### AN ABSTRACT OF THE THESIS OF

<u>Phillip M. Harris</u> for the degree of Doctor of Philosophy in <u>Fisheries Science</u> presented on <u>October 4, 2000</u>. Title: <u>Systematic Studies of the Genus Siphateles</u> (Ostariophysi: Cyprinidae) from Western North America.

## Signature redacted for privacy.

Abstract approved:

Douglas F. Markle

The minnow genus Siphateles is distributed throughout the Great Basin and adjacent drainages of western North America. Three species are currently recognized in the genus: S. alvordensis, Alvord chub, S. boraxobius, Borax Lake chub, and S. bicolor, the tui chub. S. bicolor has long been recognized as a widespread species exhibiting geographic variation. Relationships of Siphateles are investigated through phylogenetic analyses of mitochondrial cytochrome b gene sequences. Patterns of relationships within S. bicolor do not reflect current taxonomy. Sequence data support recognition of nine allopatrically distributed species currently recognized under S. bicolor: S. bicolor, S. columbianus, S. eurysomas, S. isolatus, S. mohavensis, S. newarkensis, S. obesus, S. thalasinnus and an unnamed species from Silver Lake, Oregon. Phylogenetic relationships resolved among these taxa indicate S. mohavensis forms the sister species to all remaining tui chubs. Relationships among remaining taxa were resolved as a polytomy of three species groups: an S. isolatus group containing S. isolatus + S. newarkensis; an S. bicolor group containing S. bicolor, S. columbianus, S. eurysomas, S. thalasinnus and Siphateles sp. (Silver Lake); and an S. obesus group, consisting of a polytomy of populations from California, Nevada and southcentral Oregon.

Examination of meristic and morphometric variation in the S. bicolor species group supports recognition of species proposed by the molecular phylogenetic hypothesis. Oregon populations of S. obesus exhibit temporal variation in five scale-count characters. Temporal variation occurred in all populations; comparisons among basins and three temporal groups (pre1958, 1975-1985 and 1993 collections) indicated approximately equal magnitudes of meristic variation in these two factors. No consistent trend to patterns of temporal variation was found, except within Summer Lake, where all mean scale counts were significantly different between pre1958 collections and 1975-1985 and 1993 collections. In addition, there was no correlation between mean meristic counts and average daily summer air temperatures. Consistent meristic differences in Summer Lake tui chubs may be due to the presence of S. thalassinus in this basin, either through hybridization or by biasing population characterization. Temporal variation in meristic characters is a widespread phenomena among tui chubs, and presumably among other fish species, in southern Oregon.

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## Systematic Studies of the Genus *Siphateles* (Ostariophysi: Cyprinidae) from Western North America

by

Phillip M. Harris

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### DEDICATION

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### Systematic Studies of the Genus *Siphateles* (Ostariophysi: Cyprinidae) from Western North America

### CHAPTER 1

### GENERAL INTRODUCTION

The ichthyofauna of the western United States is depauperate in species numbers when compared to the ichthyofauna east of the Continental Divide (McAllister et al., 1986). In part, this depauperate fauna results from the active geology of the western United States, which precludes the long term environmental stability associated with increasing species diversity (Ricklefs, 1989; Reeves et al., 1995). This stochastic landscape, when coupled with increasing climatic aridity since the Late Pleistocene, has reduced both long-term persistance and formation of aquatic ecosystems in this region (Smith, 1978; Smith, 1981a).

Two other factors associated with the lesser number of fish species in the western United States are the limited number of ichthyologists historically working on this fauna (Minckley and Douglas, 1991) and the influence of the Biological Species Concept (BSC; Mayr, 1942) on taxonomy during the 1950s – 1970s. Taxonomists working under the paradigm of the BSC referred morphologically-divserse, allopatric "forms" to polytypic species (usually as subspecies) because of arbitrary levels of distinctness equated with reproductive isolation (Cracraft, 1983; McKitrick and Zink, 1988; Frost and Hillis, 1990). This practice was often done without knowledge of the phylogenetic histories of these "forms," which has direct

bearing on reproductive compatibility (Rosen, 1979). The number of polytypic fish species in the western United States, many of which exhibit obvious morphological, ecological, behavioral, biochemical, molecular and life history differences, warrants reexamination of these taxa with species concepts that are based on evolutionary lineages and are phylogenetic in application (Fiero, 1986; Mayden, 1997; Mayden, 1999). Examples of polytypic species warranting such taxonomic reevaluation include tui chubs (*Siphateles bicolor* complex), speckled dace (*Rhinichthys osculus* complex), and western trout and salmon (*Oncorhynchus*).

The genus *Siphateles* (Cope, 1883) is one of the most geographically widespread groups of minnows in western North America, extending from the Columbia Basin in the north to the Mojave Desert in the south and from eastern Nevada west to the Klamath drainage in south-central Oregon and northern California. Three species are currently recognized in the genus: *S. alvordensis*, Alvord chub, *S. boraxobius*, Borax Lake chub, and *S. bicolor*, the tui chub. The taxonomy of tui chubs is poorly resolved and has a complex history. Thirteen subspecies of *S. bicolor* are described and at least 16 other populations await examination (Williams et al., 1985; Williams et al., 1989). The complex taxonomic history of *S. bicolor* is partially the result of multiple descriptions of tui chubs by early ichthyologists exploring the western United States. For example, the Lahontan tui chub (S. b. obesus) has been described in ten different genera and as eleven different species (La Rivers, 1962). Only partial synonymys have been written for a few subspecies (e.g. S. b. obesus, La Rivers, 1962; S. b. oregonensis, Bills, 1978). Additional taxonomic confusion is attributable to both intra- and inter-basin variation in trophic morphology and body shape found among many tui chub populations, a pattern documented in other western North American cyprinids (e.g. Brown et al., 1992; Douglas et al., 1999; Williams et al., 1985; Williams et al., 1989). Given the complexity of morphological variation exhibited by tui chubs, it is not surprising that S. bicolor has received minimal beta-level systematic attention. Most systematic efforts have been limited taxonomic studies of morphological and meristic variation among populations in a few basins (e.g., Bills, 1978; Hubbs et al., 1974). Although various subspecific names have been either assigned or proposed for populations of this species complex, their application has not been accompanied by a thorough analysis of either morphological or molecular variation throughout the range of the species. Chapter 2 examines variation in mtDNA cytochrome b gene sequences in the S. bicolor complex from throughout its range. Patterns of molecular variation are intrepreted in concert with variation in outgroup species suggested by Coburn and Cavender (1992) and are used to document natural diversity within the species complex and determine phylogenetic relationships among its members. Sequence data support recognition of nine

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allopatrically distributed species currently recognized under S. bicolor. These are S. bicolor, S. columbianus, S. eurysomas, S. isolatus, S. mohavensis, S. newarkensis, S. obesus, S. thalasinnus and an unnamed species from Silver Lake, Oregon. Phylogenetic patterns elucidated in this chapter are interpreted in light of the hydrologic history of the Great Basin and surrounding areas to better understand the biogeographic history of the ichthyofauna in this region.

Based on the phylogenetic hypothesis and taxonomy proposed in Chapter 2, Chapter 3 examines meristic and morphometric variation in the *S. bicolor* species group and Oregon populations of *S. obesus*. The *S. bicolor* species group consists of *S. bicolor*, *S. columbianus*, *S. eurysomas*, *S. thalasinnus* and *Siphateles* sp. (Silver Lake). Little information is available on meristic and morphometric variation in the *S. bicolor* species group, beyond the original subspecies descriptions and an unpublished Masters thesis on geographic variation in *S. bicolor oregonensis* (= *S. obesus* this study). Univariate and multivariate analyses of meristic and morphometric characters in the *S. bicolor* species group and Oregon populations of *S. obesus* support recognition of the species, and for *S. obesus* relationships among populations, proposed by the molecular phylogenetic hypothesis.

Chapter 4 examines potential causes behind temporal patterns of meristic variation in populations of *S. obesus* and *Siphateles* sp. (Silver Lake) from

southcentral Oregon. Temporal variation in meristic characters led Bills (1978) to exclude Summer Lake tui chubs from his revision of the Oregon Lakes tui chub. Results of my study indicate that temporal variation in meristic characters is a common and widespread phenomenon among tui chub populations in southcentral Oregon. Not only do meristic traits vary over time, but they tend to vary independently (with the exception of Summer Lake tui chubs) and are not related to environmental variation. In addition, the magnitude of temporal variation is approximately that of geographic variation. The implications of these results for taxonomic and conservation decisions on the icthyofauna of southern and southeastern Oregon are discussed.

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#### CHAPTER 2

### MOLECULAR PHYLOGENETIC RELATIONSHIPS OF THE GENUS SIPHATELES (OSTARIOPHYSI:CYPRINIDAE) FROM THE GREAT BASIN OF WESTERN NORTH AMERICA

### ABSTRACT

The minnow genus Siphateles is distributed throughout the Great Basin and adjacent drainages of western North America. Three species are currently recognized in the genus: S. alvordensis, Alvord chub, S. boraxobius, Borax Lake chub, and S. bicolor, the tui chub. The tui chub, S. bicolor, has long been recognized as a single, widespread species exhibiting geographic variation in morphological characters. Current taxonomy recognizes 13 subspecies of S. bicolor, and at least 16 other populations await examination. Relationships of Siphateles are investigated through phylogenetic analyses of the complete mitochondrial cytochrome b gene. Patterns of relationships within the S. bicolor species group do not reflect current taxonomy. Sequence data support recognition of nine allopatrically distributed species currently recognized under S. bicolor. These include S. bicolor, S. columbianus, S. eurysomas, S. isolatus, S. mohavensis, S. newarkensis, S. obesus, S. thalasinnus and an unnamed species from Silver Lake, Oregon. Phylogenetic relationships resolved among these taxa indicate that S. mohavensis forms the sister species to all remaining tui chubs. Relationships among remaining taxa were resolved as a polytomy of three species groups: an S. isolatus group containing S. isolatus + S. newarkensis; an S. bicolor group containing S. bicolor, S. columbianus, S. eurysomas, S. thalasinnus and Siphateles

sp. (Silver Lake); and an *S. obesus* group, consisting of a large polytomy of populations from California, Nevada and southcentral Oregon. The general correspondence between phylogenetic relationships among species and formation of the faultblock topology of the northern Great Basin provide evidence for late-Pliocene to early Pleistocene cladogenesis leading to diversification among these species.

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#### **INTRODUCTION**

The genus *Siphateles* (Cope, 1883) is one of the most geographically widespread groups of minnows in western North America. The distribution of the genus extends from the Columbia Basin in the north to the Mojave Desert in the south and from eastern Nevada west to the Klamath drainage in south-central Oregon and northern California (Fig. 2.1). These fish occupy a wide variety of habitats, from a small, thermal lake to large rivers. Many populations exist in small, isolated springs, streams or lakes and contain relatively few individuals (Hubbs and Miller, 1948; Hubbs et al., 1974). As a consequence, these fish are fragmented into many small geographic components, confined to endorheic basins shaped by the active geology of the area (Fiero, 1986; Orr et al., 1992; Grayson, 1993), without the possibility of migration or transport among those components.

Three species are currently recognized in the genus: *S. alvordensis*, Alvord chub, *S. boraxobius*, Borax Lake chub, and *S. bicolor*, the tui chub. The taxonomy of tui chubs is poorly resolved and has a complex history. Thirteen subspecies of *S. bicolor* have been described and at least 16 other populations await examination (Williams et al., 1985; Williams et al., 1989). The complex taxonomic history of *S. bicolor* is partially the result of multiple descriptions of tui chubs by early ichthyologists exploring the western United States. For example, the Lahontan tui chub (*S. b. obesa*) has been described in 10 different genera and as 11 different species (La Rivers, 1962). Only partial synonymys have been written for a few subspecies (e.g. *S. b. obesus*, La Rivers, 1962; *S. b. oregonensis*, Bills, 1978).

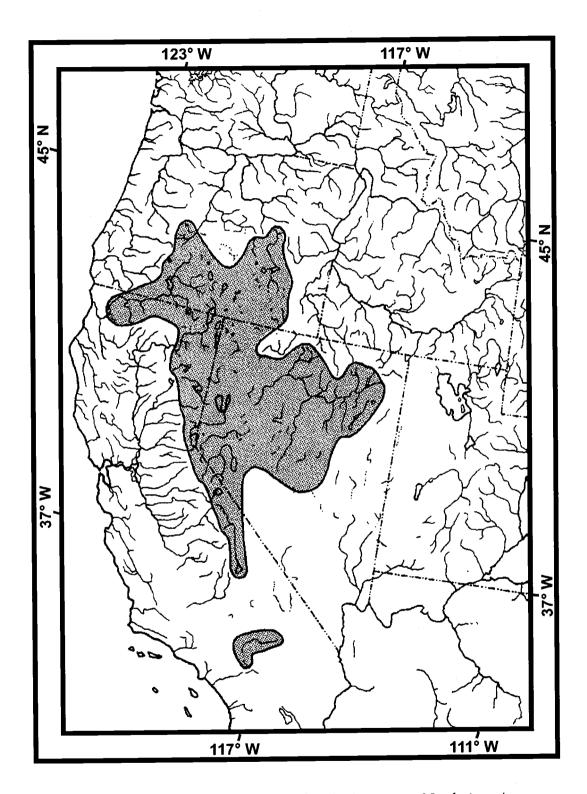


Figure 2.1. Geographic distribution of Siphateles in western North America.

Additional taxonomic confusion is attributable to both intra- and inter-basin variation in trophic morphology and body shape found among many tui chub populations, a pattern documented in other western North American cyprinids (Brown et al., 1992; Douglas et al., 1999). For example, two subspecies from Pyramid Lake, Nevada (S. b. obesus, a fluvial form, and S. b. pectinifer, a lacustrine form) are recognized on the basis of gill raker size, shape and number (Snyder, 1917). Current consensus is that S. b. obesus and S. b. pectinifer represent trophic adaptations for ecological segregation within the same population, based on studies of phenotypic plasticity in both body morphology and trophic characters in other fishes (Turner and Grosse, 1980; Lindsey, 1981; Schluter and McPhail, 1992). Despite this consensus, both subspecific epithets continue to be used in the literature (e.g. Williams et al., 1985; Williams et al., 1989). Given the complexity of morphological variation exhibited by tui chubs, it is not surprising that S. bicolor has received minimal beta-level systematic attention. Most systematic efforts have been limited taxonomic studies of morphological and meristic variation among populations in a few basins (e.g., Bills, 1978; Hubbs et al., 1974). Although various subspecific names have been either assigned or proposed for populations of this species complex, their application has not been accompanied by a thorough analysis of either morphological or molecular variation throughout the range of the species. Herein, I examine variation in the mtDNA cytochrome b gene in the S. bicolor complex from throughout its range. Patterns of molecular variation are intrepreted in concert with variation in outgroup species suggested by Coburn and

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Cavender (1992) and are used to document natural diversity within the species complex and determine phylogenetic relationships among its members. These phylogenetic patterns are interpreted in light of the hydrologic history of the Great Basin and surrounding areas to better understand the biogeographic history of the ichthyofauna in this region.

### MATERIALS AND METHODS

#### Specimens Examined

Table 2.1 lists the taxa included in this study, collecting locality, museum voucher number or collector field number and number of specimens examined. Fishes were collected using cast nets, seines, or minnow traps. At the time of collection, specimens were stored in either 95% ethanol or placed onto dry ice.

### DNA Isolation, Amplification and Sequencing

A sample of skeletal muscle was collected from each specimen, finely ground and stored in 5 ml lysis buffer solution (0.1 M Tris-HCl pH 8.0, 0.1 M EDTA, 0.01 M NaCl, and 0.5 w/v SDS, Longmire et al., 1991). DNA isolation followed the methods of Longmire et al. (1991). In brief, 0.5 mg per ml proteinase K was added and samples were incubated overnight on a tube rotator at 37°C. Samples were extracted once with 5 ml phenol saturated with Tris EDTA (TE) buffer for one hour on a tube rotator at 37°C, followed by centrifugation for seven minutes at 2,000 xg. The aqueous phase was removed and dialyzed at 4° C for 24-48 hours against three changes of the TE buffer.

I amplified and sequenced the mtDNA cytochrome b gene from genomic DNA with primers L14724 (5'-gtgacttgaaaaaccaccgttg-3'; Schmidt and Gold, 1993), L15020 (5'-gc c/t aa c/t ggcgcatcett c/t tt c/t tt-3'; Meyer, 1994), L15424N (5'-atttctttccacccatacttttc-3'; modified from Edwards et al., 1991), H15149 (5'aaactgcagcccctcagaaatatttgtcctca-3'; Kocher et al., 1989), H15669 (5'agtcctcgttgttttgaggtgtg-3', this study), and H15915 (5'aactgcagtcatctccggtttacaagac-3'; Irwin et al., 1991). PCR reactions consisted of the following: 0.1-0.5 µg genomic DNA; 5 µL 10x buffer (0.1 M tris-HCL pH 8.5, 0.015 M MgCl<sub>2</sub>, 0.5 M KCl), 5 µL dNTP mixture (2 mM each of dATP, dTTP, dCTP, dGTP in 10 mM tris-HCl, pH 7.9), 5 µL of a 10 µM solution of each of two primers, 0.5 µL of Taq polymerase (Perkin Elmer Cetus Co.), and ddH2O added for a final volume of 50  $\mu$ L. The amplification profile consisted of 95°C for 45 s, 50° C for 30 s, and 70° C for 2.5 min for 32 cycles. Double stranded PCR products were purified with QIAGEN QIAquick<sup>™</sup> PCR Purification Kits and sequenced in both directions on an Applied Biosystems Inc. 373 or 377 Automated DNA Sequencer using a Taq DyeDeoxy<sup>™</sup> Terminator Cycle Sequencing Kit (Applied Biosystems) following manufacturers recommendations.

