

Genetic relationships of tui chub populations in the northwestern Great Basin and conservation implications for the Cow Head Tui Chub

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Abstract The Cow Head Tui Chub, *Siphateles thalassinus vaccaceps*, is restricted to the small Cow Head basin in far northeastern California and northwestern Nevada. Using microsatellite DNA loci, we found extensive genetic variation among tui chub populations in the Cow Head and surrounding basins of the northwestern Great Basin. Our data support the systematic conclusions of earlier authors, while showing distinct intraspecific differences between populations in most subbasins within the pluvial basins examined. The Cow Head Tui Chub forms a distinctive population within *S. thalassinus* with greatest affinity to the tui chub in the Warner Valley into which the Cow Head Lake drains. Tui chubs from the Goose Lake and Pit River basins are genetically very similar. Warner, Abert, Summer Lake, Catlow and Guano basin tui chubs appear distinct, both from each other and from tui chubs in the other basins. However, genetic proximity and some overlap between populations in presumably isolated hydrologic basins (i.e. Summer–Goose–Warner) suggest the possibility of unrecognized hydrologic connections or anthropogenic transport across barriers. Current genetic diversity in the Cow Head Tui Chub population is similar to that of stream-

associated populations. We suggest that the Cow Head Tui Chub should remain a species of conservation concern due to its highly restricted distribution in an arid environment. Successful conservation of this unique fish will depend on stewardship of its limited habitat and maintenance of connectivity between subpopulations in a manner that ensures its long-term viability.

Keywords Tui chub · Microsatellite DNA variation · Genetic relationship · Conservation · Northwestern Great Basin

Introduction

Tui chubs (*Siphateles bicolor* Girard *sensu lato*) are a group of minnows (Cyprinidae) widely distributed in isolated aquatic systems across the Great Basin and neighboring basins in western North America (Hubbs and Miller 1948; Sigler and Sigler 1987; Moyle 2002). Since the beginning of the 20th century, persistence of many tui chub populations and other native Great Basin fish taxa has been imperiled due to increased human activities (Moyle and Williams 1990; Echelle 1991; Minckley and Douglas 1991). Numerous taxa have received state and federal protection, or are of special conservation concern (Williams et al. 1989). Despite considerable concern for their conservation, the relationships of tui chubs in the various basins are generally unresolved; many taxa remain undescribed, and limited genetic information is available to aid conservation biologists in decision-making (Harris 2001; Chen et al. 2007). This is particularly true in the Cow Head and surrounding basins of southern Oregon, northern California and northwestern Nevada, where endemic tui chubs occupy endorheic lake basins.

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The Cow Head Tui Chub (a.k.a. Cowhead Lake Tui Chub; Reid 2007), *Siphateles thalassinus vaccaceps* (*S. bicolor vaccaceps*), was recognized as a distinct taxon by Hubbs and Miller (1948) and described as a subspecies by Bills and Bond (1980). It is thought to occur only in the small Cow Head basin of northeastern California and northwestern Nevada. Cow Head basin is a perched basin (lakebed elevation 1,597 m) that drains north into Warner Valley of Oregon (Warner Lakes elevation 1,364 m). Historically, Cow Head Lake was a shallow, marshy lake with an area of approximately 11 km² when sufficient water was available. The lakebed was altered in the late 1930s to allow drainage for agricultural uses, following the extended drought of the 1920–1930s when the lake was dry. Lake habitat is now limited to the springtime, prior to seasonal drainage, and tui chubs utilize the drainage canals, perennial springs and natural channels or sloughs in the valley floor (Scoppettone and Rissler 2006). Due primarily to human alteration of the lake, restricted habitat and natural droughts, the Cow Head Tui Chub population was proposed for federal listing as “endangered” under the Endangered Species Act in 1998 (USFWS 1998); however, the proposal was withdrawn by the USFWS in 2006, based on new distribution information (Scoppettone and Rissler 2006), the signing of a conservation agreement with local stakeholders and a complete review of its conservation status (USFWS 2006).

Resolution of the Cow Head Tui Chub’s genetic variability and relationship to populations in surrounding basins is essential to conservation decisions for this species. The Cow Head and Warner basins are surrounded by six other basins containing a variety of tui chubs, most in the *S. bicolor* complex. Four members of the *S. bicolor* complex are described taxa (Cow Head, Goose Lake, Lake Abert, and Guano/Catlow Valley tui chubs), and three are generally recognized (Warner, Pit River, and Summer Lake tui chubs) but undescribed (Williams et al. 1989). It has generally been thought that the Cow Head Tui Chub would be closely related to the undescribed tui chub in Warner Valley because of the hydrologic connection between the Cow Head and Warner basins (Hubbs and Miller 1948; Bills and Bond 1980).

Using mitochondrial DNA (mtDNA) sequence data, Harris (2001) supported recognition of multiple allopatric species currently recognized under *S. bicolor* in the Cow Head and surrounding basins. He found a close relationship between tui chubs in the Cow Head, Warner and Goose Lake basins and recognized them as a single species, *S. thalassinus*, with two lineages, one in Goose Lake basin and the other in the Pluvial Warner basin (including Cow Head and Warner basins), which he referred to as *S. thalassinus* “Warner basin” pending further information. Mitochondrial DNA reflects relationships only within the

maternal lineage and is relatively slow evolving (Kocher et al. 1989; Irwin et al. 1991); therefore, it provides limited information regarding intraspecific and interpopulation variation, which is essential for conservation of taxa with limited distribution such as the Cow Head Tui Chub. For studies at the population level, a faster evolving, biparentally inherited genetic marker such as microsatellite DNA is required (Roy et al. 1994; Allen et al. 1995; Ruzzante et al. 2001).

This study uses microsatellite DNA variation to further resolve genetic relationships between the Cow Head, Warner and other geographically proximate tui chub taxa. This information will help resolve issues regarding the taxonomic and conservation status of tui chub populations in the Cow Head and surrounding basins of the northwestern Great Basin.

Materials and methods

Sample collection and DNA extraction

Tui chub samples were obtained from eight basins in the extreme northwestern Great Basin of Oregon, northeastern California and northwestern Nevada (see Fig. 1 and Table 1). Two sets of samples of Cow Head Tui Chubs were collected in sequential years (1999–2000) from Cow Head Slough, the only known population of Cow Head Tui Chubs at the time of sampling. Specimens were either

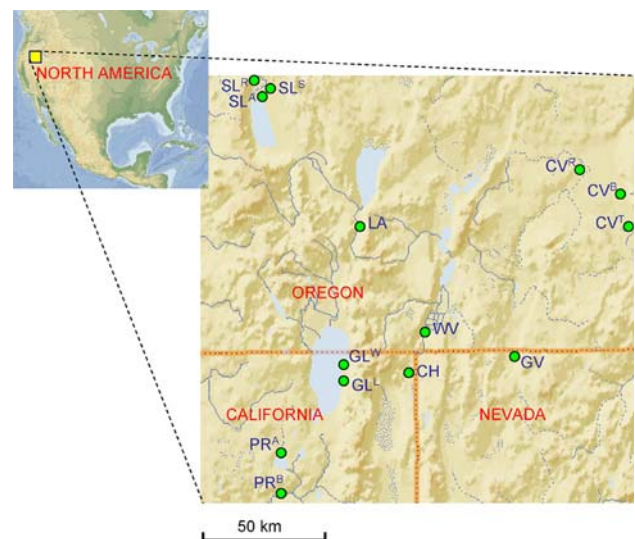


Fig. 1 Map of sampling localities for tui chubs in the Cow Head and surrounding basins in the northwestern Great Basin. This map is modified from a Microsoft Encarta map (Copyright of Microsoft Corporation 1993–2003). See Table 1 for basin and population codes

