05	-	-	-	-	0.01
209	0.03	0.08	-	-	0.04	-	0.03	0.03	-	0.10	0.06	-	0.03
213	0.04	0.22	-	0.20	0.11	0.30	0.05	0.02	-	-	0.02	-	0.07
215	-	-	-	-	0.02	-	0.07	-	-	-	-	-	0.01
217	0.02	-	0.01	-	0.04	-	0.06	-	-	-	-	-	0.02
219	-	-	-	-	0.07	0.03	0.02	0.18	-	-	-	-	0.02
221	0.04	-	0.29	0.20	0.09	0.07	0.06	0.05	-	-	-	-	0.07
223	-	-	-	-	-	-	0.03	-	-	-	-	-	<0.01
225	0.20	0.32	0.16	0.10	0.09	0.07	0.04	0.08	-	0.05	-	-	0.12
227	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01
229	0.04	-	0.03	-	0.02	0.10	0.14	0.12	-	-	-	-	0.04
233	0.03	-	-	0.10	0.13	-	0.08	0.08	0.07	-	0.04	-	0.04
237	0.05	-	-	-	0.07	0.03	0.09	0.02	-	0.03	-	0.09	0.04
241	0.03	0.09	-	-	0.13	0.03	0.05	0.07	-	-	0.08	0.04	0.05
245	-	0.01	-	-	0.02	-	0.07	0.05	0.37	0.08	-	0.08	0.04
249	-	-	-	-	-	-	0.06	0.03	-	0.03	-	0.16	0.03
253	-	-	-	-	0.02	-	0.05	0.08	-	-	-	0.16	0.03
257	-	-	0.01	-	-	-	-	-	-	0.13	-	0.11	0.02
261	-	-	0.06	-	-	0.03	0.01	0.02	-	-	-	0.14	0.03
265	0.01	0.02	0.01	-	-	-	-	-	-	-	-	0.01	0.01
269	-	-	-	-	-	-	-	-	-	-	-	0.03	<0.01
273	-	-	-	-	-	-	-	-	-	-	-	0.06	0.01
277	-	-	-	-	-	-	-	-	-	-	-	0.13	0.01
281	-	-	-	-	-	-	-	-	0.03	-	-	-	<0.01
289	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01
N	49	50	48	5	23	15	49	30	15	20	24	40	

Cyp-G3													
205	-	-	-	-	-	0.07	-	-	-	0.05	-	-	0.01
213	-	-	-	-	-	-	-	-	-	0.03	-	-	<0.01
217	0.06	-	-	-	-	-	0.01	-	-	0.08	0.13	-	0.02
221	0.18	0.38	0.37	0.50	0.17	0.07	0.26	0.11	0.71	0.08	0.06	-	0.23
223	-	0.01	-	-	-	-	-	-	-	-	-	-	<0.01
225	0.04	-	-	-	0.09	-	0.01	0.06	0.03	-	0.06	-	0.03
227	-	-	-	-	-	-	-	0.17	-	-	-	-	0.01
229	0.03	0.05	0.14	-	0.09	0.03	0.05	0.08	-	0.05	0.10	-	0.06
233	0.07	0.03	0.02	-	0.02	0.03	0.03	0.05	0.15	-	0.04	-	0.04
237	0.09	-	0.05	-	0.03	0.07	0.11	0.23	-	0.03	-	0.04	0.06
239	-	-	-	-	-	-	0.01	-	-	-	0.04	-	<0.01
241	0.06	-	-	-	0.16	0.17	0.05	-	-	0.05	0.06	0.02	0.04
243	0.01	-	-	-	-	-	-	-	-	-	-	-	<0.01
245	0.05	-	0.03	0.13	0.03	0.13	0.02	0.13	-	0.08	0.06	0.17	0.06
247	0.02	-	-	-	-	-	-	-	-	-	-	-	<0.01
249	0.02	0.03	-	-	0.05	0.03	0.05	-	-	-	0.15	0.43	0.07

251	0.01	-	-	0.06	-	-	0.02	-	-	-	0.02	-	0.01
253	0.01	0.02	-	-	0.08	0.20	0.01	-	0.03	0.13	0.02	0.12	0.04
255	0.02	-	-	-	0.03	-	0.03	0.08	-	0.03	0.02	0.01	0.02
257	-	-	0.01	-	0.06	0.07	0.02	-	-	-	-	0.05	0.02
259	0.01	-	-	0.06	0.02	0.03	0.01	-	-	0.08	-	-	0.01
261	0.01	0.03	0.02	0.06	0.05	-	0.01	-	-	-	0.08	0.01	0.02
263	0.14	-	-	0.06	0.02	0.07	0.04	0.02	-	0.08	-	-	0.03
265	0.03	-	-	-	0.03	-	0.04	-	0.03	0.03	0.02	-	0.02
267	0.02	-	-	-	-	-	-	-	-	-	-	-	<0.01
269	0.02	-	-	-	0.03	-	0.04	0.02	-	-	0.02	-	0.01
271	0.01	0.06	-	-	0.02	0.03	0.01	-	-	0.23	-	-	0.03
273	-	-	-	-	-	-	0.04	0.02	0.06	-	0.02	-	0.01
277	0.01	0.29	0.35	-	0.02	-	0.02	0.02	-	-	0.02	-	0.09
281	0.01	0.10	-	-	-	-	0.01	0.03	-	-	-	-	0.02
283	-	-	-	-	-	-	-	-	-	-	0.04	-	<0.01
285	-	-	-	-	-	-	0.02	-	-	-	-	-	<0.01
289	-	-	-	-	-	-	-	-	-	-	0.02	-	<0.01
291	-	-	-	-	-	-	0.02	-	-	-	-	-	<0.01
293	-	-	-	-	-	-	0.02	-	-	0.03	-	-	0.01
297	-	-	0.01	-	0.02	-	0.01	-	-	-	-	-	<0.01
309	0.01	-	-	-	-	-	-	-	-	-	-	0.02	<0.01
312	-	-	-	-	-	-	-	-	-	-	-	0.06	0.01
316	-	-	-	-	-	-	-	-	-	-	-	0.04	<0.01
320	-	-	-	-	-	-	-	-	-	-	-	0.02	<0.01
325	0.01	-	-	-	-	-	-	-	-	-	-	-	<0.01
327	0.01	-	-	-	-	-	-	-	-	-	-	-	<0.01
333	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01
337	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01
339	0.01	-	-	-	-	-	-	-	-	-	-	-	<0.01
371	-	-	-	0.13	-	-	-	-	-	-	-	-	<0.01
N	48	50	48	8	32	15	49	32	17	20	24	41	