Table 2.1. Taxon, locality information, catalog or field number, sample size (N) and number of unique haplotypes within sample (N') of specimens examined in this study. Formerly recognized subspecies now subsumed within *S. obesus* are placed in quotes.

Taxon	State	Basin	County	Locality	Catalog/Field No.	Ν	N'
Siphateles alvordensis	OR	Alvord	Harney	Trout Creek	PMH9317	2	1
Siphateles bicolor	OR	Klamath	Klamath	Upper Klamath Lake	OS16665	2	1
Siphateles boraxobius	OR	Alvord	Harney	Borax Lake	PMH9316	2	1
Siphateles columbianus	OR	Harney	Harney	Silver Creek	OS15577	3	3
Siphateles eurysomas	NV	Guano	Washoe	Fish Creek	OS15574	3	1
1	OR	Catlow	Harney	Roaring Springs	PMH9505	3	3
				Rock Creek	OS15635	3	3
				Skull Creek	PMH9504	3	1
				Three Mile Reservoir	OS15572	3	3
Siphateles isolatus	NV	Independence	Elko	Warm Springs Ranch	OS15622	3	2
Siphateles mohavensis	CA	Mohave	San Bernadino	China Lake Naval Sta.	BB 43, 44	2	1
Siphateles newarkensis	NV	Newark	White Pine	Circle Ranch	OS15744	2	2
1	NV	Fish Creek	Eureka	Fish Creek Springs	OS15743	2	2
Siphateles obesus	CA	Lahontan	Lassen	Eagle Lake	BB 18	1	1
	NV	Lahontan	Pershing	Humboldt River	OS15637	2	1
			Washoe	Pyramid Lake	BB 25, 28	2	2
			Mineral	Walker Lake	OS15111	2	2
		Dixie	Churchill	Casey Pond	OS15513	2	2
		Pleasant	Pershing	Pleasant Valley Spring	OS15512	2	1
		Big Smokey	Nye	Charnock Ranch Spring	OS15522	2	1
		Fish Lake	Esmeralda	Fish Lake Valley	OS15520	2	2
		Railroad	Nye	Blue Eagle Springs	OS15517	1	1
			5	Green Springs	OS15519	1	1
				Kate Springs	OS15515	1	1
				Bull Creek	OS15514	1	1
				Little Fish Lake Valley	BB LFL1, 2	2	1
"oregonensis"	OR	Abert Lake	Lake	XL Springs	OS16774	3	3

### Table 2.1. Continued.

Taxon	State	Basin	County	Locality	Catalog/Field No.	N	N'
				Brittain Springs	OS16722	3	2
				Crooked Creek	PMH9503	3	3
		Summer Lake		Ana Reservoir	OS15440	3	3
				Co.Rd. 417 Springs	OS15437	2	2
				Klippel Springs	OS15438	3	3
				Rickert Springs	OS15312	1	1
		Alkali		Hutton Springs	OS16771	3	2
"pectinifer"	NV	Lahontan	Washoe	Pyramid Lake	BB 48, 49	2	2
"snyderi"	CA	Owens	Inyo	Owens River	OS15747	2	1
Siphateles sp.	OR	Silver Lake	Lake	Buck Creek	PMH9401	3	2
Siphateles thalassinus	OR	Goose Lake	Lake	Goose Lake	BB 60, 62	2	2
1	CA	Cowhead	Modoc	Cowhead Slough	DFM9709	2	1
	OR	Warner	Lake	Hart Lake	BB 1, 2, 7	3	2
	OR	Summer Lake	Lake	Co. Rd. 417 Springs	OS15437	1	1
				Rickert Springs	OS15312	2	2
Eremichthys acros	NV	Black Rock Desert	Humboldt	Soldier Meadows	LVT1537, 1540	2	1
Gila orcutti	CA	Santa Margarita	San Diego	Rainbow Creek	OS15748	2	1
Hesperoleucus symmetricus	OR	Summer Lake	Lake	Ana River	OS15746	2	1
Relictus solitarus	NV	Ruby	Elko	Odgers Creek	OS15745	2	1

Nucleotide sequence and Phylogenetic Analyses

All DNA sequences were entered into XESEE, version 3.0 (Cabot and Beckenbach, 1989) and aligned by eye. Nucleotide variation and substitution patterns were examined using the software package MEGA (version 1.01; Kumar et al., 1993). Phylogenies were estimated by maximum parsimony analysis using the heuristic search option of PAUP 3.1 (Swofford, 1993) and PAUP\* (Swofford, 1998) with 10 replications and stepwise addition of taxa. The skewness of tree length distributions as a measure of phylogenetic information content (g1 statistic; Hillis and Huelsenbeck, 1992) was tested by generating 10,000 random trees. Bootstrap analysis (Felsenstein, 1985) employed 1,000 iterations. As an additional measure of tree stability, Bremer Decay Indices (Bremer, 1988, 1994) were calculated using TreeRot (Sorenson, 1996). Alternative tree topologies were constructed using MacClade (Maddison and Maddison, 1992).

The Evolutionary Species Concept (Simpson, 1961; Wiley, 1978) was used as the primary species concept in the recognition of evolutionary lineages. The Phylogenetic Species Concept (Eldridge and Cracraft, 1980; Cracraft, 1983), as modified by McKitrick and Zink (1988) to include the criterion of monophyly of species, however, was the operational definition used in the recognition of monophyletic groups of populations as species. In the application of the Phylogenetic Species Concept there is no distinction between species or subspecies in a polytypic species (Cracraft, 1983; Mayden, 1997, 1999; Warren, 1992). Thus, subspecies have no ontological status under this concept and are not recognized herein. Mayden and Wood (1995) and Mayden (1997, 1999) review the advantages and disadvantages of the phylogenetic species concept as a secondary, operational species concept.

### RESULTS

#### **Taxonomic Changes**

Given the observed patterns of mitchondrial DNA cytochrome *b* sequence variation and phylogenetic relationships among lineages within the *S. bicolor* species complex<sup>1</sup> presented below, the current taxonomy employed for tui chubs does not accurately reflect detectable historical patterns of cladogenesis and genealogical affinities. The following taxonomic changes are recommended for the *S. bicolor* complex to better reflect patterns of divergence among these fishes. In addition to the undescribed species from Silver Lake Valley identified in this study, at least eight species are recognized as currently masquerading under the name *Siphateles bicolor*: *S. bicolor*, *S. columbianus*, *S. eurysomas*, *S. isolatus*, *S. mohavensis*, *S. newarkensis*, *S. obesus*, and *S. thalasinnus*. Because of a multitude of names, only a partial synonomy for *S. obesus* is presented below; a complete synonomy is given in La Rivers (1962:412).

<sup>&</sup>lt;sup>1</sup> The terms "S. bicolor species complex" or "S. bicolor complex" refers to all Siphateles except S. alvordensis and S. boraxobius; "S. bicolor clade" refers to all tui chubs except S. mohavensis.

### Siphateles bicolor (Girard)

### Klamath tui chub

Algansea bicolor, Girard 1856:183 [original description; type locality: Upper

Klamath Lake, Klamath Co., Oregon].

Leucos bicolor, Jordan and Gilbert 1882:246[synopsis].

Myloleucus parovanus, Cope 1883:143 [abundance in Upper Klamath and Goose Lakes].

Rutilus bicolor, Jordan and Evermann 1896:244 [synonomy, description, distribution.]

Rutilus bicolor, Snyder 1908:94 [meristic comparison with R. columbianus, R. oregonensis, and R. thalasinnus].

Siphateles bicolor, Snyder 1919:60 [synonomy of forms].

Siphateles bicolor bicolor, Schultz 1935:379 [list of northwest fishes].

Types. Holotype: USNM 192.

Distribution: Klamath Basin of southern Oregon and northwestern California. Specimens examined from Upper Klamath Lake, Oregon.

Comments: The two *S. bicolor* examined from Upper Klamath Lake exhibited a single haplotype, which had three diagnostic nucleotide positions (positions 63 and 563 autapomorphic; position 1047 is a homoplasious, third position transition with character state shared with two *S. obesus* specimens). Girard (1856) provided minimal information and no illustration in his description of *S. bicolor*. Snyder (1908) provided a table of meristic and morphometric data on 20 specimens of *S*.

*bicolor* for comparison with *S. columbianus* and his *S. oregonensis* (= *S. obesus* this study), but did not compare *S. bicolor* with *Siphateles* sp. (Silver Lake Valley), its sister-species. *S. bicolor* has fewer lateral line and predorsal scales and fewer scale rows above and below the lateral line and around the caudal peduncle, and shorter snout and upper jaw lengths and longer dorsal fin and anal fin base lengths than it sister-species *Siphateles* sp. (Silver Lake) (Chapter 3).

#### Siphateles columbianus (Snyder)

### Columbia tui chub

Rutilus columbianus, Snyder 1908:92 [original description; type locality: Warm

Springs, near Harney Lake, Harney Co., Oregon].

Siphateles columbianus, Snyder 1917:60 [partial synonomy].

Siphateles bicolor columbianus, Schultz 1935:380 [list of northwest fishes]. Types. Holotype: USNM 55595. Paratypes: ANSP 39126 (1), 39127 (1); SU 4106 (2), 9816 (23), 28789 (5); UMMZ 139030 (6); USNM 59841 (3), 117490 (1). Distribution: Restricted by this study to the Harney Basin, Oregon. Specimens examined from Harney Lake, Oregon.

Comments: Three haplotypes were found in the three specimens of *S. columbianus* examined in this study; average sequence divergence among these haplotypes was 0.50%. *S. columbianus* can be diagnosed by seven nucleotide characters (position 936 autapomorphic; position 624 is an apomorphic third position transition and positions 714 and 1065 are apomorphic third position transversions found in two of

three specimens; position 625 is a first position transition shared only with the Fish Lake Valley population of S. obesus; and, positions 639 and 1129 are third position transitions shared only with outgroup taxa). Snyder's (1908) description of S. columbianus included meristic and morphological comparisons with S. bicolor, his S. oregonensis (= S. obesus this study) and S. thalassinus; he did not compare S. columbianus with its sister-species, S. eurysomas. S. columbianus differs from S. eurysomas in having fewer lateral line and predorsal scales and fewer scale rows above and below the lateral line (Chapter 3). Collections from the lower Columbia River (Lee et al., 1981), upper Deschutes River, Oregon (see discussion under Siphateles sp. below), central Washington (Wydoski and Whitney, 1979) and central Idaho (Simpson and Wallace, 1978) have been referred to as S. columbianus. Simpson and Wallace (1978) suggested the central Idaho populations may represent introductions. Populations from central Washington may also be introduced given their disjunct distribution. The taxonomic status of these populations remains to be tested.

### Siphateles eurysomas (Williams and Bond)

### Sheldon tui chub

Gila bicolor eurysoma, Williams and Bond 1981:223 [original description; type locality: Fish Creek, ca. 0.8 km upstream of Andy's Place, Washoe Co., Nevada].

Siphateles obesus, Hubbs and Miller 1942:Appendix G [local form in Guano Valley].

Siphateles sp., Hubbs and Miller 1948:63 [presence in Guano Valley and similarity with tui chubs in Catlow Valley].

Types. Holotype: TU 116230. Paratypes: OS 6363 (15), 6365 (4), 6445 (15), 6865 (11), 6867 (28); TU 116231 (11).

Distribution: Guano and Catlow valleys of northwestern Nevada and southeastern Oregon. Specimens examined from both valleys.

Comments: A total of eleven haplotypes in fifteen fish were identified within Guano and Catlow populations in this study; average sequence divergence among *S. eurysomas* populations ranged from 0.06-0.45%. *Siphateles eurysomas* can be diagnosed by four nucleotide characters (position 792 autapomorphic; position 90 is homoplasious but character state is only shared with *E. acros*; position 448 character state not found within *S. bicolor* clade; position 868 is a first position transition with nine of eleven specimens possessing an adenine). Hubbs and Miller (1948) noted the similarity between tui chubs from Guano and Catlow Valleys and suggested the fish were closely related.

### Siphateles isolatus (Hubbs and Miller)

#### Independence Valley tui chub

Gila bicolor isolata, Hubbs and Miller 1972:103 [original description; type locality: Warm Springs of Independence Valley, Elko Co., Nevada].

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Types. Holotype: UMMZ 186906. Paratopotypes: UMMZ 186518 (281). Distribution: This species was originally described from Warm Springs (also known as Ralph's Warm Springs) in Independence Valley, Nevada. Because of introductions of exotic fishes into Warm Springs, this species is now found in unnamed spring systems to the south of Warm Springs. Specimens examined were from this unnamed spring system.

Comments: Two haplotypes were found in the three specimens of *S. isolatus* examined in this study; average sequence divergence between these two haplotypes was 0.09%. *S. isolatus* is diagnosed by 11 nucleotide characters (positions 151, 195, 375, 580, 888, and 897 autapomorphic; positions 102, 894, and 916 are homoplasious, but unique second position [916] or third position [102, 894] transitions within the *S. bicolor* complex; positions 474 and 816 are third position transitions shared with only *G. orcutti*). Hubbs and Miller (1972) and Hubbs et al. (1974) provided limited meristic and morphometric characters in their diagnosis of this species. Diagnostic characters for this species include a distinctive black speck on midventral line at origin of lower procurrent caudal fin rays, supratemporal canal more often complete than incomplete, and a reduced number of vertebrae (modal 38 vs. 39 or 40) relative to tui chubs from Newark Valley, Fish Creek Springs, and the Lahontan basin.

# Siphateles mohavensis Snyder

# Mohave tui chub

Algansea formosa (in part), Girard 1856:183 [original description; type locality:

Merced and Mohave Rivers, California].

Leucos formosus, Jordan and Gilbert 1882:246 [synopsis].

Siphateles mohavensis, Snyder 1918:297 [original description; type locality:

Mohave River near Victor, California].

Gila (Siphateles) mohavensis, Uyeno 1961:5 [Siphateles recognized as subgenus of

Gila based on osteological examination of western cyprinids].

Gila bicolor mohavensis, Moyle 1976:164 [list of California populations].

Types. Holotype: USNM 76837. Paratypes: SU 23046 (21), 23082 (101), 33939

(153, now 145); UMMZ 139029 [ex SU 33939] (6); USNM 214 (15).

Distribution: This species was originally found in the Mohave River, California. Introduction of Arroyo chub (*Gila orcutti*) and habitat loss have extirpated this species from its original range. Refugial populations are located at Soda Springs (Lake Teunday and MC Springs), the Naval Air Station at China Lake (introduced in 1971), Camp Cady Wildlife Area and Barstow Desert Information Center. Specimens examined from China Lake Naval Air Station.

Comments: Girard's (1856) original description of *Algansea formosa* was based on specimens of *Hesperoleucus symmetricus* (Merced River) and *Siphateles mohavensis* (Mohave River). Snyder (1919) stated that both cotypes were not well enough preserved for comparison, although they were apparently suitable enough

to distinguish between the two species. Given that the Merced River was the first locality mentioned, Snyder (1919) retained A. formosa (= H. symmetricus) for the Sacremento-San Joaquin form and designated the Mohave River species as S. mohavensis. Described on the basis of differences in scale counts with S. obesus and S. thalasinnus (= S. b. formosa in Snyder), S. mohavensis was recognized as a distinct species in Uyeno's (1961) revision of Gila, in which Siphateles was reduced to a subgenus within Gila. The first reference to S. mohavensis as a subspecies was by Miller (1973) because of the apparent lack of meristic characters that separated Mohave tui chubs from S. obesus. Uyeno (1961) listed two osteological characters (presence or absence of a masticatory process and length of the hypohyals) that distinguished S. mohavensis from S. obesus. In addition, May et al. (Unpubl. Report. Genetic purity and subspecific status of the Mohave tui chub. Final report to Dept. of Navy, N68711-97-LT-70025) examined allozyme and amplified fragment length polymorphisms (AFLPs) in S. mohavensis, S. obesus (Lahontan and Owens Valley, as defined below) and S. bicolor (Upper Klamath Lake) and found fixed differences between S. mohavensis and the other tui chubs at one allozyme locus (Galactosaminidase) and an AFLP allele (Pst I-CTCG-370) not found in either Owens Valley or Lahontan tui chub samples. In this study, a single haplotype was found in the two fish examined; six nucleotide positions (297, 456, 483, 855, 948, 1056) were autapomorphies for this species. Thus, previous studies provide osteological, biochemical and nuclear DNA evidence in

conjunction with the mtDNA sequence data presented herein that support my conclusion that *S. mohavensis* should be accorded specific status.