Table 1 Tui chub taxa (*Siphateles bicolor sensu lato*) and samples collected from the Cow Head and surrounding basins of the northwestern Great Basin

Basin (code)	Taxon ^{W & M}	Taxon ^H	Sampling site (code)	Museum # (sample)	Sample size (year)
Cow Head (CH)	<i>S. b. vaccaceps</i>	<i>S. thalassinus</i> (Warner)	Cow Head Slough, Modoc Co., CA (CH)	– (fin only)	21 (1999); 25 (2000)
Warner (WV)	<i>S. b. ssp</i>	<i>S. thalassinus</i> (Warner)	Twenty Mile Slough, Lake Co., OR (WV)	OS 17847 (fin)	38 (2004)
Goose (GL)	<i>S. b. thalassinus</i>	<i>S. thalassinus</i>	Lassen Creek, Modoc Co., CA (GL ^L)	– (fin only)	20 (2003)
			Willow Creek, Modoc Co., CA (GL ^W)	– (fin only)	18 (2003)
Pit River (PR)	<i>S. b. ssp</i>	<i>S. thalassinus</i>	Pit River, west of Alturas, Modoc Co., CA (PR ^A)	OS 17852 (fin)	15 (2002)
			Big Sage Reservoir, Modoc Co., CA (PR ^B)	OS 17853 (fin)	15 (2005)
Abert (LA)	<i>S. b. oregonensis</i>	<i>S. obesa</i>	Crooked Creek, Lake Co., OR (LA)	OS 17856 (fin & tissue)	37 (2005)
Summer (SL)	<i>S. b. ssp</i>	<i>S. obesa</i> *	Ana Reservoir, Lake Co., OR (SL ^A)	OS 15440 (tissue)	18 (1995)
			County Road 417 Springs, Lake Co., OR (SL ^S)	OS 15437 (tissue)	23 (1995)
			Rickert Springs, Lake Co., OR (SL ^R)	OS 15312 (tissue)	20 (1995)
Catlow (CV)	<i>S. b. eurysomas</i>	<i>S. eurysomas</i>	Black Canyon Creek, Harney Co., OR (CV ^B)	OS 17850 (fin)	46 (2005)
			Three Mile Reservoir, Harney Co., OR (CV ^T)	OS 15572 (tissue)	8 (1995)
			Rock Creek, Harney Co., OR (CV ^R)	OS 15635 (tissue)	7 (1995)
Guano (GV)	<i>S. b. eurysomas</i>	<i>S. eurysomas</i>	Fish Creek, Humboldt Co., NV (GV)	OS 15574 (tissue)	6 (1995)

Taxon citations are W & M (Williams et al. 1989; Moyle 2002) and H (Harris 2001). Cow Head and Goose Lake specimens (designated with “–”) were not sacrificed due to conservation concerns. A small proportion of specimens in Summer Lake were found to be *S. thalassinus*, indicated by “*” (Harris 2001)

preserved whole in 100% ethanol, or pelvic fin clips were taken, with one piece (10–20 mm²) air-dried and stored in a paper envelope and another preserved in DMSO buffer. Due to conservation concerns with the Cow Head and Goose Lake tui chub populations, fish were collected with minnow traps or cast nets, fin-clipped, and released. Whole genomic DNA was extracted from tissue samples using the Qiagen DNeasyTM Tissue Kit and stored at –70°C until needed.

Laboratory methods

Ten selected microsatellite loci were employed in our study, including: Gbi-G3, Gbi-G13, Gbi-G27, Gbi-G38, Gbi-G39, Gbi-G79, and Gbi-G87 (Meredith and May 2002), and CypG3, CypG47, and CypG48 (Baerwald and May 2004). The forward primer of each primer pair was labeled with a fluorescent phosphoramidite (FAM, TET, VIC, or NED; Applied Biosystems). Microsatellite DNA was amplified via the polymerase chain reaction (PCR). Each 10 µl reaction contained: 20 mM Tris–HCl, pH 8.4,

1.5 mM MgCl₂ (3.0 mM for Gbi-G13 and Gbi-G38), 0.2 mM dNTPs, 0.5 µM reverse (unlabeled) primers, 0.025 µM forward (labeled) primers, and 0.4 units Taq DNA polymerase (Promega). Reaction mixtures were amplified using the following conditions: 96°C for 2 min, followed by 35 cycles of 95°C for 40 s, 52°C for 1 min, and 72°C for 1 min, ending with an extension of 72°C for 10 min. The PCR-generated products of microsatellite DNA were electrophoresed on a 5.5% denaturing polyacrylamide gel, and visualized on a Base Station Gel Imaging System (BioRad). Composite genotypes for individual fish were compiled by scoring co-dominant alleles at each microsatellite locus using Cartographer 1.2.6 software (BioRad).

Data analyses

The dataset included 317 tui chub samples. We used CONVERT 1.2 to compute allelic frequencies (Glaubitz 2004), and FSTAT 2.9.3 to score number of alleles for each locus and each population (Goudet 1995). We used

MICRO-CHECKER 2.2.3 to identify genotyping errors of the dataset (van Oosterhout et al. 2004), and ARLEQUIN 3.0 to test for deviations from Hardy–Weinberg equilibrium (Excoffier et al. 2005). We then classified 14 tui chub populations into eight groups corresponding to eight basins where they occur (see Table 1). We computed within-basin variation with allele richness (R_A), allele number (N_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) averaged over loci using FSTAT 2.9.3 (Goudet 1995), and scored the number of private alleles (N_P) using CONVERT 1.2 (Glaubitz 2004). R_A estimates number of alleles independent of sample size (El Mousadik and Petit 1996).

Pairwise F_{ST} and its significance test were performed with ARLEQUIN 3.0 (Excoffier et al. 2005) using 10,100 permutations. Pairwise proportions of shared alleles were computed with formula: $S = 2N_{xy}/(N_x + N_y)$, where N_{xy} is the number of alleles shared by a pair of populations, N_x is the total number of alleles found in one population (No. x), and N_y is the total number of alleles found in the other population (No. y); likewise, the proportion of unshared alleles is $1 - S$. Analysis of molecular variance (AMOVA) used ARLEQUIN 3.0 to describe partitioning of genetic variation within and among basins (Excoffier et al. 1992, 2005). Lastly, we performed a factorial correspondence analysis (FCA) to project the genetic relationships of tui chubs of multiple dimensions based on the allele counts per individual with GENETIX 4.04 program (Belkhir et al. 2003).

Results

Allele frequencies and allele numbers (N_A) for each population and locus are available in Appendix 1. Allele numbers at individual loci ranged from five (CypG47) to 37 (Gbi-G3 and Gbi-G27). The alleles at eight loci varied consistently at 4 bp, suggesting they are tetra-nucleotides, except for Gbi-G39 and Gbi-G79. The difference between most alleles of Gbi-G39 is factored by 4 bp; however, two alleles, 197 and 201, differ from their neighboring alleles at 2 bp. Gbi-G79 alleles showed irregular sizes with differences that cannot be factored by either 2 or 4 bp, which could result from a 1 bp indel in the flanking region. This indel, termed as Sb-D, was first identified in tui chub populations in the southern Great Basin, e.g., Lahontan tui chub, *S. b. obesa* (Chen et al. 2007).