Cyp-G41													
167	0.93	0.94	0.49	0.10	0.13	0.58	0.60	0.65	0.97	0.85	0.57	0.15	0.60
171	0.07	0.06	0.51	0.90	0.82	0.42	0.38	0.35	0.03	0.15	0.41	0.85	0.39
175	-	-	-	-	0.05	-	0.02	-	-	-	0.02	-	0.01
N	49	50	48	10	31	12	48	26	17	20	22	41	

Cyp-G47													
170	-	-	0.01	-	-	-	0.02	-	-	-	-	-	<0.01
174	0.01	-	-	-	-	-	0.03	-	-	-	-	-	0.01
178	0.52	0.71	0.29	0.35	0.36	0.39	0.57	0.39	0.44	0.70	0.32	-	0.43
182	0.37	0.29	0.51	0.40	0.48	0.50	0.35	0.41	0.56	0.30	0.61	1.0	0.48
186	0.06	-	0.19	0.25	0.16	0.04	0.03	0.20	-	-	-	-	0.07
190	0.02	-	-	-	-	-	-	-	-	-	-	-	<0.01
194	0.02	-	-	-	-	0.07	-	-	-	-	0.07	-	0.01
N	49	50	48	10	32	14	50	32	17	20	22	40	

Cyp-G48													
118	-	-	-	-	-	-	0.02	-	-	-	-	-	<0.01
122	-	-	-	-	0.06	-	0.06	0.02	0.18	0.13	0.02	-	0.03
126	-	-	-	-	0.02	-	0.04	-	0.03	-	-	-	0.01
130	-	-	-	-	-	0.03	-	-	-	-	-	-	<0.01
134	0.02	-	-	-	0.16	-	0.03	0.03	-	-	-	-	0.02

138	-	-	-	-	-	-	0.01	0.13	-	-	0.10	-	0.02
142	-	-	0.01	-	0.02	-	0.03	-	-	-	-	-	0.01
146	0.01	0.10	-	-	-	-	0.04	0.20	-	0.05	0.02	-	0.04
150	0.13	0.06	0.02	-	0.05	0.07	0.04	0.02	0.12	-	0.17	-	0.06
154	0.04	0.14	-	-	0.06	0.07	0.03	-	-	0.05	0.13	0.01	0.04
158	0.16	0.10	-	0.10	0.06	0.07	0.04	-	-	-	0.06	-	0.05
160	-	-	0.13	-	-	-	-	-	-	-	-	-	0.02
162	0.07	-	0.18	-	0.06	0.07	0.03	-	0.09	-	-	-	0.05
164	0.02	-	0.17	-	-	-	-	-	-	-	-	-	0.02
166	0.04	0.10	0.03	0.20	0.14	0.03	0.06	0.02	0.24	0.05	0.08	0.02	0.07
170	0.06	0.01	0.01	0.10	0.14	0.17	0.14	-	-	0.20	0.04	0.09	0.07
172	-	-	-	-	-	-	-	-	-	-	0.02	-	<0.01
174	0.03	0.23	-	0.10	0.05	0.03	0.07	0.09	0.03	0.05	0.06	0.02	0.09
176	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01
178	0.09	0.02	0.15	0.10	0.05	0.23	0.04	0.17	0.03	0.13	0.04	0.02	0.08
180	-	-	0.04	-	-	-	-	-	-	-	-	-	0.01
182	0.06	-	-	0.05	0.03	-	0.02	0.05	0.12	0.03	0.06	0.20	0.05
184	-	-	0.03	-	-	-	-	-	-	-	-	-	<0.01
186	0.09	0.01	-	0.05	0.06	0.10	0.07	-	-	0.25	-	0.10	0.01
188	0.01	-	-	-	-	-	-	-	-	-	-	-	<0.01
190	0.09	0.12	0.10	0.10	0.03	0.03	0.07	0.16	0.03	0.05	0.06	0.20	0.10
194	0.03	0.11	-	0.05	0.02	0.10	0.03	0.06	-	0.03	0.06	0.20	0.06
198	0.01	-	-	0.05	-	-	-	-	0.15	-	-	-	0.01
202	-	-	-	0.05	-	-	-	-	-	-	0.02	0.12	0.02
206	0.01	-	-	0.05	-	-	0.04	-	-	-	0.04	0.02	0.01
210	-	-	0.01	-	-	-	0.02	-	-	-	-	-	<0.01
214	-	-	0.06	-	-	-	0.01	0.02	-	-	-	-	0.01
218	-	-	0.06	-	-	-	-	-	-	-	-	-	0.01
222	-	-	-	-	-	-	-	0.05	-	-	-	-	<0.01
234	0.01	-	-	-	-	-	-	-	-	-	-	-	<0.01
262	-	-	-	-	-	-	0.01	-	-	-	-	-	<0.01
N	49	50	48	10	32	15	45	32	17	20	24	41	