Siphateles newarkensis (Hubbs and Miller)

Newark Valley tui chub

Gila bicolor newarkensis, Hubbs and Miller 1972:102 [original description; type locality: spring on alluvial slope near Diamond Peak, west side of Newark Valley, White Pine Co., Nevada].

Gila bicolor euchila, Hubbs and Miller 1972:103 [original description; type locality: Fish Creek Springs, Fish Creek Valley, White Pine Co., Nevada].

Siphateles obesus, Hubbs and Miller 1948:33 [as undescribed subspecies of S. obesus from Fish Creek Springs, Fish Creek Valley, and "springs which form remnants of Pluvial Lake Newark," Newark Valley, White Pine Co., Nevada].

Types. Holotype: UMMZ 188893. Paratopotypes: UMMZ 132185 (226). Distribution: Restricted to springs in Newark Valley and Fish Creek Valley, which is a southwestern extension of Newark Valley, White Pine Co., Nevada. Specimens examined from both localities.

Comments: Four haplotypes were found in the four specimens examined; average sequence divergence among haplotypes was 0.40%. *S. newarkensis* can be diagnosed by 13 nucleotide characters (positions 471, 729, and 777 autapomorphic; positions 132, 216 and 1005 are homoplasious but character state found in only one

outgroup taxon; positions 285, 312, 606 are unique within S. bicolor complex, while position 1044 is unique within S. bicolor clade; character state of positions 213, 390 and 1065 are shared with only one outgroup taxon plus one specimen within S. obesus). Hubbs and Miller (1972) originally described this species as two subspecies based on greater head, snout, upper jaw, mandible, and preorbital lengths and massive lips in the Fish Creek Valley population. Subsequently, these differences were attributed to midwater (Fish Creek Valley) vs. bottom (Newark Valley) feeding habits (Hubbs et al., 1974). S. newarkensis differs from S. isolatus and S. obesus in the following characters (range represents mean as a percent of standard length for males and females across samples; small ranges in S. isolatus based on comparisons between small males and females; Hubbs et al., 1974:table 27): greater predorsal length (56.3-59.0 S. newarkensis vs. 57.7-57.8 S. isolatus; 52.7-56.9 S. obesus), shorter distance between pelvic fin insertion to anal fin origin length (16.1-17.5 S. newarkensis vs. 18.4-18.5 S. isolatus; 18.4-19.7 S. obesus) and longer pectoral and pelvic fin length in males (P1 18.3-25.2, P2 16.0-18.9 S. newarkensis vs. P1 18.3-21.2, P2 16.0-16.6 S. isolatus; P1 17.4-23.4, P2 16.0-20.2 S. obesus).

#### Siphateles obesus (Girard)

#### Lahontan tui chub

Algansea obesa, Girard 1856:183 [original description; type locality: Humboldt River, Nevada].

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Siphateles vittatus, Cope 1883:146 [original description; type locality: Pyramid Lake, Washoe Co., Nevada].

Rutilus oregonensis, Snyder 1908:87 [original description; type locality: XL

Spring, Abert Lake, Lake Co., Oregon. Tui chubs from XL Springs, Chewaucan River, Hutton Springs, Summer Lake, Silver Lake and Warner Valley included in this species].

Siphateles obesus, Snyder 1917:60 [partial synonomy].

Siphateles oregonensis, Snyder 1917:60 [partial synonomy].

Leucidius pectinifer, Snyder 1917:64 [original description; type locality: Pyramid

Lake, Washoe Co., Nevada].

Siphateles bicolor obesus, Schultz 1935:379 [list of northwest fishes].

Siphateles bicolor oregonensis, Schultz 1935:380 [list of northwest fishes].

Siphateles obesus pectinifer, Hubbs and Miller 1948:37 [Lake Tahoe fish fauna].

Siphateles bicolor pectinifer, Shapovalov and Dill 1950:386 [checklist of

California fishes].

Gila bicolor snyderi, Miller 1973:1 [original description; type locality: irrigation canal and ditches about 8 mi. S of Bishop near Keough Hot Springs, Inyo Co., California].

Types. USNM 193 (1), 194 (1).

Distribution: Lahontan Basin and adjacent basins in Nevada and California; Owens Valley, California; Railroad Valley, Nevada; and Abert Lake, Summer Lake and Hutton Springs of southcentral Oregon. Specimens examined from all localities.

Comments: A total of 42 haplotypes were found in the 49 specimens of S. obesus examined in this study; average sequence divergence among these haplotypes was 0.84% (range 0-3.3%; see below). S. obesus can be diagnosed from 4 nucleotide characters (positions 597 and 943 autapomorphic [Owens Valley specimen exhibited a T to C transition at position 943); positions 681 and 699 homoplasious but are unique third position transitions within S. bicolor complex). Three previously recognized subspecies (oregonensis, pectinifer and snyderi) are subsumed within S. obesus, making this species the most geographically widespread and morphologically variable tui chub. No comprehensive examination of morphological variation in S. obesus, as defined herein, exists. Inclusion of the Abert Lake, Summer Lake and Hutton Springs populations from southcentral Oregon in this species is somewhat problematic, particularly the sister-group relationship between ((Abert + Hutton Springs) + Railroad Valley, NV) populations given their disjunct distributions (see Discussion). The paraphyletic relationships among the populations formerly recognized as S. b. oregonensis (Snyder, 1908) resolved by the molecular data supports Bills' (1978) results based on meristic and morphometric data. Bills (1978) restricted oregonensis to tui chubs from Abert Lake (XL Springs and Chewaucan River); he did not provide names for the other nominal subspecies. The name oregonensis remains available should further evidence exclude Abert Lake tui chubs from S. obesus.

## Siphateles thalasinnus (Cope)

## Goose Lake tui chub

Myloleucus thalassinus, Cope 1883:144 [original description; type locality: Goose

Lake, Lake Co., Oregon].

Myloleucus parovanus, Cope 1883:143 [abundance in Klamath and Goose Lakes].

Rutilus bicolor, Jordan and Evermann 1896:244 [synopsis].

Rutilus thalassinus, Snyder 1908:86 [discussion of meristic and morphometric

differences with other Oregon populations].

*Rutilus bicolor*, Rutter 1908:135 [meristic variation in northern California populations].

Siphateles formosus, Snyder 1919:60 [partial synonomy].

Siphateles bicolor, Evermann and Clark 1931:55 [distributional list].

- Siphateles obesus, Hubbs and Miller 1948:62 [Cowhead Lake tui chub as undescribed subspecies].
- Siphateles obesus thalassinus, Hubbs and Miller 1948:70 [description of Goose Lake fauna].

Siphateles bicolor formosa, Bond 1961:28 [key to Oregon fishes].

Gila bicolor, Moyle 1976:21 [as undescribed subspecies].

Gila bicolor vaccaceps, Bills and Bond 1980:320 [original description; type

locality: Cowhead Lake, Modoc Co., California].

Types. Holotype: ANSP 19848.

Distribution: Goose Lake of southcentral Oregon and northeastern California,

upper Pit River, tributary of Sacramento River system, Cowhead Lake, Modoc Co., California and Warner Valley, Lake Co., Oregon. Introduced into Summer Lake (see Comments). Specimens examined from Goose Lake, Cowhead Lake, Warner Valley and Summer Lake.

Comments: Eight haplotypes were found in the ten S. thalassinus examined in this study: average sequence divergence among these haplotypes was 0.26%; average sequence divergence among Goose Lake and Warner Basin haplotypes was 0.36%. S. thalassinus can be diagnosed with four nucleotide characters (position 904 is autapomorphic; positions 174, 228, 960 are homoplasious but character state is only shared with outgroup taxa). I recognize two lineages within S. thalassinus, one lineage occurring in Goose Lake and Summer Lake (see below) and one lineage in Cowhead Lake and Warner Valley (collectively called Warner Basin). Although these two lineages are reciprocally monophyletic, tui chubs from Warner Basin are diagnosed by a single homoplasy. S. thalassinus exhibits the greatest range of intraspecific meristic variation in the S. bicolor species group; no meristic or morphometric characters separate this species from other members of the species group (Chapter 3). Examination of meristic characters for the three populations comprising S. thalassinus, however, indicates that Cowhead Lake tui chubs have higher scale and fin ray counts than either Goose Lake or Warner Valley tui chubs. Comparison of principal component scores for Cowhead Lake tui chubs with those of the other species in meristic space indicates that this population would minimally overlap with S. obesus, S. eurysomas and Siphateles sp. (Silver Lake),

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and would not overlap with either *S. bicolor* or *S. columbianus* (Chapter 3). Rather than recognize a species based on a single, homoplasious nucleotide character, I suggest that tui chubs from Warner Valley + Cowhead Lake be referred to as *S. thalassinus* "Warner Basin." This name employs the utility of a subspecies designation without implying any ontological status to the names (cf. Frost, 1995; Frost et al., 1998). The name *vaccaceps* remains available should further evidence warrant recognition of either Warner Valley + Cowhead Lake, or just Cowhead Lake populations, as a distinct species.

Three fish collected in Summer Lake (two from Rickert Springs and one from Co. Rd. 417 Springs) shared complete sequence identity with *S. thalassinus* from Goose Lake. Average sequence divergence between *S. thalassinus* and Summer Lake *S. obesus* was 2.28% (data not shown). This suggests potential long-term sympatry of *S. thalassinus* and *S. obesus* in Summer Lake Basin. Additional sampling of tui chubs from Summer Lake populations is needed to determine the incidence and distribution of *S. thalassinus* in Summer Lake Basin. If the two species are sympatric, future research should attempt to determine if they are syntopic and partitioning the habitat and whether a hybrid zone is forming in areas of syntopy.

#### Siphateles sp.

# Silver Lake tui chub

Leucus bicolor (in part), Jordan and Gilbert 1882:143 [synopsis].

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Myloleucos bicolor (in part), Cope 1883:143 [synopsis].

Rutilus bicolor (in part), Jordan and Evermann 1896:244 [synopsis].

Rutilus oregonensis (in part), Snyder 1908:87 [original description; type locality:

XL Spring, Abert Lake, Lake Co., Oregon].

Rutilus formosus (in part), Fowler 1913:70 [list of type specimens].

Siphateles oregonensis, Snyder 1917:60 [partial synonomy].

Siphateles bicolor oregonensis, Schultz 1935:380 [list of northwest fishes].

Siphateles obesus oregonensis, Hubbs and Miller 1948:74 [list of fishes in Fort

Rock Basin].

Distribution: Presently confined to Bridge Creek, Buck Creek, Thompson Reservoir and Silver Creek in Silver Lake Valley, Oregon. Specimens examined from Buck Creek.

Comments: Two haplotypes were found in the three specimens examined; average sequence divergence among these haplotypes was 0.09%. *Siphateles* sp. (Silver Lake) can be diagnosed from four nucleotide characters (position 867 autapomorphic; positions 432, 522, 906 are homoplasious with character state shared with *S. obesus*, but found in no other member of the *S. bicolor* group). This species was originally described as *S. oregonensis* (Snyder 1908) from Silver Lake, Summer Lake, Abert Lake, Hutton Springs and Warner Valley. Bills (1978) examined meristic and morphometric variation in all populations of *S. oregonensis sensu* Snyder (1908) and concluded that tui chubs from Silver Lake were an undescribed subspecies based on fewer number of scales from the lateral line to

pelvic fin insertion and shorter pelvic fin length relative to XL Springs tui chubs and fewer number of scales around the caudal peduncle relative to Chewaucan River tui chubs. Bills did not examine *S. bicolor* from Klamath Lake. Phylogenetic analysis in this study resolved *S. bicolor* as the sister taxon to *Siphateles* sp. (Silver Lake). Meristic and morphometric comparisons are given under *S. bicolor*.

### Sequence Data and Duplicated Haplotypes

Mitochondrial DNA sequences were obtained initially from a total of 100 individual fish, including outgroups. Individuals with duplicate haplotypes were removed from the data matrix to simplify computations and reduce the number of unresolved trees that would be produced with this artifact included in analyses. The number of unique haplotypes found within each species or population is given in Table 2.1. The final data matrix used for phylogenetic analysis contained 1140 bp of sequence for 78 specimens, or 88,920 bp (see Appendix).

#### Compositional Bias and Saturation

Analysis of complete mtDNA cytochrome b gene sequences among 78 specimens yielded 295 variable sites, with 218 of these sites being phylogenetically informative. Of the informative sites, 35 (12.5%) were at the first codon position, 3 (1.3%) were at the second codon position, and 180 (82.5%) were at the third codon position. Nucleotide composition was typical of that found in other actinopterygian fishes (data not shown; Lydeard and Roe, 1997). Differences in nucleotide composition among and within species were most evident at the third codon position, which exhibited the anti-G bias as reported in other studies (Table 2.2; Lydeard and Roe, 1997; Meyer, 1993). Chi-square tests for homogeneity of base frequences across taxa for all three codon positions failed to detect significant differences among taxa (Codon 1:  $\chi^2 = 4.31$ ; Codon 2:  $\chi^2 = 0.29$ ; Codon 3:  $\chi^2 =$ 33.58; df = 231, P = 1.0), suggesting differences in base composition would not significantly influence phylogenetic analyses.

Pairwise comparisons of absolute number of transitions and transversions versus genetic distance (uncorrected for multiple hits) did not indicate saturation of transitions or transversions at any of the three codon positions. At the first codon position, transitions slightly outnumber transversions at genetic distances less than about 0.03%, after which transitions outnumber transversions (Fig. 2.2A). For the

	Position								
Nucleotide	First	Second	Third						
A	24.1 (0.003)	20.0 (0.0005)	37.3 (0.008)						
С	25.3 (0.003)	24.9 (0.0008)	31.9 (0.008)						
G	25.9 (0.003)	13.7 (0.0008)	8.9 (0.007)						
Т	24.7 (0.003)	41.3 (0.0004)	21.9 (0.008)						
Compositional Bias Index	0.016	0.213	0.255						

Table 2.2. Base composition (and standard deviation) at the first, second and third codon positions.

Note: Compositional bias index is calculated as  $C = (2/3) \Sigma |c_i - 0.25|$  where C is the compositional bias index and  $c_i$  the frequency of the *i*th base (Irwin et al., 1991).

second codon position, genetic distance values were <0.09% and no pattern was apparent in nucleotide substitutions (data not shown). For the third codon position, transitions exhibit a linear increase and outnumber transversions at all genetic distance values (Fig. 2.2B). The gap in genetic distance values between approximately 0.10 - 0.20% reflects comparisons among the *S. bicolor* complex and remaining taxa, as well as comparisons among outgroup taxa.

### Phylogenetic Analysis

Phylogenetic analyses of the mtDNA sequences was performed with nucleotide characters unordered and unweighted as a consequence of no indication of saturation of nucleotides in the three codon positions. This analysis resulted in 600 equally parsimonious trees of 532 steps (CI = 0.626, RI = 0.858, RC = 0.537). The  $g_1$  value (-0.473) indicated the presence of significant phylogenetic signal (P < 0.01) in the data, based on the critical values presented in Hillis and Huelsenbeck (1992). The large number of equally parsimonious trees resulted from instability of branching order among some taxa. Because it is possible for heuristic searches to become trapped within a local optimum, even with random addition of branches (Maddison, 1991; Page, 1993), a reduced data set of one randomly selected individual per population was analyzed. Topologies resulting from this analysis did not differ from topologies found in analyses of the complete data set.

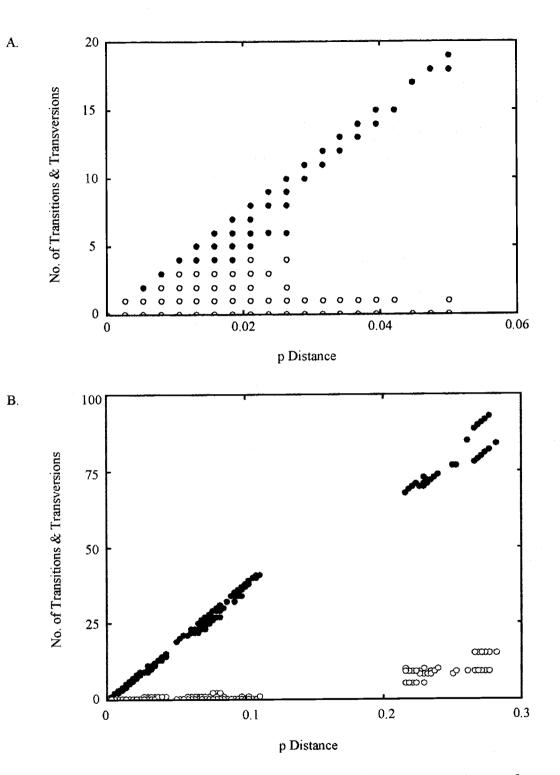


Figure 2.2. Absolute number of transitions and transversion versus p distance for all pairwise comparisons. A) Codon 1; B) Codon 3. Solid circles = transitions; open circles = transversions.

The strict consensus tree of the complete data set is shown in Figure 2.3.

*Siphateles* formed a monophyletic group; this topology was supported in 62% of bootstrap replicates. Within *Siphateles*, *S. alvordensis* and *S. boraxobius* formed a monophyletic group sister to the *S. bicolor* complex.