Our microsatellites revealed extensive genetic variation within and among tui chub populations in the northwestern Great Basin. Heterozygosities varied greatly from locus to locus and from population to population. Eleven of 140 MICRO-CHECKER tests showed evidence for null alleles ($p \leq 0.01$), five of which were associated with Gbi-G3. Eight of 134 tests of Hardy–Weinberg equilibrium indicated

Table 2 Genetic diversity averaged over nine microsatellite loci in tui chub populations from the various basins (See Table 1 for basin codes)

Index	Basin							
	CH	WV	GL	PR	LA	SL	CV	GV
H_O	0.62	0.67	0.73	0.59	0.70	0.69	0.61	0.61
H_E	0.67	0.73	0.76	0.68	0.75	0.74	0.63	0.57
R_A	3.88	4.62*	4.99*	4.34	4.46	4.45	3.54	2.83
N_A	7.22	13.78*	14.44*	10.56	10.11	12.44*	7.56	3.00
N_P	0.22	0.67	1.00	0.78	1.11	0.33	1.00	0.22
N	46	33–38	36–38	28–30	37	59–61	59–61	4–6

Asterisk indicates significant difference between Cow Head and another basin ($\alpha = 1\%$; two tailed t-test; paired two sample for means). The Guano tui chub was excluded in significance tests due to its small sample size. H_O , H_E , R_A , N_A , and N_P are the average values of observed heterozygosity, expected heterozygosity, allele richness, number of alleles, and number of private alleles, respectively. Sample size (N) varied at some loci due to amplification difficulties with some specimens

significant deviation ($p \leq 0.01$), five of which involved the same positions as null alleles at Gbi-G3 (see Appendix 1). Thus, we excluded this locus from subsequent population genetic analyses.

Excluding the small collection from Guano Basin, both observed heterozygosity (H_O) and expected heterozygosity (H_E) were similar among basins, ranging from 0.59 to 0.73 and 0.63 to 0.76, respectively (Table 2). Allele richness (R_A) and average allele number (N_A) varied from 3.54 to 4.99 and 7.22 to 14.44, respectively. The Cow Head Tui Chub population had the lowest numbers of alleles ($N_A = 7.22$) among the seven basins with similar sample sizes. However, Cow Head shared 85% of its alleles with Warner basin, 68% with Goose basin, and 42–65% with other basins. Cow Head had only two private alleles, both at low frequencies (275 at CypG3, frequency = 0.03; 229 at CypG48, frequency = 0.01). One Cow Head allele (277 at Gbi-G27, frequency = 0.18) was uniquely shared with Warner basin.

Pairwise F_{ST} values (0.03–0.27) were all statistically significant ($p \leq 0.001$), and proportions of alleles that were not shared among basins ranged from 0.29 to 0.69, while comparisons with the small sample ($n = 6$) from Guano Basin were excluded (Table 3). Concordantly, the Cow Head population showed closest relation to the Warner population ($F_{ST} = 0.13$; $1 - S = 0.42$). F_{ST} -based AMOVA indicated that 79.2% of the molecular variation occurs within populations, 18.5% among basins and 2.3% among populations within basins.

FCA showed that individual tui chubs from the same basin were genetically similar and formed discrete clusters. Although the first three principal axes (factors) explained relatively small amounts of variation of 4.43%, 3.01%, and

Table 3 F_{ST} values (below the diagonal) and proportions of unshared alleles (above the diagonal) for pairwise comparisons between tui chub populations in the various basins (See Table 1 for basin codes)

	N	CH	WV	GL	PR	LA	SL	CV	GV
CH	46	–	0.42	0.54	0.66	0.56	0.53	0.53	0.80
WV	38	0.13	–	0.29	0.38	0.44	0.31	0.64	0.76
GL	38	0.21	0.10	–	0.36	0.52	0.33	0.63	0.77
PR	40	0.23	0.12	0.03	–	0.57	0.46	0.69	0.84
LA	37	0.22	0.17	0.13	0.18	–	0.44	0.66	0.73
SL	43	0.26	0.18	0.15	0.19	0.18	–	0.64	0.76
CV	61	0.20	0.19	0.24	0.27	0.25	0.27	–	0.68
GV	6	0.19	0.20	0.24	0.28	0.23	0.24	0.18	–

All F_{ST} comparisons were statistically significant ($p \leq 0.001$)

2.96%, respectively, the FCA projection generally reflected the spatial pattern of distributions of tui chub populations in the northwestern Great Basin. Seven clusters were formed, representing: Catlow, Guano, Abert, Summer, Warner, Goose/Pit River and Cow Head basins (Fig. 2). The Guano basin tui chubs clustered near to Catlow; Lake Abert appeared distinct, but clustered near to Summer Lake; Warner Valley formed a relatively discrete cluster, exhibiting some overlap with tui chubs from Goose and Pit River basins, which showed the highest level of overlap, and the distinct Cow Head cluster showed greatest affinity to the Warner.

Discussion

Genetic relationships of tui chub populations in the northwestern Great Basin

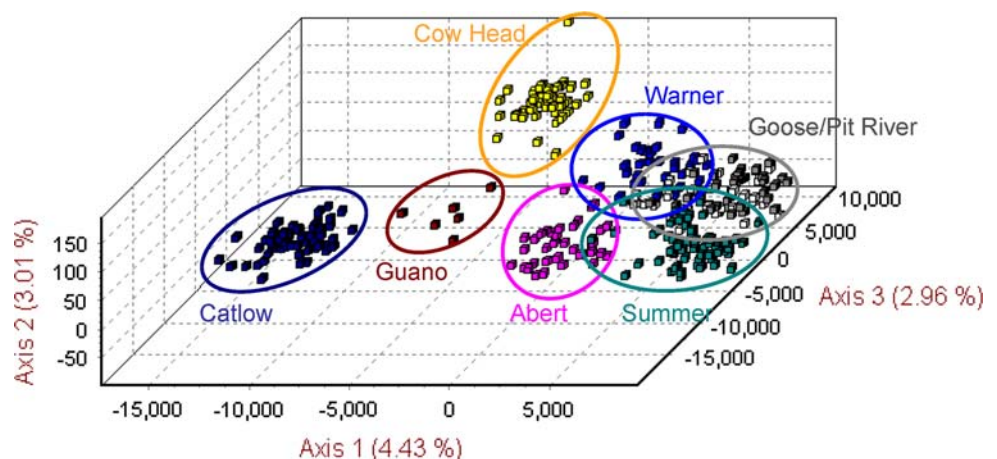
Our microsatellite data generally support systematic distinctions among tui chub populations in the northwestern Great Basin as proposed by earlier authors, while showing

distinct intraspecific differences between most subbasins within the pluvial basins examined (Cope 1883; Hubbs and Miller 1948; Harris 2001). In our study area, Harris’s (2001) recent mitochondrial DNA sequence data supported recognition of three allopatric species, i.e., *Siphateles thalassinus*, *S. obesa* and *S. eurysomas*, all previously recognized as subspecies under *S. bicolor*. *S. thalassinus* is restricted to the Pluvial Warner (including Cow Head) and neighboring Goose Lake basins; *S. obesa* is restricted (regionally) to the Pluvial Lake Chewaucan basin (including Summer and Abert subbasins); and *S. eurysomas* is restricted to the Pluvial Guano basin (including Guano and Catlow basins).