Within the *S. bicolor* complex, *S. mohavensis* and the *S. bicolor* clade were reciprocally monophyletic. Autapomorphies for *S. mohavensis* included five transitions and one transversion. There were three distinct species groups in a trichotomy within the *S. bicolor* clade: *S. isolatus* group consisting of *S. newarkensis* and *S. isolatus*; *S. bicolor* group consisting of Oregon populations, excluding Abert Lake, Summer Lake and Hutton Springs of southcentral Oregon; and *S. obesus*, consisting of populations from the Lahontan Basin of Nevada and California, populations in basins adjacent to the Lahontan Basin, populations from Railroad Valley in southcentral Nevada, Owens Valley in California, and Abert Lake, Summer Lake and Hutton Springs of southcentral Oregon.

The *S. isolatus* group was resolved as monophyletic in all trees; this topology was recovered in 89% of bootstrap replicates. In 38% of the trees, this group was basal to a clade of the *S. bicolor* group + *S. obesus*. Phylogenetic analysis using *S. alvordensis* and *S. boraxobius* as functional outgroups (Watrous and Wheeler, 1981) also produced a topology in which the *S. isolatus* group was basal to a clade of the *S. bicolor* group + *S. obesus* (2,700 trees, 306 steps, CI = 0.71). The *S. isolatus* group consisted of two reciprocally monophyletic species, *S. isolatus* and *S. newarkensis*, supported in 100% of bootstrap replicates.

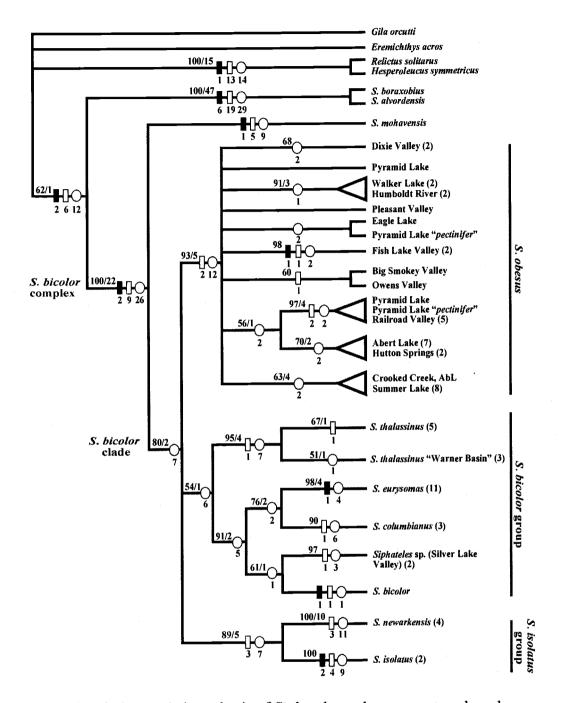


Figure 2.3. Phylogenetic hypothesis of *Siphateles* and outgroup taxa based on mtDNA cytochrome *b* sequence data. Numbers before "/" bootstrap values; numbers following Bremer decay index. Numbers in parentheses are the number of haplotypes found within a species or population. Dark bars are tagged with the number of unique (CI = 1.0) transversions; open bars are tagged with the number of unique transitions. Open circles are tagged with the number of transitions and transversions that show homoplasy along a particular internode.

The *S. bicolor* group was also resolved as monophyletic in all trees; this topology was recovered in 54% of bootstrap replicates. All topologies recovered two clades within the *S. bicolor* group: one consisting of *S. thalassinus* and a second clade consisting of *Siphateles* sp. (Silver Lake) + *S. bicolor* sister to a clade of *S. columbianus* + *S. eurysomas*. The monophyly of these two clades was supported in 95% and 91% of bootstrap replicates, respectively. All topologies depicted populations comprising the formerly recognized subspecies "oregonensis" as paraphyletic, with specimens from Abert Lake, Summer Lake and Hutton Springs occurring in *S. obesus* (Fig. 2.3).

Populations of *S. obesus* formed a monophyletic group in all trees; the monophyly of *S. obesus* was supported in 93% of bootstrap replicates. *S. obesus* consisted of a large polytomy of populations from Nevada and California found in the Lahontan Basin, populations from Nevada in basins adjacent to the Lahontan Basin, populations from Railroad Valley in southcentral Nevada, and populations from Abert Lake, Summer Lake and Hutton Springs of Oregon. Within the polytomy, populations from Abert Lake and Hutton Springs of southcentral Oregon were monophyletic and sister to populations from Railroad Valley from southcentral Nevada plus one *S. obesus* and one "*pectinifer*" from Pyramid Lake, Nevada. This topology was supported in 56% of bootstrap replications. Monophyly of Abert Lake + Hutton Springs populations and Railroad Valley populations was supported in 70% and 97% of bootstrap replicates, respectively. Other monophyletic groups contained within this polytomy of populations included Dixie Valley (68% bootstrap support), Walker Lake + Humboldt River (91%), Fish Lake Valley (98%), and Owens Valley + Big Smokey Valley (60%) (Fig. 2.3).

Despite lack of *a priori* justification for weighting transversions over transitions or by codon positions, I performed several weighted analyses to determine if the position of the Abert Lake, Summer Lake and Hutton Springs specimens would change under different phylogenetic assumptions. My analyses included weighting transversions 2x and 3x transitions, weighting codon positions 1 and 2 2x, 3x, 5x and 10x relative to codon position 3, irrespective of nucleotide composition, and calculating the maximum likelihood scores for each nucleotide site to use as a character weight in parsimony analysis. These additional analyses did not change the phylogenetic position of these populations in the resultant topologies from that of the unweighted analyses.

### Genetic Divergence of Clades

Kimura's "two parameter" distances (Kimura, 1980) were calculated based on the terminal species groups identified in the phylogenetic analyses. Because these groups often represent multiple haplotypes, average distances among groups are presented in Table 2.3. Pairwise percent sequence differences of *S. alvordensis* and *S. boraxobius* with members of the *S. bicolor* complex ranged from 9.67-10.81%; between *S. alvordensis* and *S. boraxobius* the sequence divergence was 0.35%. Within the *S. bicolor* complex, percent sequence differences ranged from 0.36-4.05%. The greatest amounts of interspecific divergence were found in comparisons of *S. mohavensis* (2.95-4.05%), *S. isolatus* (2.86-4.05%) and *S. newarkensis* (2.86-3.84%) with other tui chub populations. Populations within *S. obesus* exhibited the greatest range of sequence divergence (0-3.30%; data not shown). Limiting comparisons to only Nevada and California populations within *S. obesus*, intraspecific sequence divergence ranged from 0-1.30%. Sequence divergence ranged from 0-3.30% among specimens from Oregon within *S. obesus*. Sequence divergence of Oregon specimens with other members of *S. obesus* ranged from 0.44-3.26%.

#### DISCUSSION

Phylogenetic analysis of mtDNA cytochrome *b* sequence data from Siphateles supports other phylogenetic studies based on morphology (Coburn and Cavender, 1992) and mtDNA 12S and 16S rRNA sequences (Simons and Mayden, 1998) that Siphateles is monophyletic and not a member of the genus Gila. The sister group to Siphateles was unresolved in this analysis. Relictus and Eremichthys, considered by Coburn and Cavender (1992) to be monophyletic and sister to Siphateles, did not form a monophyletic group, and neither taxa were sister to Siphateles in any topology. Instead, Relictus and Hesperoleucus formed a monophyletic group and were part of a basal polytomy that included Eremichthys, Gila and Siphateles. These results agree, in general, with Simons and Mayden's (1998) conclusion that relationships among several basal members of their Orthodon clade of western minnows (i.e., Eremichthys, Lavinia, Ptychocheilus, and

Species	Species														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. S. mohavensis															
2. S. isolatus	4.05														
3. S. newarkensis	3.84	2.86													
4. S. obesus	3.25	3.60	3.78												
5. S. eurysomas	3.16	3.38	3.59	2.52											
6. S. columbianus	3.19	3.45	3.56	2.74	1.10										
7. Siphateles sp. (Silver Lake)	3.21	3.07	3.42	2.25	1.18	1.26									
8. S. bicolor	2.97	3.12	3.28	2.53	1.04	1.12	0.67								
9. S. thalassinus	2.95	2.90	3.26	2.68	1.92	1.91	1.71	1.58							
10. S. thalassinus "Warner Basin"	2.98	2.90	3.25	2.63	1.79	1.78	1.77	1.63	0.36						
11. S. boraxobius	10.13	10.60	10.16	9.67	9.91	10.13	9.98	10.14	10.49	10.25					
12. S. alvordensis	10.33	10.81	10.37	9.86	10.00	10.23	10.08	10.24	10.59	10.35	0.35				
13. R. solitarus	9.41	10.81	9.86	8.95	8.78	9.13	8.64	8.79	9.03	8.91	10.36	10.24			
14. H. symmetricus	10.59	11.50	11.54	10.85	11.11	11.05	10.65	11.03	10.41	10.39	12.16	12.04	4.17		
15. <i>E. acros</i>	8.70	10.41	10.05	9.14	9.80	9.79	9.68	9.84	9.19	9.18	12.03	12.24	9.01	10.25	
16. G. orcutti	11.69	12.05	12.17	11.86	12.32	12.51	11.96	12.11	11.93	11.79	12.54	12.76	9.90	10.84	11.3

Table 2.3. Percent pairwise genetic distances among *Siphateles* species and outgroups based on Kimura's (1980) "two parameter" model.

Siphateles) remain largely unresolved. Additional data, including a total evidence (Kluge, 1989) approach combining available molecular data from Simons and Mayden (1998) and this study with the morphological data of Coburn and Cavender (1992), are needed to resolve relationships among these taxa.

## Taxonomy of Siphateles bicolor Complex

Patterns of variation in the cytochrome *b* sequence data support recognition of nine species in the *S. bicolor* complex: *S. bicolor*, *S. columbianus*, *S. eurysomas*, *S. isolatus*, *S. mohavensis*, *S. newarkensis*, *S. obesus*, *S. thalasinnus*, and *Siphateles* sp. (Silver Lake) (Fig. 2.3). Division of the traditionally recognized polytypic *S. bicolor* into these species, and recognition of species groups with strong geographic components, reflects more realistic levels of divergence within this species complex. In addition, recognition of these species provides the requisite historical framework to conduct a thorough analysis of morphological variation in this group that will further assist in diagnosing these species.

An exception to the recovery of species groups with a coherent geographic component include the populations of the *S. obesus* group from Abert Lake, Summer Lake and Hutton Springs. Tui chubs from these areas were not resolved as a monophyletic group but share a unique third position transversion at position 441 (no amino acid substitution). Because transversions at the third position of the codon are rare (Irwin et al., 1991), and in this case occurs in populations from adjacent subbasins, this suggests the possibility that this transversion is a shared evolutionary event that occurred in the common ancestor of these populations. To explore this possibility, I used MacClade (Maddison and Maddison, 1992) to construct alternative topologies where this clade formed a monophyletic group and was placed at four locations on the tree (Fig. 2.3): 1) in the current position of Summer Lake specimens; 2) as sister to the Railroad Valley + Pyramid Lake specimens (current position of Abert Lake + Hutton Springs specimens); 3) as sister to S. thalassinus in the S. bicolor group because of the geographic proximity of the basins in which these populations occur; and 4) as part of a basal polytomy with S. obesus, the S. bicolor group and the S. isolatus group. I used Wilcoxon's signed-rank test (Templeton, 1983a, 1983b) as implemented in PAUP\* (Swofford, 1998) to compare these alternative topologies to the strict consensus tree presented in Fig. 2.3. Alternative topologies 1 and 2 were not significantly different from the most parsimonious tree (P = 0.18 and 0.41, respectively); alternative topologies 3 and 4, however, were significantly different from the most parsimonious tree (P < P0.05 for both alternatives). Thus, while placing populations from Abert Lake, Summer Lake and Hutton Springs into a monophyletic group does not differ significantly from the strict consensus tree, there is no support for the placement of these populations as a monophyletic group outside of S. obesus. Further data from a faster evolving portion of the mitochondrial genome (e.g. the control region) is needed to test the monophyly of these populations.

Biogeography and Speciation of Siphateles

The Great Basin of the western United States is characterized by a series of long, narrow, north-south trending faultblock mountain ranges alternating with broad basins. The extensional tectonics responsible for the current topography began in the Miocene and extended into the Pliocene and occurred in two distinct phases. The first phase was between 20-10 million years ago (mya) and produced the crustal fractures of eastern Oregon, the western Snake River Graben and opening the Nevada-Oregon rift (Orr et al., 1992). The second phase occurred during the last 10 million years and resulted in the current faultblock mountain and basin topography (Orr et al., 1992). Climatic cycles throughout the Pliocene and Pleistocene alternately filled and dried the large lakes in these basins (Benson, 1978; Benson et al., 1990; Benson et al., 1992). Periods of maximum lake elevation resulted in surface water connections among some lakes, particularly in southeastern Oregon and the Lahontan Basin of Nevada. These connections were ultimately severed during the more arid conditions of the late Pleistocene and Quaternary. The result of these geologic and climatic events is a complex mosaic of aquatic habitats whose faunal elements have uncertain phylogenetic affinities. Phylogenetic diversification within the S. bicolor complex indicates patterns of peripheral isolation and vicariance among these species (Figs. 2.4 and 2.5) that corresponds with several aspects of the known geological history of the Great Basin, providing some insight into patterns of drainage relationships. The distribution and potential isolating mechanisms for individual species groups are

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discussed below. Following the convention of Hubbs and Miller (1948), the term "Pluvial Lake" is used to denote lakes that are not recent in origin (i.e., since the end of the Pleistocene; for example, Pluvial Lake Lahontan). For lakes of recent origin, "Lake" follows the specific name.

### <u>S. alvordensis + S. boraxobius</u>

The oldest cladogenetic event within *Siphateles* is the divergence of *S. alvordensis* + *S. boraxobius* in the Alvord Desert of southeastern Oregon (Fig. 2.4). Hubbs and Miller (1948) postulated a connection between the Alvord and Lahontan basins based on physiographic evidence. Given the amount of genetic divergence between these two species and the *S. bicolor* complex (average genetic distance = 10.23%), however, this speciation event likely involved a peripheral isolation or vicariant event from the ancestral *Siphateles* form, possibly corresponding to the formation of the Nevada-Oregon rift during the early Miocene (Orr et al., 1992).

#### S. mohavensis

The next divergence within the *S. bicolor* complex involved *S. mohavensis* and the ancestor of the *S. bicolor* clade (Fig. 2.4). Miller (1946), Hubbs and Miller (1948) and Morrison (1965) postulated a late Pleistocene connection between Pluvial Lake Owens (containing *S. obesus*) and Mohave via the Amaragosa River and Pluvial Lake Manly in Death Valley. Smith (1981b) suggested that *S. mohavensis* was either a rapidly differentiating Pleistocene colonist or a relict of

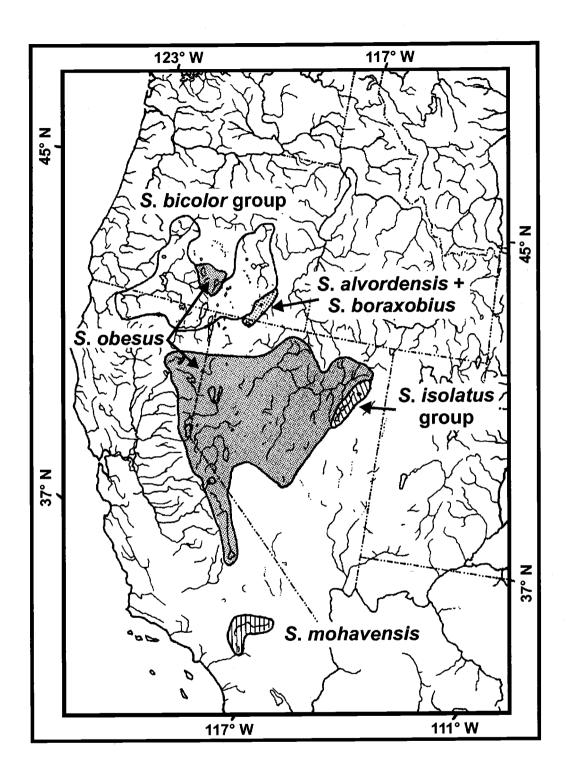


Figure 2.4. Geographic distribution of species and species groups within *Siphateles* identified in phylogenetic analysis of mtDNA cytochrome b gene.