In our results, Cow Head Tui Chubs formed a clearly distinctive population within *Siphateles thalassinus*, with greatest affinity to the Warner population. Earlier authors have distinguished the Cow Head population morphologically from tui chubs of the Warner and Goose basins (Hubbs and Miller 1948; Bills and Bond 1980). However, tui chubs show high meristic and morphological variability, often associated with geographical, ecological and temporal settings, thereby increasing the uncertainties of systematic relationships based on morphology (Harris 2001; Moyle 2002). Harris’s mitochondrial DNA data showed very little distinction between Warner ($n = 3$ specimens) and Cow Head ($n = 2$) populations, which he combined and distinguished as a separate lineage within *S. thalassinus*. We recommend maintaining the Cow Head population as a distinct subspecific taxon within *S. thalassinus* until such time as the systematic relationship of the Goose and Warner tui chubs is better resolved through phylogenetic analysis.

Although the Warner and Goose basins are separated by the Warner Mountain Range, our results support Harris’s inclusion of the Warner, Cow Head and Goose Lake tui chubs within a group referred to as *S. thalassinus*. Warner and Goose populations shared a high number of alleles and showed the lowest inter-basin F_{ST} values of the populations

Fig. 2 Orthogonal plot of FCA of individual tui chubs from the northwestern Great Basin, on which degrees of genetic variation are explained by three principal axes, Axis 1, Axis 2 and Axis 3. Each square represents an individual tui chub from a specific basin: Cow Head (yellow), Warner (blue), Goose Lake (white), Pit River (gray), Abert (fuchsia), Summer Lake (teal), Catlow (navy) and Guano (maroon)



examined, slightly lower than even Cow Head-Warner (two subbasins with a direct hydrological connection) and substantially lower than conspecific population pairs only isolated since late Pleistocene pluvial lake withdrawals (i.e. Guano–Catlow and Abert–Summer). The genetic similarity between the Warner and Goose populations in both Harris's mtDNA and in more rapidly evolving microsatellites is noteworthy and contradicts Negrini's (2002) presumed hydrologic isolation of these two basins since the Pliocene or earlier. Future research should explore the possibility of an unrecognized and relatively recent hydrologic connection or anthropogenic transport of tui chubs, and perhaps other fishes, between the two basins.

Upper Pit River populations (undescribed and not considered by Harris) were genetically almost indistinguishable from Goose Lake populations and should also be included under *S. thalassinus*. Currently, Goose Lake is a disjunct subbasin of the upper Pit River. As recently as 1868, Goose Lake occasionally overflowed into the upper Pit River, and the two fish faunas share most taxa (Hubbs and Miller 1948; Moyle and Daniels 1982). Snyder (1908) and Rutter (1908) considered tui chub populations in Goose Lake and the upper Pit River to be similar, although Hubbs et al. (1979) provisionally listed the population in the upper Pit River as an undescribed subspecies. Hubbs et al. (1979) also listed the tui chubs in Hat Creek (tributary to the lower Pit River, below Pit River Falls) as an undescribed species, and when Rutter (1908) compared populations from above and below Pit River Falls, he noted substantial differences in lateral line scale counts. Material from the lower Pit River was not available for this study, and the relationship between the upper and lower Pit River populations remains unresolved.

Although Lake Abert and Summer Lake subbasins were united in the Pluvial Lake Chewaucan, they have been isolated from each other for at least 10,000–12,000 years (Negrini 2002). Their tui chub populations have been consistently distinguished based on both morphological and genetic data (Harris 2001; this study). Harris (2001) found that the Lake Abert and Hutton Springs populations formed a monophyletic clade distinct from the Summer Lake tui chub, although he included both under *S. obesa*, a generally more southern taxon, while recognizing the unresolved geographic discontinuity between Oregon and Nevada populations of this species. The Summer Lake basin population also appears distinct in our results, although it clusters near to Goose/Pit. Harris (2001) found that three of his Summer Lake specimens were identical to Goose *S. thalassinus* in cytochrome *b* sequence, suggesting the presence of both taxa in the Summer basin or some historic gene flow between populations in these two hydrologically isolated basins. Our samples came from the same museum lots used in Harris's (2001) study. Unfortunately, we do not

know which were identified as *S. thalassinus* by Harris (2001) according to mitochondrial haplotype. The Summer Lake populations have been subject to at least three eradication actions by management agencies in 1958, 1961 and 1970, suggesting that the presence of *S. thalassinus* alleles in the basin may be due to relatively recent transport of bait or forage fish from the nearby Goose basin rather than historic sympatry. Full resolution of Summer Lake basin's complex tui chub composition will require a targeted study utilizing a broader geographic sample set from Summer Lake basin, together with genetic data and morphological identification of phenotypes.

The Guano and Catlow populations have been generally considered a single taxon, with most authors including them under *Siphateles (Gila) bicolor eurysomas*, while recognizing some degree of distinctiveness (Hubbs and Miller 1948; Williams and Bond 1981). Harris (2001) included both populations under *S. eurysomas*, based on both morphological and genetic data. Our results also support similarity of the Catlow population to the Guano population, *S. eurysomas (sensu stricto)*. Catlow and Guano valleys share a low divide in the north that probably provided a hydrologic connection in the late Pleistocene (Hubbs and Miller 1948), with the Guano population currently occupying the Fish Creek drainage in the extreme south of the basin (Sheldon National Antelope Refuge, Nevada) and historical records from Piute and Guano creeks to the north (Williams and Bond 1981).

Conservation implications for the Cow Head Tui Chub

Population genetic analysis is a powerful tool in evaluating the taxonomic and conservation status of rare and endangered species and assisting in management decisions (Avice 2004; Wayne and Morin 2004; Allendorf and Luikart 2007). Our findings indicate that the Cow Head Tui Chub clearly represents a distinctive, geographically isolated population within the *S. thalassinus* complex. Presently, the taxon is restricted in range to the small Cow Head basin and dependent on limited habitat in spring-fed streams, a 1-km permanent drainage canal, intermittent reaches of Cow Head Slough and intervening stream channels between permanent spring sources (Scopettone and Rissler 2006; USFWS 2006). Though the Cow Head population has apparently maintained itself in this setting throughout the Holocene and for nearly 80 years since the original draining of Cow Head Lake in the 1920s, it showed somewhat reduced genetic diversity compared to larger lake-associated populations, principally reflected by lower allele number and richness. Nevertheless, overall allele diversity was within the range of other regional populations and did not indicate substantial loss of genetic resiliency. Furthermore, our samples were from the single

known population recognized at the time of collection (Cow Head Slough; Scoppettone and Rissler 2006), and examination of newly discovered spring-associated populations along with the larger canal population (unsampled; Scoppettone and Rissler 2006) may increase the diversity estimate of the overall population.

In general, fish are highly fecund organisms that are able to recover their population size in a relatively short period of time when conditions are favorable; however, the loss of genetic diversity due to genetic drift through periodic bottlenecks cannot be quickly restored in proportion to population size (Bonnell and Selander 1974; Franklin 1980; O'Brien et al. 1983). We have observed such an example in the endangered Mohave tui chub populations (*Siphateles bicolor mohavensis*; Chen 2006; unpublished Ph.D. Dissertation). Small and declining populations are particularly vulnerable to environmental degradation, natural catastrophes, and climate change (Allendorf and Luikart 2007). Although the original proposal to list the Cow Head Tui Chub under the Endangered Species Act was withdrawn by the USFWS in 2006, based on new distribution information, the signing of a conservation agreement with local stakeholders and a complete review of its conservation status (USFWS 2006), we suggest that

the Cow Head Tui Chub remain a species of conservation concern due to its highly restricted distribution in an arid environment with various deterministic and stochastic risks that include the potential loss of genetic variability. Successful conservation of the Cow Head Tui Chub will depend on stewardship of its limited habitat in a manner that ensures its long-term viability. Cooperative efforts should be continued to prevent loss of habitat in the Cow Head basin, manage available habitat at the ecosystem level, and maintain connectivity among the various sub-populations to preserve the overall genetic diversity of the Cow Head Tui Chub.

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Appendix 1 Allele frequencies for the 10 microsatellite DNA loci examined (See Table 1 for sampling site and basin codes)

	CH	WV	GL ^L	GL ^w	PR ^A	PR ^B	LA	SL ^A	SL ^S	SL ^R	CV ^B	CV ^T	CV ^R	GV
Gbi-G3														
228	–	–	0.03	–	–	–	–	–	–	–	–	–	–	–
236	–	0.12	0.15	0.08	0.07	0.61	–	–	–	0.03	–	–	–	–
240	–	0.01	–	0.03	0.13	–	–	–	–	–	–	–	–	–
244	–	0.04	0.05	0.11	–	–	–	0.03	–	–	–	–	–	–
248	0.14	–	0.05	–	–	–	0.01	–	–	–	–	–	–	–
252	0.02	0.01	0.03	0.03	–	–	0.03	–	–	–	–	–	–	–
256	–	0.11	0.10	0.03	0.03	–	–	–	–	–	–	–	–	–
260	0.12	0.22	0.08	–	0.20	–	–	–	–	–	–	–	–	–
264	0.10	0.19	0.08	0.06	0.03	0.07	0.04	–	–	0.16	–	–	–	–
268	0.21	0.09	–	–	–	–	0.04	–	0.19	0.13	–	–	–	–
272	0.10	0.03	0.10	0.11	0.10	–	0.03	0.17	0.31	0.03	–	–	–	–
276	0.02	0.01	0.03	–	–	0.07	0.05	0.03	–	–	–	–	–	–
280	0.08	0.01	0.05	0.03	0.03	–	0.22	–	0.05	0.03	–	–	–	–
284	–	0.01	0.05	0.03	0.13	–	0.14	–	–	–	–	–	–	–
288	0.11	0.01	0.08	–	0.07	0.04	–	0.06	0.12	0.05	–	–	–	–
292	0.05	–	–	0.03	–	–	0.19	–	–	0.08	0.07	–	–	–
296	0.02	–	0.05	0.11	–	–	0.04	–	–	–	–	–	–	–
300	–	–	0.05	0.25	–	–	–	–	–	–	–	–	–	–
304	–	–	0.03	–	–	0.11	–	0.03	–	–	0.01	–	–	–
308	–	0.01	–	0.03	–	–	0.09	–	–	–	0.01	–	–	–
312	0.03	–	0.03	–	0.03	–	0.01	–	–	–	0.04	0.13	0.14	–
316	–	0.03	–	0.06	0.07	0.11	0.11	–	0.10	0.03	0.03	0.25	–	–

Appendix 1 continued

	CH	WV	GL ^L	GL ^w	PR ^A	PR ^B	LA	SL ^A	SL ^S	SL ^R	CV ^B	CV ^T	CV ^R	GV
320	–	0.01	–	–	–	–	–	0.06	0.02	–	0.10	0.13	0.29	–
324	–	–	–	–	0.10	–	–	–	0.02	–	0.04	0.06	–	0.08
328	–	–	–	–	–	–	–	0.11	–	0.05	–	–	–	–
332	–	–	–	–	–	–	–	0.22	0.05	0.13	0.09	0.25	–	–
336	–	–	–	–	–	–	–	–	0.07	0.13	0.07	–	0.07	0.25
340	–	–	–	–	–	–	–	0.14	–	0.03	0.30	0.06	0.43	0.50
344	–	–	–	0.03	–	–	–	–	0.02	–	0.07	0.13	0.07	0.17
348	–	–	–	–	–	–	–	–	0.02	–	0.08	–	–	–
352	–	0.01	–	–	–	–	–	–	–	–	0.07	–	–	–
356	–	–	–	–	–	–	–	0.08	–	0.05	0.02	–	–	–
360	–	0.05	–	–	–	–	–	–	–	0.05	0.01	–	–	–
372	–	–	–	–	–	–	–	0.03	–	–	–	–	–	–
376	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–
384	–	–	–	–	–	–	–	–	0.02	–	–	–	–	–
400	–	–	–	–	–	–	–	0.06	–	–	–	–	–	–
H _O	0.85	0.59	0.85	0.61	0.87	0.21	0.73	0.67	0.71	0.74	0.85	1.00	0.71	0.67
H _E	0.89	0.89 ^{‡,§}	0.95	0.91 ^{‡,§}	0.92	0.67 ^{‡,§}	0.89	0.90 ^{‡,§}	0.88	0.94 ^{‡,§}	0.87	0.88	0.77	0.73
N _A	12	18	17	15	12	6	13	12	12	15	15	7	5	4
N _S	46	37	20	18	15	14	37	18	21	19	46	8	7	6
Gbi-G13														
206	1.00	0.99	0.68	0.78	0.97	0.93	0.58	0.33	0.17	0.23	1.00	1.00	1.00	1.00
210	–	0.01	0.33	0.22	0.03	0.07	–	0.11	0.30	0.05	–	–	–	–
214	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–
222	–	–	–	–	–	–	0.16	0.53	0.43	0.70	–	–	–	–
226	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–
230	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–
234	–	–	–	–	–	–	0.07	–	–	–	–	–	–	–
238	–	–	–	–	–	–	–	0.03	–	0.03	–	–	–	–
250	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–
254	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–
266	–	–	–	–	–	–	0.04	–	0.09	–	–	–	–	–
H _O	–	0.03	0.55	0.44	0.07	0.00	0.59	0.61	0.70	0.45	–	–	–	–
H _E	–	0.05	0.45	0.40	0.13	0.19	0.63	0.66	0.70	0.47	–	–	–	–
N _A	1	2	2	2	2	2	9	4	4	4	1	1	1	1
N _S	46	38	20	18	15	15	37	18	23	20	46	8	7	6
Gbi-G27														
165	–	–	–	0.03	–	–	–	–	–	–	–	–	–	–
169	–	0.28	0.42	0.22	0.53	0.57	–	0.22	0.07	0.10	–	–	–	–
173	–	–	0.03	–	0.03	–	–	–	–	–	–	–	–	–
177	–	–	–	–	0.03	–	–	–	–	–	–	–	–	–
181	–	0.01	0.03	–	–	–	–	–	–	–	–	–	–	–
185	–	–	–	–	–	–	0.04	–	–	–	0.38	0.44	0.64	–
189	–	–	–	–	–	–	0.14	–	0.04	–	0.02	0.19	–	–
193	–	–	–	–	–	–	0.08	–	–	–	0.54	0.38	0.36	0.42
197	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–
217	–	–	0.08	0.08	–	–	0.01	0.03	–	–	–	–	–	–
221	–	–	0.08	0.03	–	–	0.11	–	–	–	–	–	–	0.08
225	0.03	0.01	–	–	–	0.03	–	–	–	0.15	0.04	–	–	–