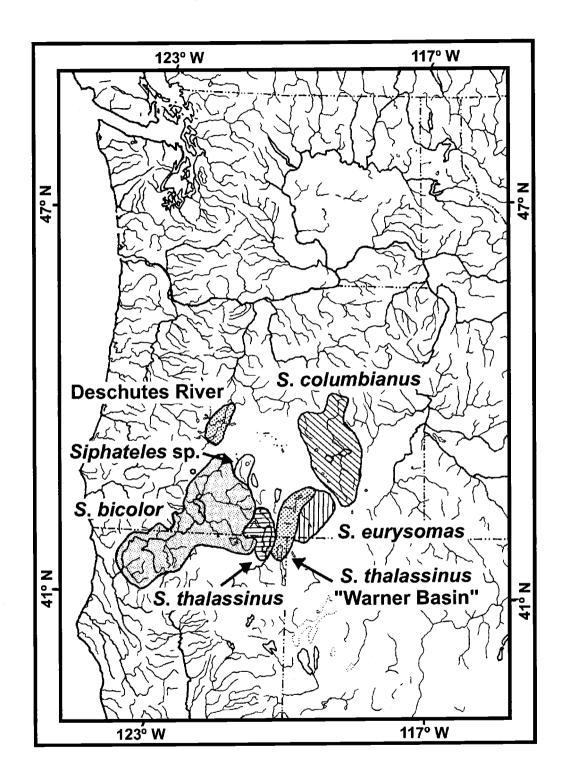


Figure 2.5. Geographic distribution of *S. bicolor* species group in Oregon identified in phylogenetic analysis of mtDNA cytochrome *b* gene.

Pliocene north-south connections. This speciation event likely involved vicariance during the late Pliocene or early Pleistocene because *S. mohavensis* forms the sister group to the *S. bicolor* clade and subsequent cladogenetic events in the *S. bicolor* clade are correlated with late Pleistocene events such as the periodic connection/isolation of Lahontan Basin populations.

#### <u>S. isolatus group</u>

Siphateles isolatus and S. newarkensis have not been recognized previously as a monophyletic group independent of S. obesus (Figs. 2.3 and 2.4). Hubbs and Miller (1948) postulated a connection between Pluvial Lake Newark and the Humboldt River of the Lahontan Basin via Huntington Creek during the late Pleistocene. Snyder et al. (1964) and Snyder (pers. comm. cited in Hubbs et al. 1974) did not support this view based on their inability to confirm discharge location. In addition, the altitude of the highest recognizable shoreline was found below the divide between Newark and Huntington Valleys. Hubbs et al. (1974) recognized, however, that S. newarkensis was more differentiated than tui chubs from northward basins with direct connections to the Humboldt River and conceded that these fish may have been isolated prior to the late Pleistocene.

*Siphateles isolatus* occupies spring habitats remnant from Pluvial Lake Clover (Hubbs and Miller, 1948; Hubbs et al., 1974). Based on field observations, Pluvial Lake Clover was apparently endorheic during the late Pleistocene (Hubbs and Miller, 1948). Hubbs et al.(1974) speculated on a connection with the Humboldt River through a northern pass in the western arm of the lake during a "pluvial period sufficiently remote" that shoreline features from the lake are now obscured. Empirical data supporting such a connection is lacking, however.

Regardless of the exact nature of connections with the Humboldt River, phylogenetic analysis indicates that the ancestor of the *S. isolatus* group occurred in the upper Humboldt River and adjacent basins and was subsequently isolated from the ancestor of the *S. bicolor* group + *S. obesus*. Given the degree of genetic differentiation of the *S. isolatus* group with other tui chubs, isolation of these species probably occurred during the late Pliocene or early Pleistocene. The topography of the Humboldt River contains no physical barriers to dispersal (La Rivers, 1962). It is probable that general trends towards increasing aridity that occurred throughout the Late Pliocene and Pleistocene reduced lake levels (Morrison, 1961; Morrison, 1965), thus cutting off surface water connections and isolating populations within these basins.

#### <u>S. bicolor group</u>

Two monophyletic lineages were recognized within the *S. bicolor* group: *S. thalasinnus* and a clade containing ((*S. eurysomas* + *S. columbianus*)(*S. bicolor* + *Siphateles* sp. (Silver Lake))) (Fig. 2.3). No phylogenetic support was found for the existence of two races of tui chubs in Oregon based on gill raker counts, as suggested by Minckley et al. (1986).

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Formation of the faultblock topography of southeastern Oregon initiated with the second phase of extensional tectonics beginning some 10 mya (Orr et al., 1992) potentially served as the initial vicariant event isolating not only these two lineages, but also species within these lineages (Fig. 2.5). The vicariant event separating Goose Lake and Warner Basin *S. thalassinus* is somewhat problematic given that the low genetic divergence values between Goose Lake and Warner Basin populations (0.36%) is not concordant with formation of the Warner Mountains during the Miocene. Headwater transfer between Goose Lake and Warner Basin is an unlikely possibility because the steep topography of the Warner Mountains and the proclivity of tui chubs to occupy low-gradient habitats. Hubbs and Miller (1948) discussed likely north-south connections between Warner Basin and Pluvial Lake Meinzer, but found no potential east-west connection between Goose Lake and Warner Basin. Thus, the vicariant or dispersal event, and the timing of that event, between the two basins remains speculative.

The ancestor of the ((*S. eurysomas* + *S. columbianus*)(*S. bicolor* + *Siphateles* sp. (Silver Lake))) clade had an extensive range in eastern Oregon, being distributed from the Harney to Klamath basins. Silver Lake is the remnant of Pluvial Lake Fort Rock, which drained into the Deschutes River of the Columbia Basin during the late Pliocene or early Pleistocene (Allison, 1940; Allison, 1979). The vicariant event isolating the *S. bicolor* + *Siphateles* sp. (Silver Lake) clade from the *S. columbianus* + *S. eurysomas* clade potentially occurred with the severing of the connection between Pluvial Lake Fort Rock and the Deschutes River. An important, unanswered question is the relationship of upper Deschutes River tui chubs with other members of the S. bicolor group. If Deschutes River tui chubs fall within S. columbianus, then this population may be part of what was once a larger, more widespread S. columbianus that encompasses the Columbia River Basin, rather than restricted to the Malhuer Basin as defined herein. However, if Deschutes River tui chubs are part of the S. bicolor + Siphateles sp. (Silver Lake) clade, in either a basal position to these two species or as sister to Siphateles sp. (Silver Lake), this would provide faunal evidence supporting a connection between Pluvial Lake Fort Rock and the Deschutes River (Allison, 1940; Allison, 1979). An S. bicolor + Deschutes River clade would suggest a past connection between the Klamath Basin and Deschutes River; geologic evidence supporting such a connection is lacking (Allison, 1940; Hubbs and Miller, 1948). The sister group relationship of S. bicolor + Siphateles sp. (Silver Lake), however, supports previously hypothesized connections between Klamath Lake and Silver Lake via stream capture from Sycan Marsh of the upper Klamath Basin (Hubbs and Miller, 1948). The sister group relationship of S. columbianus + S. eurysomas supports the contention of Hubbs and Miller (1948) of a connection between Catlow and Harney basins. Minckley et al. (1986) speculated that the directionality of the connection was from Catlow Basin into Harney Basin because of the precipitous spill that would have occurred at the gap between the two basins.

## S. obesus and the Evolution of S. thalassinus

The *S. obesus* polytomy, excluding the Oregon and Railroad Valley populations, supports a late Pleistocene connection among populations of the Lahontan Basin and adjacent basins (Fig. 2.4). An exception to this pattern among Nevada populations is the Fish Lake Valley population in southwestern Nevada, which exhibits as many apomorphies and high bootstrap values as found in some species of the *S. bicolor* group. Hubbs and Miller (1948) suggested a possible connection with the Walker Lake arm of Pluvial Lake Lahontan via two intervening valleys that now contain salt marshes. They also speculated that the fish could have been introduced as bait from populations in Owens Valley, California. This speculation seems unlikely given the number of apomorphies characterizing Fish Lake Valley tui chubs and relationship of Owens Valley with Big Smokey Valley tui chubs.

As discussed above, alternative topologies with specimens from Abert Lake, Summer Lake and Hutton Springs as a monophyletic lineage within *S. obesus*, either as a sister group to Railroad Valley + two Pyramid Lake specimens or as a separate lineage within *S. obesus*, were not significantly different from the topology of the strict consensus tree. Regardless of their exact placement within *S. obesus*, the inclusion of these populations demonstrates that *S. obesus* once had a continuous distribution from central Oregon south to the Lahontan Basin of Nevada (Fig. 2.6A). The distribution of *S. obesus* in Oregon and California at that time would have included Goose Lake, Pluvial Lake Meinzer (immediately to the east of

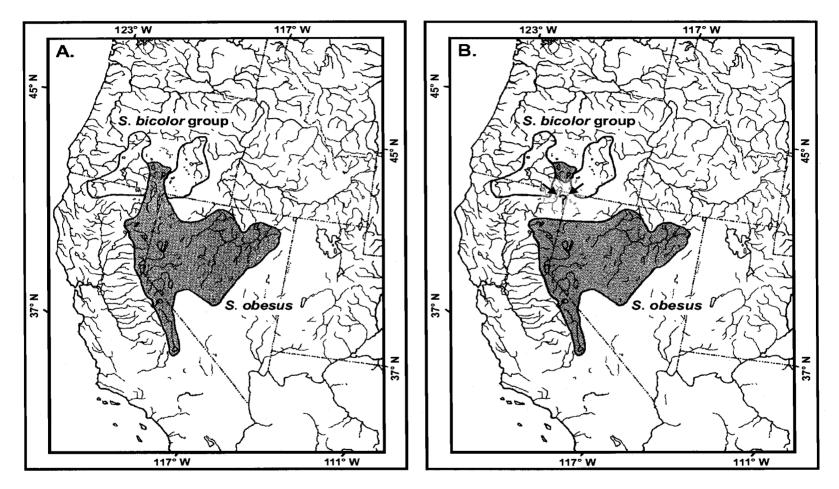


Fig. 2.6. Biogeographic hypotheses for the *S. bicolor* species group and *S. obesus*. A) Distribution of *S. obesus* prior to disjunction of northern and southern populations; B) Subsequent distribution of *S. obesus* and potential dispersal paths of members of the *S. bicolor* species group into Goose Lake.

Goose Lake Basin and south of Warner Basin), Warner Basin, Abert Lake, Summer Lake and Hutton Springs. A vicariant event(s), such as the initial and continuing formation of the faultblock topography in southeastern Oregon (Orr et al., 1992), climatic changes resulting in increasing aridity and dessication of Goose Lake, Warner Basin and Pluvial Lake Meinzer (Grayson, 1993; Hubbs and Miller, 1948; Orr et al., 1992), or a combination of both, interrupted the continuous distribution of this species and produced the current disjunct distribution (Fig. 2.4).

The evolution of *S. thalassinus* is somewhat problematic. Phylogenetic relationships of the *S. bicolor* species group (Fig. 2.3), coupled with the current distribution of these species (Fig. 2.4), indicates that the ancestor of this clade was widespread and sympatric with *S. obesus* in Goose Lake and Warner Basin. This distribution suggests the possibility of sympatric speciation via ecological segregation and assortative mating resulting in the evolution of *S. thalassinus* (Wiley, 1981). The generalized ecology of tui chubs, and their *r*-selected type of life history strategy, would seem to preclude sympatric speciation as a viable explanation. Wiley and Mayden (1985) found no examples of sympatric speciation via ecological segregation in North American fishes.

If *S. obesus* and the ancestor of *S. thalassinus* were allopatric, then dispersal of the ancestral *S. thalassinus* into Goose Lake and Warner Basin would have to come from either Catlow Valley to the east or Klamath Basin to the west (Fig. 2.6B). As noted above, however, the primary direction of flow from Catlow and

Guano Valleys is to the north into Harney Basin (Hubbs and Miller, 1948; Minckley et al., 1986). In addition, genetic divergence values between *S. thalassinus* and *S. columbianus* or *S. eurysomas* are greater than those for *S. thalassinus* and *S. bicolor* or *Siphateles* sp. (Silver Lake) (Table 2.3); also no apomorphic homoplasies were found in the *S. bicolor* species group that would provide limited support for such a relationship.

In contrast, two homoplasious, apomorphic nucleotide characters (positions 984 and 1035; Appendix 1) provide potential support for an *S. bicolor* + *Siphateles* sp. (Silver Lake) +*S. thalassinus* clade. If valid, the ancestor of this clade probably originated in the Klamath Basin and potentially gained access into Goose Lake through the low elevation headwaters of the Sprague River (Klamath Basin) and Drews Creek (Goose Lake Basin). Hubbs and Miller (1948) suggested a possible stream connection between the Klamath Basin and Goose Lake and the Pit River across the "interior platform" of the Modoc Plateau, which was subsequently obscured by the Modoc Lava Flows (McKee et al., 1983).

#### <u>S. bicolor group + S. obesus</u>

In 38% of the most parsimonious trees recovered in this analysis, the *S. isolatus* group was basal to an *S. bicolor* group + *S. obesus* clade. Hubbs and Miller (1948) hypothesized an early Pleistocene connection between Pluvial Lakes Modoc (Klamath Basin) and Lahontan across an "interior platform" in northeastern

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California and southcentral Oregon. Taylor (1960; 1985) and Taylor and Smith (1981) listed a number of extant and extinct molluscan and fish taxa distributed from the Lahontan Basin across northeastern California to the Klamath Basin. This repeated faunal pattern may be a remnant of when the Snake River flowed across southeastern and southcentral Oregon, northwestern Nevada, and northeastern California (Wheeler and Cook, 1954; Axelrod, 1958; 1962; Christensen, 1966; Hay, 1976; Taylor, 1985). Minckley et al. (1986) hypothesized that the connection to the Klamath Basin was severed more than 10 mya; this timing coincides with the formation of the volcanic Modoc Plateau 5-10 mya (McKee et al., 1983). Other studies examining phylogenetic relationships of molluscs and fishes distributed across this region are need to corroborate and/or refine the biogeographic hypotheses outlined above and further elucidate the complex history of aquatic ecosystems in the Great Basin.

## APPENDIX. DATA MATRIX OF ALIGNED MITOCHONDRIAL CYTROCHROME *B* SEQUENCE DATA.

	4
	1 0
S. alvordensis	ATGGCAAGCCTACGAAAAACTCATCCGCTAATAAAAATCG
S. boraxobius	
S. mohavensis	CA
S. obesus	
Walker Lake OS15111-1	
Walker Lake OS15111-2	CCA
Humboldt River OS15637-1	
Humboldt River OS15637-3	A
Pleasant Valley OS15512-2	CA
Dixie Valley OS15513-1	A
Dixie Valley OS15513-2	A
EagleLake BB18	A
Pyramid Lake BB25	G
- Pyramid Lake BB28	A
"pectinifer" BB48	A
"pectinifer" BB49	A
"snyderi" OS15747-1	A
Fish Lake Valley OS15520-1	A
Fish Lake Valley OS15520-2	A
Bull Creek OS15514-1	A
Kate Spr OS15515-2	A
Blue Eagle Spr. OS15517-2	
Green Spring OS15519-2	A
Little Fish Lake BB1	A
Big Smoky Valley OS15522-1	A
Abert Lake OS16774-1	CCA
Abert Lake OS16774-2	CCA
Abert Lake OS16774-3	CCA
Abert Lake OS16722-1	CCA
Abert Lake OS16722-2	CCA
Abert Lake PMH9503-1	CCA
Abert Lake PMH9503-2	CCA
Abert Lake PMH9503-3	CCA
Hutton Spring PMH9320-1	CCA
Hutton Spring PMH9320-3	CCA
Rickert Spring OS15312-33	
County Road 417 OS15437-47	
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Klippel Spring OS15438-16	
Klippel Spring OS15438-17 Klippel Spring OS15438-18	
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4 0 1 S. isolatus OS15622-1 .....C..C..A..... S. isolatus OS15622-3 .....C..C..A...... S. newarkensis OS15743-1 .....C...C...A...... S. newarkensis OS15743-2 S. newarkensis OS15744-1 S. newarkensis OS15744-2 .....A..... S. eurysomas OS15572-1 S. eurysomas OS15572-2 .....C..C..A...... S. eurysomas OS15572-3 .....C...C...A...... S. eurysomas OS15635-1 eurysomas OS15635-2 Aurusomas OS15635-3 CCA .

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S. eurysomas PMH9504-3	A
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S. eurysomas PMH9505-3	A
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S. columbianus OS15577-4	
S. columbianus OS15577-6	
S. columbianus OS15577-8	CA
Siphateles sp. PMH9401-1	
Siphateles sp. PMH9401-2	CCA
<i>S. bicolor</i> OS16665-1	CCA
S. bicolor OS16665-2	CCA
S. thalassinus BB60	CCA
S. thalassinus BB62	CCA
S. thalassinus OS15312-32	CCA
S. thalassinus OS15312-32	CCA
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	CCA
S. thalassinus BBW1	
S. thalassinus BBW2	CCA
S. thalassinus DFM979-1	CCA
R. solitarus OS15745-1	T.
H. symmetricus OS15746-1	T.
E. acros LVT1537	CCC
G. orcutti OS15748-1	A

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S. isolatus OS15622-1	.TC.	
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S. newarkensis OS15743-1	.TC.	
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S. eurysomas OS15635-3	.TAA	
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S. eurysomas PMH9505-1	.TAAAAAA	
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S. eurysomas PMH9505-3	.TAAA	
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S. columbianus OS15577-4	.TAA	
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S. thalassinus OS15437-48	.TAAAA.	
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S. thalassinus BBW2	.TAAA.	
S. thalassinus DFM979-1	.TAAA.	
R. solitarus OS15745-1	.TAA	
H. symmetricus OS15746-1	.TAA	
E. acros LVT1537	AAAC	
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Walker Lake OS15111-2	ΤΑΤ
Humboldt River OS15637-1	ΤΑΤ
Humboldt River OS15637-3	ΤΑΤ
Pleasant Valley OS15512-2	ΤΑΤ
Dixie Valley OS15513-1	ΤΑΤ
Dixie Valley OS15513-2	TAT
EagleLake BB18	TAT
Pyramid Lake BB25	TAT
Pyramid Lake BB28	TAT
"pectinifer" BB48	TAT
"pectinifer" BB49	TAT
"snyderi" OS15747-1	TAT
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Kate Spr OS15515-2	ТА.Т
Blue Eagle Spr. OS15517-2 Green Spring OS15519-2	ТА.Т
Little Fish Lake BB1	ТА.Т
Big Smoky Valley OS15522-1	ТА.Т
Abert Lake OS16774-1	ТАТ
Abert Lake OS16774-2	ТАТ
Abert Lake OS16774-3	ТАТ
Abert Lake OS16722-1	ТАТ
Abert Lake OS16722-2	ТАТ
Abert Lake PMH9503-1	ТАТ
Abert Lake PMH9503-2	ΤΑΤ
Abert Lake PMH9503-3	ΤΑΤ
Hutton Spring PMH9320-1	ΤΑΤ
Hutton Spring PMH9320-3	ΤΑΤ
Rickert Spring OS15312-33	ΤΑΤ
County Road 417 OS15437-47	ΤΑΤ
County Road 417 OS15437-49	ΤΑΤ
Klippel Spring OS15438-16	ΤΑΤ
Klippel Spring OS15438-17	ΤΑΤ
Klippel Spring OS15438-18	ΤΑΤ
Ana Reservoir OS15440-2	TAT
Ana Reservoir OS15440-4	ΤΑΤ
S. isolatus OS15622-1	TT.
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S. newarkensis OS15743-1	TAT
S. newarkensis OS15743-2	TAT
S. newarkensis OS15744-1	TAT

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S. eurysomas OS15635-2	TG	AT
S. eurysomas OS15635-3	TG	AT
S. eurysomas PMH9504-3	Τ	AT
S. eurysomas PMH9505-1	TG	AT
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S. eurysomas PMH9505-3		AT
S. eurysomas OS15574-4		AT
S. columbianus OS15577-4	Τ	AT
S. columbianus OS15577-6		AT
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Siphateles sp. PMH9401-1		AT
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S. bicolor OS16665-1		AT
S. bicolor OS16665-2	Τ	AT
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S. thalassinus BB62		AT
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S. thalassinus OS15437-48		AT
S. thalassinus BBW1		AT
S. thalassinus BBW2		AT
S. thalassinus DFM979-1		AT
R. solitarus OS15745-1		ATG
H. symmetricus OS15746-1		ATG
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G. orcutti OS15748-1		AGG

1 1 6 2 Ω 1 TTAATTACCCAAATTCTGACCGGGTTATTTTTAGCCATGC S. alvordensis S. boraxobius .....A.....A. S. mohavensis S. obesus .....A. Walker Lake OS15111-1 .....A. Walker Lake OS15111-2 .....A.....A. Humboldt River OS15637-1 .....A.....A. Humboldt River OS15637-3 .....A...C.....A. Pleasant Valley OS15512-2 Dixie Valley OS15513-1 .....A. Dixie Vallev OS15513-2 .....A....A. EagleLake BB18 .....A. Pvramid Lake BB25 .....A....A. Pyramid Lake BB28 .....A. "pectinifer" **BB48** .....A....A. "pectinifer" BB49 "snyderi" OS15747-1 .....A....A. Fish Lake Valley OS15520-1 .....A. Fish Lake Valley OS15520-2 .....A.....A.....A.....A.....A. Bull Creek OS15514-1 .....A. Kate Spr OS15515-2 .....A....A. Blue Eagle Spr. OS15517-2 .....A....A. Green Spring OS15519-2 Little Fish Lake BB1 .....A....A. Big Smoky Valley OS15522-1 .....A. Abert Lake OS16774-1 .....A....A. Abert Lake OS16774-2 .....A. Abert Lake OS16774-3 .....A....A. Abert Lake OS16722-1 .....A. Abert Lake OS16722-2 .....A....C......A. Abert Lake PMH9503-1 Abert Lake PMH9503-2 .....A. Abert Lake PMH9503-3 Hutton Spring PMH9320-1 .....A.....A. Hutton Spring PMH9320-3 .....A....A. .....A.....A. Rickert Spring OS15312-33 .....A....A. County Road 417 OS15437-47 .....A...C.....A. County Road 417 OS15437-49 .....A. Klippel Spring OS15438-16 .....A....A. Klippel Spring OS15438-17 .....A.....A. Klippel Spring OS15438-18 .....A....A. Ana Reservoir OS15440-2 .....A....C.....A. Ana Reservoir OS15440-4 .....A....C.....A....A.... S. isolatus OS15622-1 S. isolatus OS15622-3 .....G.....A...C...... S. newarkensis OS15743-1 .....G.....A...C...... S. newarkensis OS15743-2

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S. columbianus OS15577-6	A.
S. columbianus OS15577-8	A.
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Siphateles sp. PMH9401-2	ACA.
S. bicolor 0S16665-1	ACA.
<i>S. bicolor</i> 0S16665-2	AACA.
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S. thalassinus BB62	ACAA.
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S. thalassinus OS15437-48	ACAA.
S. thalassinus BBW1	AACAA.
S. thalassinus BBW2	A
S. thalassinus DFM979-1	A
R. solitarus OS15745-1	AAAGCGA.
H. symmetricus OS15746-1	AAAGCGA.
E. acros LVT1537	GAAAGA.
G. orcutti OS15748-1	GGA.

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s.	columbianus OS15577-4				
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s.	thalassinus DFM979-1		T	.T	AA.
R.	solitarus OS15745-1			.T	.A.
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2 2 4 0 0 1 CCACATCTGCCGAGACGTAAATTATGGCTGACTCATCCGA S. alvordensis S. boraxobius S. mohavensis S. obesus Walker Lake OS15111-1 Walker Lake OS15111-2 Humboldt River OS15637-1 Humboldt River OS15637-3 Pleasant Vallev OS15512-2 Dixie Valley OS15513-1 Dixie Vallev OS15513-2 EagleLake BB18 Pyramid Lake BB25 Pyramid Lake BB28 "pectinifer" **BB48** "pectinifer" **BB49** "snyderi" OS15747-1 Fish Lake Valley OS15520-1 Fish Lake Vallev OS15520-2 Bull Creek OS15514-1 Kate Spr OS15515-2 Blue Eagle Spr. OS15517-2 Green Spring OS15519-2 Little Fish Lake BB1 Big Smoky Valley OS15522-1 Abert Lake OS16774-1 Abert Lake OS16774-2 Abert Lake OS16774-3 Abert Lake OS16722-1 Abert Lake OS16722-2 Abert Lake PMH9503-1 Abert Lake PMH9503-2 Abert Lake PMH9503-3 Hutton Spring PMH9320-1 Hutton Spring PMH9320-3 Rickert Spring OS15312-33 County Road 417 OS15437-47 County Road 417 OS15437-49 Klippel Spring OS15438-16 Klippel Spring OS15438-17 Klippel Spring OS15438-18 Ana Reservoir OS15440-2 Ana Reservoir OS15440-4 S. isolatus OS15622-1 S. isolatus OS15622-3 S. newarkensis OS15743-1 S. newarkensis OS15743-2 S. newarkensis OS15744-1

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S. bicolor OS16665-1	T		
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2 2 4 8 Ω 1 AGCCTACACGCTAATGGAGCATCCTTCTTCTTCATCTGTA S. alvordensis S. boraxobius S. mohavensis S. obesus Walker Lake OS15111-1 Walker Lake OS15111-2 Humboldt River OS15637-1 Humboldt River OS15637-3 ..... Pleasant Valley OS15512-2 Dixie Valley OS15513-1 Dixie Valley OS15513-2 ..... EagleLake BB18 Pyramid Lake BB25 Pyramid Lake BB28 .....T....C..C.... "pectinifer" **BB48** "pectinifer" BB49 "snyderi" OS15747-1 Fish Lake Valley OS15520-1 ....C..C.... Fish Lake Valley OS15520-2 ....T....C..C......... Bull Creek OS15514-1 .....T....C...C.............. Kate Spr OS15515-2 ....T....C..C.... Blue Eagle Spr. OS15517-2 .....T....C...C.... Green Spring OS15519-2 ....T....C..C................. Little Fish Lake BB1 .....C...C..... Big Smoky Valley OS15522-1 ....т....с..с................. Abert Lake OS16774-1 .....T....C..C.... Abert Lake OS16774-2 .....T....C..C......... Abert Lake OS16774-3 .....T....C..C.... Abert Lake OS16722-1 .....T....C...C............... Abert Lake OS16722-2 .....T....C..C........ Abert Lake PMH9503-1 Abert Lake PMH9503-2 ....T..C..C..C.... .....T....C..C.... Abert Lake PMH9503-3 Hutton Spring PMH9320-1 ....T....C..C.... .....T....C..C.... Hutton Spring PMH9320-3 .....T..C..C..C.... Rickert Spring OS15312-33 .....T..C..C..C.... County Road 417 OS15437-47 .....T..C..C..C...... County Road 417 OS15437-49 ....T..C..C..C....... Klippel Spring OS15438-16 .....T..C..C..C...... Klippel Spring OS15438-17 .....T..C..C..C.... Klippel Spring OS15438-18 ....T..C..C..C.... Ana Reservoir OS15440-2 .....T..C..C..C.... Ana Reservoir OS15440-4 .....T....C..C........ S. isolatus OS15622-1 S. isolatus OS15622-3 .....T....C..C.... ....T....C..C.... S. newarkensis OS15743-1 .....T....C...C.... S. newarkensis OS15743-2 ....T....C..C.... S. newarkensis OS15744-1

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S. eurysomas OS15635-1	TCC	
S. eurysomas OS15635-2	TCC	
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S. eurysomas PMH9504-3	TCC	
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S. thalassinus OS15312-31	TCC	
S. thalassinus OS15437-48	TCC.	
S. thalassinus BBW1	TCC	
S. thalassinus BBW2	TCC	
S. thalassinus DFM979-1	TCC	
<i>R. solitarus</i> OS15745-1	TTCGGG	
H. symmetricus OS15746-1		
E. acros LVT1537	GTCA	
G. orcutti OS15748-1	TCGA	

3 2 2 8 1 0 TTTACATACACATTGCCCGAGGCCTATATTACGGGTCGTA S. alvordensis S. boraxobius S. mohavensis S. obesus Walker Lake OS15111-1 ....T......A.. Walker Lake OS15111-2 ....T......A.. Humboldt River OS15637-1 ....T......A.. Humboldt River OS15637-3 Pleasant Valley OS15512-2 Dixie Valley OS15513-1 Dixie Valley OS15513-2 EagleLake BB18 Pvramid Lake BB25 Pyramid Lake BB28 "pectinifer" BB48 ....T.....A.. "pectinifer" BB49 "snyderi" OS15747-1 Fish Lake Valley OS15520-1 Fish Lake Valley OS15520-2 ....T.....A.. Bull Creek OS15514-1 ....T.....A.. Kate Spr OS15515-2 Blue Eagle Spr. OS15517-2 ....T.....A.. ....T.....A.. Green Spring OS15519-2 ....T.....A.. Little Fish Lake BB1 Big Smoky Valley OS15522-1 ....T.....A.. XL Spring PMH9318-1 XL Spring PMH9318-2 XL Spring PMH9318-3 ....T.....A.. Brittain Spring PMH9319-1 ....T.....A.. Brittain Spring PMH9319-2 Hutton Spring PMH9320-1 ....Т.....А.. Hutton Spring PMH9320-3 ....T......A.. Abert Lake PMH9503-1 ....Т.....А.. Abert Lake PMH9503-2 ....T......A.. Abert Lake PMH9503-3 ....T......A.. Rickert Spring OS15312-33 County Road 417 OS15437-47 ....T.....A.. ....T......A.. County Road 417 OS15437-49 Klippel Spring OS15438-16 Klippel Spring OS15438-17 ....T.....A.. Klippel Spring OS15438-18 Ana Reservoir OS15440-2 ....T.....A.. Ana Reservoir OS15440-4 S. isolatus OS15622-1 ....T.....A.. S. isolatus OS15622-3 ....C....A.. S. newarkensis OS15743-1 S. newarkensis OS15743-2 ....C....A.. S. newarkensis OS15744-1

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S. newarkensis OS15744-2	_	CA.	
S. eurysomas OS15572-1	T		
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S. eurysomas OS15635-1	T		
S. eurysomas OS15635-2	T		
S. eurysomas OS15635-3	T		
S. eurysomas PMH9504-3	T		
S. eurysomas PMH9505-1	T		
S. eurysomas PMH9505-2	T	CTA.	
S. eurysomas PMH9505-3	T		
S. eurysomas OS15574-4	T		
S. columbianus OS15577-4	T	A.	
S. columbianus OS15577-6	T	CTA.	
S. columbianus OS15577-8	T		
Siphateles sp. PMH9401-1	T	CTA.	
Siphateles sp. PMH9401-2	T	CTA.	•
S. bicolor OS16665-1	T	CTA.	•
S. bicolor OS16665-2	T	CTA.	•
S. thalassinus BB60	T	CTA.	•
S. thalassinus BB62	T	CTA.	•
S. thalassinus OS15312-32	T	CTA.	•
S. thalassinus OS15312-31	T	CTA.	•
S. thalassinus OS15437-48	T		
S. thalassinus BBW1	T	CTA.	•
S. thalassinus BBW2	T	CTA.	•
S. thalassinus DFM979-1	T	CTA.	•
<i>R. solitarus</i> OS15745-1	T	CTA.	•
H. symmetricus OS15746-1	· · · · T · · · · · T · · · · · · · · ·		
E. acros LVT1537	TTC		
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S. alvordensis	TCTTTATAAAGAAACCTGAAATATTGGTGTCGTCCTACTC
S. boraxobius	
S. mohavensis	CA
S. obesus	
Walker Lake OS15111-1	CA
Walker Lake OS15111-2	CA
Humboldt River OS15637-1	CA
Humboldt River OS15637-3	CA
Pleasant Valley OS15512-2	CA
Dixie Valley OS15513-1	СА
Dixie Valley OS15513-2	СА
-	CGA
EagleLake BB18	CGA
Pyramid Lake BB25	CG
Pyramid Lake BB28	
"pectinifer" BB48	CAA.
"pectinifer" BB49	CA
"snyderi" OS15747-1	CA
Fish Lake Valley OS15520-1	
Fish Lake Valley OS15520-2	CA
Bull Creek OS15514-1	CA
Kate Spr OS15515-2	CA
Blue Eagle Spr. OS15517-2	CAG
Green Spring OS15519-2	CA
Little Fish Lake BB1	CA
Big Smoky Valley OS15522-1	CA
Abert Lake OS16774-1	CAA
Abert Lake OS16774-2	CAA
Abert Lake OS16774-3	CA
Abert Lake OS16722-1	CA
Abert Lake OS16722-2	CA
Abert Lake PMH9503-1	CA
Abert Lake PMH9503-2	CA
Abert Lake PMH9503-3	CG
Hutton Spring PMH9320-1	CGAA
Hutton Spring PMH9320-3	CGA
Rickert Spring OS15312-33	
County Road 417 OS15437-47	
County Road 417 0S15437-49	CAA.
Klippel Spring OS15438-16	CG
Klippel Spring OS15438-17	CAA.
Klippel Spring OS15438-18	CG
Ana Reservoir OS15440-2	CG
Ana Reservoir OS15440-4	CG
S. isolatus OS15622-1	CAA
S. isolatus OS15622-3	CAA
S. newarkensis OS15743-1	CAA
S. newarkensis OS15743-2	CAA

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S. eurysomas OS15635-2	CGG		
S. eurysomas OS15635-3	CGG		
S. eurysomas PMH9504-3	CGG		
S. eurysomas PMH9505-1	CGG		
S. eurysomas PMH9505-2	CGG		
S. eurysomas PMH9505-3			
S. eurysomas OS15574-4	CGG		
S. columbianus OS15577-4	CGG		
S. columbianus OS15577-6	CGG		
S. columbianus OS15577-8	CGG		
Siphateles sp. PMH9401-1	CGG		
Siphateles sp. PMH9401-2	CGG		
S. bicolor OS16665-1	CGG		
S. bicolor OS16665-2	CGG		
S. thalassinus BB60	CGG		
S. thalassinus BB62	CGG		
S. thalassinus OS15312-32	CGG		
S. thalassinus OS15312-31	CGG		
S. thalassinus OS15437-48	CGG		
S. thalassinus BBW1	CGG	A	
S. thalassinus BBW2	CGG		
S. thalassinus DFM979-1	CG		••••
R. solitarus OS15745-1	CG		
H. symmetricus OS15746-1	CG		
E. acros LVT1537	CG		
G. orcutti OS15748-1	С	GCG	