Appendix 1 continued

	CH	WV	GL ^L	GL ^w	PR ^A	PR ^B	LA	SL ^A	SL ^S	SL ^R	CV ^B	CV ^T	CV ^R	GV
229	–	0.03	–	–	–	–	0.12	–	–	–	–	–	–	–
233	–	0.09	–	0.03	0.10	–	0.04	–	–	–	–	–	–	0.33
237	–	0.09	–	0.06	–	0.07	–	–	0.07	0.05	–	–	–	0.17
241	0.08	0.01	–	0.06	0.07	–	0.01	0.03	0.07	0.05	–	–	–	–
245	–	0.01	0.03	–	–	–	0.16	0.11	0.04	0.08	–	–	–	–
249	0.04	0.04	–	0.03	–	–	0.19	0.03	0.09	–	–	–	–	–
253	0.04	0.04	–	–	0.03	–	0.05	0.03	0.09	–	–	–	–	–
257	–	0.01	0.06	–	0.03	–	–	0.06	0.11	0.10	–	–	–	–
261	–	0.04	0.03	0.06	–	0.03	0.01	0.11	–	0.15	–	–	–	–
265	–	0.01	0.03	0.08	0.07	0.13	–	–	0.07	0.10	–	–	–	–
269	0.02	0.01	–	0.06	0.03	–	–	0.17	0.09	0.08	–	–	–	–
273	0.16	0.01	0.03	0.06	–	–	–	0.08	–	0.03	–	–	–	–
277	0.18	0.04	–	–	–	–	–	–	–	–	–	–	–	–
281	0.20	0.04	0.06	0.06	–	0.07	–	–	0.02	–	–	–	–	–
285	0.05	0.01	–	0.03	–	–	0.03	–	0.07	–	–	–	–	–
289	0.09	0.03	–	–	–	0.10	–	–	0.13	–	–	–	–	–
293	0.09	0.04	0.03	–	0.07	–	–	0.06	0.02	–	–	–	–	–
297	0.01	0.01	–	0.06	–	–	–	0.03	–	0.13	–	–	–	–
301	–	–	0.03	–	–	–	–	–	–	–	–	–	–	–
305	–	0.01	0.03	0.06	–	–	–	0.03	–	–	–	–	–	–
309	–	0.01	–	–	–	–	–	–	0.04	–	–	–	–	–
313	–	0.01	–	–	–	–	–	0.03	–	–	–	–	–	–
317	–	0.04	0.03	0.03	–	–	–	–	–	–	–	–	–	–
321	–	0.03	–	–	–	–	–	–	–	–	–	–	–	–
329	–	0.01	0.03	–	–	–	–	–	–	–	–	–	–	–
H _O	0.70	0.82	0.78	0.78	0.73	0.73	0.95	0.83	0.96	0.70	0.48	0.88	0.14	0.83
H _E	0.89§	0.90	0.82	0.93	0.71	0.66	0.89	0.91	0.94	0.92‡,§	0.58	0.68	0.58	0.74
N _A	12	27	16	17	10	7	13	14	15	11	5	3	2	4
N _S	46	38	18	18	15	15	37	18	23	20	46	8	7	6
Gbi-G38														
268	–	–	0.03	0.03	–	0.37	–	–	–	0.03	–	–	–	–
272	–	–	0.03	–	–	–	–	0.03	–	–	0.03	–	–	–
276	–	–	0.03	0.03	–	–	–	–	0.04	0.08	–	–	–	–
280	0.20	–	0.05	–	–	–	0.01	0.03	0.02	–	0.07	–	–	–
284	–	–	0.03	–	0.07	–	0.01	0.06	0.28	0.22	–	–	–	0.42
288	–	–	0.03	0.06	–	–	0.03	0.03	0.02	0.17	0.01	–	–	–
292	–	0.05	–	0.03	–	0.03	0.07	–	–	–	0.41	0.31	0.57	–
296	0.07	0.08	0.03	0.03	0.13	–	0.18	–	0.04	0.08	0.07	0.06	0.07	–
300	0.08	0.14	0.05	0.11	–	0.03	0.12	0.03	–	–	0.12	0.19	0.14	–
304	0.10	0.05	–	–	0.03	–	0.14	0.03	–	–	0.03	–	–	–
308	0.27	0.07	–	–	0.07	–	0.03	0.08	0.13	0.03	–	–	–	–
312	0.22	0.03	0.08	0.06	–	–	0.09	0.19	0.04	0.11	–	–	–	–
316	0.07	0.11	–	0.03	–	–	0.07	0.06	0.04	0.08	–	–	–	–
320	–	0.04	–	–	–	–	0.08	0.08	–	–	–	–	–	–
324	–	0.17	0.10	0.03	–	–	0.09	–	–	–	–	–	–	–
328	–	0.04	0.10	0.03	0.03	–	0.04	0.19	0.15	0.06	–	–	–	–
332	0.01	0.09	0.10	0.08	0.13	–	–	–	0.13	0.03	–	–	–	–
336	–	0.07	–	–	–	0.07	0.01	0.03	–	–	–	–	–	–

Appendix 1 continued

	CH	WV	GL ^L	GL ^w	PR ^A	PR ^B	LA	SL ^A	SL ^S	SL ^R	CV ^B	CV ^T	CV ^R	GV
340	–	–	0.05	0.08	0.03	–	0.03	0.03	–	0.03	–	–	–	–
344	–	0.03	0.08	0.03	0.03	–	–	–	0.04	0.08	0.03	–	–	–
348	–	–	–	0.08	0.03	0.10	–	–	–	–	–	–	–	–
352	–	0.01	0.05	0.06	–	–	–	0.03	0.02	–	–	–	–	0.33
356	–	0.01	0.03	0.06	0.07	0.07	–	0.11	0.02	–	0.12	0.31	0.14	0.25
360	–	0.01	0.05	0.06	0.03	0.03	–	–	–	–	–	0.06	–	–
364	–	–	–	–	–	0.20	–	–	–	–	–	–	–	–
368	–	–	0.03	0.03	0.10	0.07	–	–	–	–	–	–	–	–
372	–	–	0.03	0.03	0.07	0.03	–	–	–	–	0.08	–	0.07	–
376	–	–	0.03	0.06	0.07	–	–	–	–	–	0.01	–	–	–
384	–	–	–	–	0.10	–	–	–	–	–	–	0.06	–	–
388	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–
392	–	–	0.03	–	–	–	–	–	–	–	0.01	–	–	–
404	–	–	0.03	0.03	–	–	–	–	–	–	–	–	–	–
H _O	0.78	0.89	1.00	0.89	0.73	0.87	0.95	0.89	0.83	0.89	0.72	0.88	0.57	0.83
H _E	0.83	0.92	0.96	0.97	0.95§	0.85	0.91	0.92	0.87	0.90	0.80	0.81	0.75	0.71
N _A	8	16	22	21	15	10	15	15	13	12	13	6	5	3
N _S	46	38	20	18	15	15	37	18	23	18	46	8	7	6
Gbi-G39														
175	–	–	–	–	–	–	–	–	0.07	0.05	–	–	–	–
179	–	0.30	–	–	–	–	–	–	–	–	–	–	–	–
183	–	–	–	0.08	–	–	0.09	0.50	0.54	0.58	–	–	–	–
187	–	–	0.03	0.03	–	0.03	0.01	–	–	–	–	–	–	–
191	–	–	0.18	0.03	–	–	–	0.06	0.15	0.13	–	–	–	–
195	–	0.12	0.18	0.11	0.17	0.03	–	0.28	0.09	0.10	–	–	–	–
197	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–
199	–	0.04	0.20	0.11	0.20	0.10	–	0.08	–	–	–	–	–	–
201	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–
203	–	–	0.13	0.06	0.30	0.30	–	–	0.09	0.05	–	–	–	–
207	–	0.20	0.18	0.28	0.07	–	0.09	0.06	0.07	0.05	–	–	–	–
211	–	0.04	0.08	0.14	0.13	0.07	0.01	–	–	–	–	–	–	–
215	–	0.04	–	0.03	0.03	0.03	–	–	–	–	–	–	–	–
219	0.01	–	–	–	–	–	0.31	–	–	–	0.01	–	–	–
223	0.07	0.03	–	0.03	–	–	–	–	–	–	0.22	–	0.43	0.08
227	0.26	0.04	–	0.03	–	–	0.23	0.03	–	–	0.18	0.36	0.36	0.92
231	0.33	0.07	–	–	–	–	–	–	–	–	0.10	0.07	–	–
235	0.17	0.07	–	–	0.07	–	0.04	–	–	–	–	–	–	–
243	–	0.04	–	0.03	0.03	–	0.03	–	–	–	–	–	–	–
247	0.11	0.01	–	0.03	–	0.03	0.01	–	–	–	0.11	0.29	–	–
251	0.04	0.01	–	–	–	–	–	–	–	–	0.01	–	–	–
255	0.01	–	0.03	0.03	–	0.33	–	–	–	–	–	–	–	–
259	–	–	0.03	–	–	–	–	–	–	–	–	–	–	–
267	–	–	–	–	–	0.07	–	–	–	–	0.10	0.07	0.07	–
271	–	–	–	–	–	–	–	–	–	–	0.17	0.07	–	–
275	–	–	–	–	–	–	–	–	–	0.05	0.01	–	–	–
303	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
307	–	–	–	–	–	–	–	–	–	–	0.04	0.14	0.07	–
311	–	–	–	–	–	–	–	–	–	–	0.02	–	0.07	–

Appendix 1 continued

	CH	WV	GL ^L	GL ^w	PR ^A	PR ^B	LA	SL ^A	SL ^S	SL ^R	CV ^B	CV ^T	CV ^R	GV
H _O	0.74	0.87	0.70	1.00	0.67	0.80	0.81	0.83	0.61	0.60	0.93	1.00	1.00	0.17
H _E	0.79	0.85	0.88	0.89	0.86	0.82	0.83	0.68	0.68	0.68	0.86	0.81	0.73	0.32
N _A	8	13	9	14	8	9	11	6	6	7	12	6	5	2
N _S	46	38	20	18	15	15	37	18	23	20	46	7	7	6
Gbi-G79														
206	–	0.36	0.05	0.09	0.03	0.10	–	0.06	0.15	0.13	–	–	–	–
209	–	–	–	–	–	–	0.11	0.14	0.28	0.08	–	–	–	–
210	–	–	0.05	0.03	0.07	0.07	–	–	–	–	–	–	–	–
213	–	–	–	–	–	–	–	–	–	–	0.03	–	0.14	–
214	–	0.01	–	0.03	–	–	0.30	–	–	0.05	–	–	–	–
217	0.39	0.16	–	–	–	–	–	–	0.09	0.03	0.13	–	0.14	–
218	–	–	0.08	–	–	–	–	–	0.07	0.08	–	–	–	–
221	0.14	0.01	0.03	–	–	–	–	–	–	–	–	–	–	–
222	–	0.03	0.33	0.41	0.37	0.60	–	0.22	0.17	0.13	–	–	–	–
225	–	0.01	0.05	0.06	–	–	–	–	–	0.03	0.29	0.06	0.21	0.42
226	–	0.16	0.13	0.09	0.13	0.10	0.04	–	–	–	–	–	–	–
229	0.18	–	–	–	–	–	0.01	–	–	0.03	0.04	–	–	0.58
230	–	0.01	0.10	0.12	–	–	0.22	0.06	–	0.05	–	–	–	–
233	0.02	0.01	–	0.12	–	–	–	0.03	–	–	0.02	–	–	–
234	–	0.14	0.08	–	0.30	0.07	–	0.08	–	–	–	–	–	–
237	0.05	–	–	–	–	–	0.05	0.14	0.17	0.18	–	–	–	–
238	–	0.04	–	–	–	–	0.12	–	–	–	–	–	–	–
241	0.09	0.01	0.05	–	–	–	–	0.19	0.07	0.20	–	0.06	–	–
242	–	0.03	–	–	–	–	0.12	0.06	–	–	–	–	–	–
245	–	–	0.08	0.06	0.03	0.07	–	0.03	–	0.03	0.01	–	–	–
246	–	–	–	–	–	–	0.03	–	–	–	–	–	–	–
249	0.11	0.01	–	–	–	–	–	–	–	0.03	0.36	0.63	0.14	–
253	0.01	–	–	–	0.07	–	–	–	–	–	0.11	0.25	0.36	–
H _O	0.76	0.79	0.80	0.82	0.60	0.47	0.73	0.78	0.83	0.80	0.78	0.50	0.57	0.83
H _E	0.78	0.82	0.86	0.80	0.80	0.67	0.83	0.89	0.85	0.91	0.77	0.58	0.88	0.53
N _A	8	14	11	9	7	6	9	10	7	13	8	4	5	2
N _S	46	38	20	17	15	15	37	18	23	20	46	8	7	6
Gbi-G87														
160	–	–	0.08	0.08	–	0.13	–	0.06	–	–	–	–	–	–
164	–	0.01	0.20	0.25	0.32	0.17	0.11	0.11	–	0.03	–	–	–	–
168	–	–	0.03	–	–	–	–	–	–	–	–	–	–	–
172	–	–	–	–	–	–	0.09	–	–	–	–	–	–	–
176	–	0.03	–	–	–	–	–	–	–	–	–	–	–	–
180	–	0.25	0.03	0.08	0.04	0.13	–	–	–	0.10	–	–	–	0.08
184	–	0.09	0.05	0.08	0.21	0.07	–	–	–	0.10	–	–	–	–
188	–	0.12	–	–	–	–	0.14	0.64	0.80	0.45	–	–	–	0.42
192	–	0.03	–	0.03	0.07	–	0.04	–	–	0.13	–	–	–	0.08
196	0.01	0.04	0.03	0.11	–	–	–	–	–	–	0.65	0.88	0.71	–
200	0.04	0.01	–	–	–	–	–	–	–	–	0.11	0.13	0.14	–
204	0.22	0.13	0.03	–	0.04	–	0.01	–	–	–	–	–	–	–
208	0.12	0.11	0.10	0.03	–	–	–	–	–	0.03	–	–	–	–
212	0.03	0.05	0.13	0.06	–	–	–	0.03	–	0.03	–	–	–	–
216	0.45	0.08	0.10	–	0.07	0.07	0.16	–	–	–	–	–	–	–