4 3 0 6 0 1 CTTTTAGTTATAATAACCGCCTTCGTGGGCTACGTACTTC S. alvordensis S. boraxobius S. mohavensis S. obesus Walker Lake OS15111-1 Walker Lake OS15111-2 .....C..T..T....C. Humboldt River OS15637-1 Humboldt River OS15637-3 Pleasant Valley OS15512-2 ....G.....C..T..T....C. Dixie Valley OS15513-1 Dixie Valley OS15513-2 ....G.....C..T..T....C. EagleLake BB18 ....C..T..T....C. Pyramid Lake BB25 Pyramid Lake BB28 "pectinifer" BB48 "pectinifer" **BB49** ....G.....C..T..T....C. "snyderi" OS15747-1 Fish Lake Valley OS15520-1 .....C..T..T....C. Fish Lake Valley OS15520-2 Bull Creek OS15514-1 Kate Spr OS15515-2 .....C..T..T....C. ....C..T..T....C. Blue Eagle Spr. OS15517-2 Green Spring OS15519-2 Little Fish Lake BB1 Big Smoky Valley OS15522-1 ....C..T..T....C. Abert Lake OS16774-1 Abert Lake OS16774-2 ....C..T..T....C. Abert Lake OS16774-3 Abert Lake OS16722-1 .....C. Abert Lake OS16722-2 Abert Lake PMH9503-1 Abert Lake PMH9503-2 Abert Lake PMH9503-3 .....C..T..T....C. Hutton Spring PMH9320-1 Hutton Spring PMH9320-3 Rickert Spring OS15312-33 County Road 417 OS15437-47 ....G.....C..T..T....C. County Road 417 OS15437-49 Klippel Spring OS15438-16 Klippel Spring OS15438-17 Klippel Spring OS15438-18 Ana Reservoir OS15440-2 Ana Reservoir OS15440-4 .....C..T..T....C. S. isolatus OS15622-1 ....C..T..T....C. S. isolatus OS15622-3 .....C. S. newarkensis OS15743-1

S. newarkensis OS15743-2

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S. newarkensis OS15744-1	- C
S. newarkensis OS15744-2	C.
S. eurysomas OS15572-1	GC.
S. eurysomas OS15572-2	G
S. eurysomas OS15572-3	G
S. eurysomas OS15635-1	G
S. eurysomas OS15635-2	G
S. eurysomas OS15635-3	G
S. eurysomas PMH9504-3	G
S. eurysomas PMH9505-1	G
S. eurysomas PMH9505-2	G
S. eurysomas PMH9505-3	G
S. eurysomas OS15574-4	G
S. columbianus OS15577-4	G
S. columbianus OS15577-6	G
S. columbianus OS15577-8	GCTTC.
Siphateles sp. PMH9401-1	G
Siphateles sp. PMH9401-2	GCTTC.
S. bicolor OS16665-1	GCTTC.
S. bicolor OS16665-2	G
S. thalassinus BB60	G
S. thalassinus BB62	GCTTC.
S. thalassinus OS15312-32	G
S. thalassinus OS15312-31	G
S. thalassinus OS15437-48	G
S. thalassinus BBW1	G
S. thalassinus BBW2	G
S. thalassinus DFM979-1	GCTTC.
R. solitarus OS15745-1	
H. symmetricus OS15746-1	G
E. acros LVT1537	
G. orcutti OS15748-1	GG

4 4 4 0 0 1 CATGAGGCCAAATATCTTTTTGAGGCGCCACAGTAATTAC S. alvordensis S. boraxobius S. mohavensis S. obesus Walker Lake OS15111-1 ....G..T....... Walker Lake OS15111-2 Humboldt River OS15637-1 ....G..T..... Humboldt River OS15637-3 Pleasant Valley OS15512-2 Dixie Valley OS15513-1 Dixie Valley OS15513-2 EagleLake BB18 Pyramid Lake BB25 Pvramid Lake BB28 "pectinifer" BB48 "pectinifer" BB49 "snyderi" OS15747-1 Fish Lake Valley OS15520-1 Fish Lake Valley OS15520-2 .....G.....G.... Bull Creek OS15514-1 Kate Spr OS15515-2 Blue Eagle Spr. OS15517-2 Green Spring OS15519-2 Little Fish Lake BB1 Big Smoky Valley OS15522-1 Abert Lake OS16774-1 Abert Lake OS16774-2 Abert Lake OS16774-3 Abert Lake OS16722-1 Abert Lake OS16722-2 Hutton Spring PMH9320-1 Hutton Spring PMH9320-3 Abert Lake PMH9503-1 Abert Lake reek PMH9503-2 Abert Lake PMH9503-3 .....G.....G.... Rickert Spring OS15312-33 County Road 417 OS15437-47 County Road 417 OS15437-49 Klippel Spring OS15438-16 Klippel Spring OS15438-17 Klippel Spring OS15438-18 Ana Reservoir OS15440-2 Ana Reservoir OS15440-4 S. isolatus OS15622-1 S. isolatus OS15622-3 S. newarkensis OS15743-1 S. newarkensis OS15743-2 

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s.	newarkensis OS15744-1	G	
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s.	eurysomas OS15572-1	GG	•
s.	eurysomas OS15572-2	GG	
s.	eurysomas OS15572-3	GG	•
s.	eurysomas OS15635-1	GG	•
s.	eurysomas OS15635-2	GG	•
s.	eurysomas OS15635-3	GG	
s.	eurysomas PMH9504-3	GG	
s.	eurysomas PMH9505-1	GG	
s.	eurysomas PMH9505-2	GG	
s.	eurysomas PMH9505-3	GG	
s.	eurysomas OS15574-4	GG	
s.	columbianus OS15577-4	GA	
s.	columbianus OS15577-6	GG	
s.	columbianus OS15577-8	GG	
	phateles sp. PMH9401-1	GG	
-	phateles sp. PMH9401-2	GG	
5.	bicolor OS16665-1	GG	
s.	<i>bicolor</i> 0S16665-2	GG	
5. S.	thalassinus BB60	G	
5. S.	thalassinus BB62	GG	
з. S.	thalassinus OS15312-32	G	
	thalassinus OS15312-32	GG	
S.		GG	
<i>S</i> .	thalassinus OS15437-48	······································	•
<i>S</i> .	thalassinus BBW1	GG	
<i>S</i> .	thalassinus BBW2	GG	
S.	thalassinus DFM979-1	G	٠

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H. symmetricus OS15746-1

R. solitarus OS15745-1

E. acros LVT1537 G. orcutti OS15748-1

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S. alvordensis	-	ACCACTCCCTT	ATATAGGAGACACTCTT
S. boraxobius			•••••
S. mohavensis	C	GA	C
S. obesus			
Walker Lake OS15111-1	GCT.G	G	C
Walker Lake OS15111-2			C
Humboldt River OS15637-1			C
Humboldt River OS15637-3			C
Pleasant Valley OS15512-2			C
Dixie Valley OS15513-1			C
Dixie Valley OS15513-2			C
EagleLake BB18	CT.G	G	C
Pyramid Lake BB25	GCT.G	G	C
Pyramid Lake BB28			C
"pectinifer" BB48			C
"pectinifer" BB49			
"snyderi" OS15747-1			C
Fish Lake Valley OS15520-1			C
Fish Lake Valley OS15520-2	GCT.G	G	C
Bull Creek OS15514-1	GCT.G	G	C
Kate Spr OS15515-2			C
Blue Eagle Spr. OS15517-2			C
			C
Green Spring OS15519-2			
Little Fish Lake BB1			C
Big Smoky Valley OS15522-1			C
Abert Lake OS16774-1			C
Abert Lake OS16774-2	CCT.G	.,G	C
Abert Lake OS16774-3	CCT.G	G	C
Abert Lake OS16722-1			C
Abert Lake OS16722-2			C
Hutton Spring PMH9320-1			
Hutton Spring PMH9320-3			C
Abert Lake PMH9503-1			C
Abert Lake PMH9503-2			C
Abert Lake PMH9503-3	.CCT.G	G	C
Rickert Spring OS15312-33	CCT.G	G	C
County Road 417 OS15437-47			
County Road 417 OS15437-49			C
Klippel Spring OS15438-16			C
Klippel Spring OS15438-17			C
Klippel Spring OS15438-18			C
Ana Reservoir OS15440-2			C
Ana Reservoir OS15440-4	CCT.G	G	C
S. isolatus OS15622-1	CT	G	TC
<i>S. isolatus</i> OS15622-3			
<i>S. newarkensis</i> OS15743-1			GC
S. newarkensis OS15743-2	· · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·

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S. newarkensis OS15744-1	- CTGGGG
S. newarkensis OS15744-2	CTG
S. eurysomas OS15572-1	CGGC
S. eurysomas OS15572-2	CG
S. eurysomas OS15572-3	CG
S. eurysomas OS15635-1	CG
S. eurysomas OS15635-2	CG
S. eurysomas OS15635-3	CGGC
S. eurysomas PMH9504-3	CGGC
S. eurysomas PMH9505-1	CGGC
S. eurysomas PMH9505-2	CG
S. eurysomas PMH9505-3	CG
S. eurysomas OS15574-4	CG
S. columbianus OS15577-4	CTG
S. columbianus OS15577-6	CTG
S. columbianus OS15577-8	CTG
Siphateles sp. PMH9401-1	CTG
Siphateles sp. PMH9401-2	CTGC
S. bicolor OS16665-1	CTGC
S. bicolor OS16665-2	CTGG
S. thalassinus BB60	CTG
S. thalassinus BB62	CTGC
S. thalassinus OS15312-32	CTGC
S. thalassinus OS15312-31	CT
S. thalassinus OS15437-48	CTGC
S. thalassinus BBW1 S. thalassinus BBW2	CTGC CTGC
S. thalassinus BBW2 S. thalassinus DFM979-1	CTGC
	CTT.GCGGC
R. solitarus OS15745-1 H. symmetricus OS15746-1	CTT.GAGC
E. acros LVT1537	GCTT
<i>G. orcutti</i> 0S15748-1	GC
G. OICULLI OBIJ/40-1	U

5 4 2 8 Ω 1 S. alvordensis **GTTCAATGAATTTGAGGAGGCTTCTCAGTAGACAACGCAA** S. boraxobius S. mohavensis ..C.....C....G............... S. obesus Walker Lake OS15111-1 .....C...A.G..... Walker Lake OS15111-2 .....C...A.G..... Humboldt River OS15637-1 .....C...A.G..... Humboldt River OS15637-3 ....C...A.G.... .....C....G..... Pleasant Valley OS15512-2 Dixie Valley OS15513-1 .....C...A.G..... Dixie Valley OS15513-2 EagleLake BB18 ....C....G...... ....G....C....G.....G.....G.... Pyramid Lake BB25 Pyramid Lake BB28 "pectinifer" ....G....C...A.G......G..... BB48 "pectinifer" BB49 .....C...A.G..... .....C.....G..... "snyderi" OS15747-1 Fish Lake Valley OS15520-1 Fish Lake Valley OS15520-2 ....C....G..... Bull Creek OS15514-1 ....G....C....G.....G.....G.... Kate Spr OS15515-2 Blue Eagle Spr. OS15517-2 ....G....C....G.....G.....G.... ....G....C....G.....G.....G.... Green Spring OS15519-2 ....G....C...A.G......G...... Little Fish Lake BB1 .....C....G...... Big Smoky Valley OS15522-1 Abert Lake OS16774-1 ....C....G...... Abert Lake OS16774-2 .....C....G...... Abert Lake OS16774-3 Abert Lake OS16722-1 .....C....G.................... Abert Lake OS16722-2 Abert Lake PMH9503-1 Abert Lake PMH9503-2 Abert Lake PMH9503-3 Hutton Spring PMH9320-1 ....C....G...... Hutton Spring PMH9320-3 .....C....G.................. Rickert Spring OS15312-33 .....C....G.................. County Road 417 OS15437-47 County Road 417 OS15437-49 ....C....G..... Klippel Spring OS15438-16 Klippel Spring OS15438-17 ....C....G..... Klippel Spring OS15438-18 ....C....G..... Ana Reservoir OS15440-2 ....C....G..................... Ana Reservoir OS15440-4 .....C...A.G..... S. isolatus OS15622-1 S. isolatus OS15622-3 .....C...A.G..... S. newarkensis OS15743-1 ....C....G..... S. newarkensis OS15743-2

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S. newarkensis OS15744-1	CA.G	
S. newarkensis OS15744-2	CA.G	
S. eurysomas OS15572-1	CG	• • •
S. eurysomas OS15572-2	CG	• • •
S. eurysomas OS15572-3	CA.G	• • •
S. eurysomas OS15635-1	CA.G	
S. eurysomas OS15635-2	CG	
S. eurysomas OS15635-3	CG	
S. eurysomas PMH9504-3	CG	
S. eurysomas PMH9505-1	CA.G	
S. eurysomas PMH9505-2	CA.G	
S. eurysomas PMH9505-3	CA.G	
S. eurysomas OS15574-4	CG	
S. columbianus OS15577-4	CG	
S. columbianus OS15577-6	CG	
S. columbianus OS15577-8	CG	
Siphateles sp. PMH9401-1	CG	
Siphateles sp. PMH9401-2	CG	
S. bicolor OS16665-1	CG	
S. bicolor OS16665-2	CG	
S. thalassinus BB60	CG	
S. thalassinus BB62	CG	
S. thalassinus OS15312-32	CG	
S. thalassinus OS15312-31	CG	
S. thalassinus OS15437-48	CG	
S. thalassinus BBW1		
S. thalassinus BBW2	CG	
S. thalassinus DFM979-1	CG	
R. solitarus OS15745-1	CA.G	
H. symmetricus OS15746-1	TT	
E. acros LVT1537	G	
G. orcutti OS15748-1	СААСТ	

5 5 2 6 0 1 CGCTAACACGATTCTTCGCCTTCCATTTCCTCCTGCCTTT S. alvordensis S. boraxobius S. mohavensis S. obesus Walker Lake OS15111-1 Walker Lake OS15111-2 Humboldt River OS15637-1 Humboldt River OS15637-3 Pleasant Valley OS15512-2 Dixie Valley OS15513-1 Dixie Valley OS15513-2 EagleLake BB18 Pyramid Lake BB25 Pvramid Lake BB28 "pectinifer" BB48 "pectinifer" BB49 "snyderi" OS15747-1 Fish Lake Valley OS15520-1 Fish Lake Valley OS15520-2 Bull Creek OS15514-1 Kate Spr OS15515-2 Blue Eagle Spr. OS15517-2 Green Spring OS15519-2 .C....C..... Little Fish Lake BB1 Big Smoky Valley OS15522-1 Abert Lake OS16774-1 Abert Lake OS16774-2 Abert Lake OS16774-3 Abert Lake OS16722-1 Abert Lake OS16722-2 Abert Lake PMH9503-1 .C.....A.... Abert Lake PMH9503-2 Abert Lake PMH9503-3 Hutton Spring PMH9320-1 Hutton Spring PMH9320-3 Rickert Spring OS15312-33 .C.....A.... County Road 417 OS15437-47 .C....A.... County Road 417 OS15437-49 Klippel Spring OS15438-16 .C.....A.... Klippel Spring OS15438-17 .C.....A.... Klippel Spring OS15438-18 Ana Reservoir OS15440-2 Ana Reservoir OS15440-4 S. isolatus OS15622-1 .C....T..... .C....T...... S. isolatus OS15622-3 S. newarkensis OS15743-1 S. newarkensis OS15743-2 

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S. newarkensis OS15744-1	.TT	
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S. eurysomas OS15572-2	.TA	
S. eurysomas OS15572-3	.TA	
S. eurysomas OS15635-1	.TA	
S. eurysomas OS15635-2	.TA	
S. eurysomas OS15635-3	.TA	
S. eurysomas PMH9504-3	.TA	
S. eurysomas PMH9505-1	.TA	
S. eurysomas PMH9505-2	.TA	
S. eurysomas PMH9505-3	.TA	
S. eurysomas OS15574-4	.TA	
S. columbianus OS15577-4	.TA	
S. columbianus OS15577-6	.TA	
S. columbianus OS15577-8	.TA	•
Siphateles sp. PMH9401-1	.CAA	•
Siphateles sp. PMH9401-2	.CA	•
S. bicolor OS16665-1	.T	•
S. bicolor OS16665-2	.TA	•
S. thalassinus BB60	.TA	•
S. thalassinus BB62	.TA	•
S. thalassinus OS15312-32	.TA	•
S. thalassinus OS15312-31	.TA	
S. thalassinus OS15437-48	.TA	•
S. thalassinus BBW1	.TA	•
S. thalassinus BBW2	.TA	•
S. thalassinus DFM979-1	.TA	
R. solitarus OS15745-1	.AC	
H. symmetricus OS15746-1		
E. acros LVT1537	.AG	
<i>G. orcutti</i> 0S15748-1	.AG.ACG.	•