Appendix 1 continued

	CH	WV	GL ^L	GL ^w	PR ^A	PR ^B	LA	SL ^A	SL ^S	SL ^R	CV ^B	CV ^T	CV ^R	GV
220	0.04	0.01	0.10	0.03	–	–	0.01	–	0.07	0.03	–	–	–	–
224	–	0.01	0.03	0.08	–	0.03	0.03	0.03	0.04	0.03	–	–	–	0.17
228	0.09	0.01	0.08	0.08	0.04	0.13	0.08	0.11	0.04	0.10	–	–	–	–
232	–	–	–	–	0.11	–	0.16	0.03	0.04	–	0.08	–	–	0.25
236	–	–	–	0.06	–	–	0.16	–	–	–	0.02	–	–	–
240	–	–	–	0.03	–	–	–	–	–	–	0.09	–	0.07	–
244	–	–	0.05	–	0.11	–	–	–	–	–	0.05	–	0.07	–
248	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–
260	–	–	–	–	–	0.20	–	–	–	–	–	–	–	–
264	–	–	–	–	–	0.07	–	–	–	–	–	–	–	–
H _O	0.76	0.82	1.00	1.00	0.79	0.80	0.51	0.67	0.39	0.75	0.50	0.25	0.43	1.00
H _E	0.74	0.89	0.92	0.91	0.85	0.89	0.89 ^{‡,§}	0.61	0.39	0.77	0.57	0.23	0.49	0.79
N _A	8	16	14	13	9	9	11	7	5	10	6	2	4	5
N _S	46	38	20	18	14	15	37	18	23	20	46	8	7	6
CypG3														
191	–	–	0.18	0.03	–	0.17	–	–	–	–	–	–	–	–
195	–	0.59	0.20	0.28	0.43	0.33	–	0.33	0.57	0.61	–	–	–	–
199	–	0.09	0.05	0.14	0.27	–	–	0.17	0.26	0.14	–	–	–	–
203	–	0.02	0.10	0.14	–	0.03	0.84	0.06	0.09	0.06	–	–	–	–
207	0.17	0.02	0.05	0.03	–	–	0.09	0.08	–	–	–	–	–	–
211	0.08	0.05	0.13	0.06	–	–	0.04	0.06	–	–	–	–	–	–
215	0.42	0.06	0.20	0.19	0.23	0.23	–	0.14	0.09	0.08	–	–	–	–
219	0.25	0.08	–	–	0.03	0.17	0.03	0.14	–	0.03	–	–	–	–
223	0.04	0.06	0.08	0.03	0.03	0.07	–	–	–	–	–	–	–	–
227	–	–	–	0.06	–	–	–	–	–	–	–	–	–	–
235	–	–	0.03	0.03	–	–	–	–	–	0.08	–	–	–	–
239	–	0.03	–	0.03	–	–	–	–	–	–	–	–	–	–
241	–	0.02	–	–	–	–	–	–	–	–	–	–	–	–
247	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13
251	–	–	–	–	–	–	–	–	–	–	0.04	0.13	0.08	–
255	–	–	–	–	–	–	–	–	–	–	0.15	0.31	0.33	–
259	–	–	–	–	–	–	–	0.03	–	–	0.12	0.31	0.25	–
263	–	–	–	–	–	–	–	–	–	–	0.28	0.25	0.25	0.63
267	–	–	–	–	–	–	–	–	–	–	0.40	–	0.08	0.13
271	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13
275	0.03	–	–	–	–	–	–	–	–	–	–	–	–	–
H _O	0.54	0.42	0.80	0.83	0.60	0.80	0.22	0.72	0.61	0.39	0.63	0.75	0.67	0.75
H _E	0.74 [§]	0.64 ^{‡,§}	0.88	0.86	0.71	0.80	0.32	0.83	0.61	0.64	0.73	0.81	0.85	0.64
N _A	6	10	9	11	5	6	4	8	4	6	5	4	5	4
N _S	46	33	20	18	15	15	37	18	23	18	46	8	6	4
CypG47														
169	–	0.33	0.93	0.94	0.83	1.00	0.58	0.44	0.22	0.35	–	–	–	–
173	0.80	0.59	0.03	–	–	–	–	–	–	–	0.59	0.69	0.58	0.67
177	–	0.05	0.05	0.06	0.17	–	0.07	0.53	0.78	0.63	0.41	0.31	0.42	0.33
181	0.20	0.03	–	–	–	–	0.34	–	–	–	–	–	–	–
185	–	–	–	–	–	–	0.01	0.03	–	0.03	–	–	–	–
H _O	0.39	0.45	0.15	0.11	0.33	–	0.65	0.33	0.35	0.55	0.61	0.63	0.50	0.33
H _E	0.34	0.55	0.19	0.16	0.34	–	0.56	0.59	0.35	0.53	0.49	0.46	0.62	0.64

Appendix 1 continued

	CH	WV	GL ^L	GL ^w	PR ^A	PR ^B	LA	SL ^A	SL ^S	SL ^R	CV ^B	CV ^T	CV ^R	GV
N _A	2	4	3	2	2	1	4	3	2	3	2	2	2	2
N _S	46	38	20	18	15	15	37	18	23	20	46	8	6	6
CypG48														
121	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–
125	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–
129	–	0.01	–	–	–	0.03	–	–	–	–	–	–	–	–
133	–	–	–	0.15	–	–	–	–	–	0.03	–	–	–	–
137	–	0.07	–	–	–	–	–	–	–	–	–	–	–	–
141	–	0.03	–	0.15	0.08	–	–	–	–	–	–	–	–	–
145	–	0.06	0.21	0.03	0.04	0.03	–	0.06	0.11	0.08	–	–	–	–
149	–	0.01	0.13	0.09	0.08	0.13	–	–	0.15	0.05	–	–	–	–
153	–	0.16	0.03	0.12	0.12	0.03	0.04	–	–	–	–	–	–	–
157	–	0.10	–	–	0.12	0.10	–	–	–	–	–	–	–	–
161	–	0.09	–	–	0.04	–	0.01	–	–	–	–	–	–	–
165	–	0.01	–	–	–	–	0.18	0.03	–	0.03	–	–	–	–
169	–	0.03	0.08	0.03	–	0.17	0.04	–	–	0.10	–	–	–	–
173	0.03	0.06	0.03	–	0.15	–	0.07	–	–	–	0.02	–	–	–
177	0.09	0.03	0.05	0.09	–	–	0.05	0.17	0.15	–	0.08	–	–	–
181	–	0.06	–	–	–	–	0.04	0.25	–	0.08	0.05	–	–	–
185	0.12	0.03	0.11	–	0.08	–	0.04	0.22	0.22	0.08	0.13	0.21	0.25	–
189	–	0.01	–	–	0.08	0.13	0.31	–	0.07	0.15	0.11	0.14	0.08	–
193	–	0.06	0.05	–	0.08	0.13	0.03	0.08	0.11	0.25	0.07	–	0.08	–
197	0.09	0.03	0.08	0.09	–	0.07	0.01	–	–	–	–	0.14	0.08	–
201	0.09	0.01	0.08	0.03	–	0.17	–	0.03	0.04	0.03	0.03	0.14	0.17	–
205	0.15	0.01	–	0.12	–	–	0.01	0.06	–	–	0.21	–	0.08	0.13
209	0.05	0.07	0.05	–	–	–	0.08	0.06	0.13	0.13	0.13	–	–	0.13
213	0.08	0.01	–	0.06	–	–	–	–	–	–	0.05	0.29	0.08	–
217	0.02	0.01	0.03	–	–	–	0.04	–	–	–	0.12	–	0.17	0.25
221	0.09	–	0.03	0.03	–	–	–	0.03	0.02	–	–	0.07	–	–
225	0.18	–	–	–	–	–	0.04	0.03	–	–	–	–	–	0.50
229	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–
233	–	–	0.03	–	–	–	–	–	–	–	–	–	–	–
245	–	–	–	0.03	–	–	–	–	–	–	–	–	–	–
253	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–
257	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–
265	–	–	0.03	–	–	–	–	–	–	–	–	–	–	–
H _O	0.91	0.91	0.74	0.76	0.77	0.87	0.86	0.72	0.91	0.95	0.89	0.86	1.00	0.75
H _E	0.90	0.94	0.93	0.94	0.95	0.92	0.86	0.87	0.88	0.89	0.89	0.87	0.92	0.75
N _A	12	22	15	13	13	10	15	11	9	12	11	6	8	4
N _S	46	34	19	17	13	15	37	18	23	20	46	7	6	4

Observed (H_O) and expected heterozygosities (H_E), total number of alleles (N_A), and sample size (N_S) are summarized for each locus, with missing alleles indicated by “–”. P-value ≤ 0.01 of Hardy–Weinberg equilibrium tests indicated by ‡ on H_E, and evidence of null alleles (α = 1%) indicated by § on H_E

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