6 5 0 6 0 1 TGTTATCGCCGGCGCGACCATCCTACACTTACTATTTCTA S. alvordensis S. boraxobius S. mohavensis S. obesus .....T....CT.. Walker Lake OS15111-1 .....T....CT.. Walker Lake OS15111-2 .....T....CT.. Humboldt River OS15637-1 Humboldt River OS15637-3 Pleasant Valley OS15512-2 .....T....CT.. .....T....CT.. Dixie Valley OS15513-1 .....T....CT.. Dixie Valley OS15513-2 .....T....CT.. EagleLake BB18 Pvramid Lake BB25 .....T....CT.. Pvramid Lake BB28 "pectinifer" BB48 "pectinifer" BB49 .....T....CT.. "snyderi" OS15747-1 Fish Lake Valley OS15520-1 .....T....CT.. .....T....CT.. Fish Lake Valley OS15520-2 Bull Creek OS15514-1 Kate Spr OS15515-2 .....T....CT.. Blue Eagle Spr. OS15517-2 .....T....CT.. Green Spring OS15519-2 Little Fish Lake BB1 Big Smoky Valley OS15522-1 Abert Lake OS16774-1 Abert Lake OS16774-2 .....T....CT.. Abert Lake OS16774-3 .....T....CT.. Abert Lake OS16722-1 Abert Lake OS16722-2 .....T....CT.. Abert Lake PMH9503-1 Abert Lake PMH9503-2 Abert Lake PMH9503-3 Hutton Spring PMH9320-1 .....T....CT.. Hutton Spring PMH9320-3 .....T....CT.. Rickert Spring OS15312-33 .....T....CT.. County Road 417 OS15437-47 .....T....CT.. County Road 417 OS15437-49 .....T....CT.. Klippel Spring OS15438-16 .....T....CT.. Klippel Spring OS15438-17 Klippel Spring OS15438-18 Ana Reservoir OS15440-2 Ana Reservoir OS15440-4 S. isolatus OS15622-1 S. isolatus OS15622-3 S. newarkensis OS15743-1 S. newarkensis OS15743-2

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S. newarkensis OS15744-1	T	.т.
S. newarkensis OS15744-2	T	.т.
S. eurysomas OS15572-1	T	.т.
S. eurysomas OS15572-2		.т.
S. eurysomas OS15572-3	T	.т.
S. eurysomas OS15635-1	T	.т.
S. eurysomas OS15635-2	T	.т
S. eurysomas OS15635-3	T	.т.
S. eurysomas PMH9504-3	T	.т.
S. eurysomas PMH9505-1	T	.т.
S. eurysomas PMH9505-2	T	.т.
S. eurysomas PMH9505-3		.т.
S. eurysomas OS15574-4	T	.т.
S. columbianus OS15577-4	T	
S. columbianus OS15577-6	T	.т
S. columbianus OS15577-8	T	.т
Siphateles sp. PMH9401-1	T	.т
Siphateles sp. PMH9401-2	T	.т
S. bicolor OS16665-1	G	.т
<i>S. bicolor</i> OS16665-2	GT	.т
S. thalassinus BB60	T	.т
S. thalassinus BB62		.т
S. thalassinus OS15312-32	T	.т
S. thalassinus OS15312-31	T	.т
S. thalassinus OS15437-48		.т
S. thalassinus BBW1	T	.т
S. thalassinus BBW2	T	
S. thalassinus DFM979-1	T	.т
R. solitarus OS15745-1	G	
H. symmetricus OS15746-1	TAT	
E. acros LVT1537	GT	
G. orcutti OS15748-1		

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S. alvordensis	CACGAGACCGGCTCAAACAACCCGGCCGGGCTGAACTCTG
S. boraxobius	GG
S. mohavensis	ATGG
S. obesus	
Walker Lake OS15111-1	ATGG
Walker Lake OS15111-2	ATGG
Humboldt River OS15637-1	ATGG
Humboldt River OS15637-3	ATGG
Pleasant Valley OS15512-2	ATGG
Dixie Valley OS15513-1	ATGG
Dixie Valley OS15513-2	ATGGAA
EagleLake BB18	ATGG
Pyramid Lake BB25	ATGG
Pyramid Lake BB28	ATGG
"pectinifer" BB48	ATGG
"pectinifer" BB49	ATGG
"snyderi" OS15747-1	ATGG
	ATGGCG.A
Fish Lake Valley OS15520-1	ATGGCG.A
Fish Lake Valley OS15520-2	ATGG
Bull Creek OS15514-1	
Kate Spr 0S15515-2	ATGGAA
Blue Eagle Spr. 0S15517-2	ATGGAA
Green Spring OS15519-2	ATGG
Little Fish Lake BB1	ATGGAA.
Big Smoky Valley OS15522-1	ATGGAA.
Abert Lake OS16774-1	ATGG
Abert Lake OS16774-2	ATGG
Abert Lake OS16774-3	ATGG
Abert Lake OS16722-1	ATGG
Abert Lake OS16722-2	ATGG
Abert Lake PMH9503-1	ATGG
Abert Lake PMH9503-2	ATGG
Abert Lake PMH9503-3	ATGGA
Hutton Spring PMH9320-1	ATG
Hutton Spring PMH9320-3	ATG
Rickert Spring OS15312-33	ATGG
County Road 417 OS15437-47	ATGG
County Road 417 OS15437-49	ATGG
Klippel Spring OS15438-16	ATGGGAA
Klippel Spring OS15438-17	ATGG
Klippel Spring OS15438-18	ATGGA
Ana Reservoir OS15440-2	ATGG
Ana Reservoir OS15440-4	ATGG
<i>S. isolatus</i> OS15622-1	ATGG
<i>S. isolatus</i> OS15622-3	ATGG
S. newarkensis OS15743-1	
S. newarkensis OS15743-2	

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S. newarkensis OS15744-1	т ТGGАА
S. newarkensis OS15744-1 S. newarkensis OS15744-2	
	ATAG
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S. eurysomas OS15572-3	ATAGG
S. eurysomas OS15635-1	ATAGG
S. eurysomas OS15635-2	ATAGG
S. eurysomas OS15635-3	
S. eurysomas PMH9504-3	ATAG
S. eurysomas PMH9505-1	ATAG
S. eurysomas PMH9505-2 S. eurvsomas PMH9505-3	ATAG
	ATAG
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	ATAGACC.
S. columbianus OS15577-6	
S. columbianus OS15577-8	ATAGACC.
Siphateles sp. PMH9401-1	ATAG
Siphateles sp. PMH9401-2	ATAG
S. bicolor OS16665-1	ATAG
S. bicolor OS16665-2	ATAG
S. thalassinus BB60	ATAGAA
S. thalassinus BB62	ATAGAA
S. thalassinus OS15312-32	ATAGGAA
S. thalassinus OS15312-31	ATAGGAA
S. thalassinus OS15437-48	ATAGAA
S. thalassinus BBW1	ATAGAAA
S. thalassinus BBW2	ATAGAA
S. thalassinus DFM979-1	ATAGAAA
R. solitarus OS15745-1	AAC
H. symmetricus OS15746-1	AA
E. acros LVT1537	AGGAAA
G. orcutti OS15748-1	AGGGCAAT

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S. alvordensis	ATGCGGATAAAGTTTCTTTCCATCCATACTTCTCATACAA
S. boraxobius	· · · · · · · · · · · · · · · · · · ·
S. mohavensis	ACT
S. obesus	
Walker Lake OS15111-1	T
Walker Lake OS15111-2	
Humboldt River OS15637-1	ACT
Humboldt River OS15637-3	
Pleasant Valley OS15512-2	
Dixie Valley OS15513-1	
Dixie Valley OS15513-2	T
EagleLake BB18	T
Pyramid Lake BB25	
Pyramid Lake BB28	
"pectinifer" BB48	T
"pectinifer" BB49	T
"snyderi" OS15747-1	T
Fish Lake Valley OS15520-1	
Fish Lake Valley OS15520-2	
Bull Creek OS15514-1	
Kate Spr OS15515-2	
Blue Eagle Spr. OS15517-2	
Green Spring OS15519-2	
Little Fish Lake BB1	
Big Smoky Valley OS15522-1	
Abert Lake OS16774-1	
Abert Lake OS16774-2	
Abert Lake OS16774-3	
Abert Lake OS16722-1	
Abert Lake OS16722-2	
Abert Lake PMH9503-1	
Abert Lake PMH9503-2	
Abert Lake PMH9503-3	
Hutton Spring PMH9320-1	
Hutton Spring PMH9320-3	
Rickert Spring OS15312-33	T
County Road 417 OS15437-47	
County Road 417 OS15437-49	ACT
Klippel Spring OS15438-16	ACT
Klippel Spring OS15438-17	ACT
Klippel Spring OS15438-18	ACT
Ana Reservoir OS15440-2	ACT
Ana Reservoir OS15440-4	ACT
S. isolatus OS15622-1	ACT
<i>S. isolatus</i> 0515622-3	ACT
<i>S. newarkensis</i> 0S15743-1	ACT
S. newarkensis OS15743-2	ACT
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S. newarkensis OS15744-1	T	••
S. newarkensis OS15744-2	T	••
S. eurysomas OS15572-1		••
S. eurysomas OS15572-2	T	
S. eurysomas OS15572-3		
S. eurysomas OS15635-1	T	
S. eurysomas OS15635-2		
S. eurysomas OS15635-3		
S. eurysomas PMH9504-3		
S. eurysomas PMH9505-1		
S. eurysomas PMH9505-2	T	
S. eurysomas PMH9505-3		
S. eurysomas OS15574-4	AC	
S. columbianus OS15577-4	T	
S. columbianus OS15577-6	T	
S. columbianus OS15577-8		
Siphateles sp. PMH9401-1	T	
Siphateles sp. PMH9401-2	T	
S. bicolor OS16665-1	T	
S. bicolor OS16665-2	T	
S. thalassinus BB60	ACT	
S. thalassinus BB62	C	
S. thalassinus OS15312-32	ACT	
S. thalassinus OS15312-31	T	
S. thalassinus OS15437-48	ACT	
S. thalassinus BBW1	T	
S. thalassinus BBW2	ACT	
S. thalassinus DFM979-1		
<i>R. solitarus</i> OS15745-1		
H. symmetricus OS15746-1	A.CCC	
E. acros LVT1537	ACC	
G. orcutti OS15748-1	CA	••

7 6 2 8 0 1 GGACCTTCTTGGCTTCGTGCTAATACTACTAGCCCTTACA S. alvordensis S. boraxobius A....C.....A....T..T...C... S. mohavensis S. obesus Walker Lake OS15111-1 ....C....C....C....C... Walker Lake OS15111-2 ....C....C....C.... Humboldt River OS15637-1 Humboldt River OS15637-3 .....C.....C.....C.... Pleasant Vallev OS15512-2 Dixie Valley OS15513-1 Dixie Valley OS15513-2 EagleLake BB18 Pyramid Lake BB25 Pyramid Lake BB28 ....T.C.....C.... "pectinifer" BB48 "pectinifer" BB49 "snyderi" OS15747-1 Fish Lake Valley OS15520-1 Fish Lake Valley OS15520-2 ....C....C....C.... Bull Creek OS15514-1 Kate Spr OS15515-2 .....C.....C.....C.... Blue Eagle Spr. OS15517-2 Green Spring OS15519-2 Little Fish Lake BB1 Big Smoky Valley OS15522-1 ....C....C....C.... Abert Lake OS16774-1 Abert Lake OS16774-2 ....C....C....C.... Abert Lake OS16774-3 ....C....C....C.... Abert Lake OS16722-1 Abert Lake OS16722-2 ....C....C....C.... Abert Lake PMH9503-1 Abert Lake PMH9503-2 Abert Lake PMH9503-3 ....C....C....C.... Hutton Spring PMH9320-1 Hutton Spring PMH9320-3 ....C....C....C.... Rickert Spring OS15312-33 County Road 417 OS15437-47 ....C....C....C.... County Road 417 OS15437-49 Klippel Spring OS15438-16 ....C....C....C.... Klippel Spring OS15438-17 Klippel Spring OS15438-18 ....C....C.....C.... Ana Reservoir OS15440-2 Ana Reservoir OS15440-4 S. isolatus OS15622-1 A....C....A....T..... S. isolatus OS15622-3 A....T.....C... S. newarkensis OS15743-1 S. newarkensis OS15743-2 A....C....C....A.....T.....C...

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S. newarkensis OS15744-1	ACATC
S. newarkensis OS15744-2	ATC.A
S. eurysomas OS15572-1	ACATC
S. eurysomas OS15572-2	ACATC
S. eurysomas OS15572-3	ACATC
S. eurysomas OS15635-1	ACATC
S. eurysomas OS15635-2	ACATC
S. eurysomas OS15635-3	ACATC
S. eurysomas PMH9504-3	ACATC
S. eurysomas PMH9505-1	ACATC
S. eurysomas PMH9505-2	ACATC
S. eurysomas PMH9505-3	ACATC
S. eurysomas OS15574-4	ACATC
S. columbianus OS15577-4	ACATC
S. columbianus OS15577-6	ACA
S. columbianus OS15577-8	ACA
Siphateles sp. PMH9401-1	ACATC
Siphateles sp. PMH9401-2	ACATC
S. bicolor OS16665-1	ACATC
S. bicolor OS16665-2	ACATC
S. thalassinus BB60	ACATC
S. thalassinus BB62	ACATC
S. thalassinus OS15312-32	ACATC
S. thalassinus OS15312-31	ACATC
S. thalassinus OS15437-48	ACATC
S. thalassinus BBW1	ACA
S. thalassinus BBW2	ACATC
S. thalassinus DFM979-1	ACATC
R. solitarus OS15745-1	ATC
H. symmetricus OS15746-1	ATCC
E. acros LVT1537	AT
G. orcutti OS15748-1	AC

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S. alvordensis	TCATTAGCATTATTCTCCCCCAACCTATTAGGTGACTCGG
S. boraxobius	
	ТА.
S. mohavensis	· · · · · · · · · · · · · · · · · · ·
S. obesus	
Walker Lake OS15111-1	GCA.
Walker Lake OS15111-2	GCA.
Humboldt River OS15637-1	GCA.
Humboldt River OS15637-3	GCA.
Pleasant Valley OS15512-2	GCA.
Dixie Valley OS15513-1	GCA.
Dixie Valley OS15513-2	
EagleLake BB18	
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Pyramid Lake BB25	
Pyramid Lake BB28	
"pectinifer" BB48	A.
"pectinifer" BB49	
"snyderi" OS15747-1	GCA.
Fish Lake Valley OS15520-1	GCA.
Fish Lake Valley OS15520-2	GCA.
Bull Creek OS15514-1	GCA.
Kate Spr OS15515-2	GCA.
Blue Eagle Spr. OS15517-2	GC
Green Spring OS15519-2	GC
Little Fish Lake BB1	GCA.
Big Smoky Valley OS15522-1	
Abert Lake OS16774-1	
Abert Lake OS16774-2	GCA.
Abert Lake OS16774-3	GCA.
Abert Lake OS16722-1	GCA.
Abert Lake OS16722-2	GCA.
Abert Lake PMH9503-1	GCA.
Abert Lake PMH9503-2	CA.
Abert Lake PMH9503-3	GCA.
Hutton Spring PMH9320-1	GC.GA.
Hutton Spring PMH9320-3	GC.GA.
Rickert Spring OS15312-33	CA.
County Road 417 OS15437-47	A.
-	T
County Road 417 0S15437-49	
Klippel Spring OS15438-16	CA.
Klippel Spring OS15438-17	A.
Klippel Spring OS15438-18	CA.
Ana Reservoir OS15440-2	A.
Ana Reservoir OS15440-4	CA.
S. isolatus OS15622-1	A.
S. isolatus OS15622-3	A.
S. newarkensis OS15743-1	GA.
S. newarkensis OS15743-2	GTA.

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S. newarkensis OS15744-1	GTA.
S. newarkensis OS15744-2	GTTA.
S. eurysomas OS15572-1	CA.
S. eurysomas OS15572-2	CA.
S. eurysomas OS15572-3	CA.
S. eurysomas OS15635-1	CA.
S. eurysomas OS15635-2	C
S. eurysomas OS15635-3	C
S. eurysomas PMH9504-3	C
S. eurysomas PMH9505-1	CA.
S. eurysomas PMH9505-2	C
S. eurysomas PMH9505-3	C
S. eurysomas OS15574-4	CA.
S. columbianus OS15577-4	C
S. columbianus OS15577-6	A.
S. columbianus OS15577-8	CA.
Siphateles sp. PMH9401-1	CA.
Siphateles sp. PMH9401-2	CA.
S. bicolor OS16665-1	CA.
S. bicolor OS16665-2	CA.
S. thalassinus BB60	A.
S. thalassinus BB62	A.
S. thalassinus OS15312-32	A.
S. thalassinus OS15312-31	A.
S. thalassinus OS15437-48	A.
S. thalassinus BBW1	A.
S. thalassinus BBW2	A.
S. thalassinus DFM979-1	A.
R. solitarus OS15745-1	G
H. symmetricus OS15746-1	G
E. acros LVT1537	AGC.CA.
G. orcutti OS15748-1	TCTTTGGTA.

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S. alvordensis	AAAATTTTATTCCAGCAAACCCCCTAGTCACTCCCCCACA
S. boraxobius	
S. mohavensis	CC
S. obesus	
Walker Lake OS15111-1	CG
Walker Lake OS15111-2	CG
Humboldt River OS15637-1	CGCC
Humboldt River OS15637-3	CGC
Pleasant Valley OS15512-2	CG
Dixie Valley OS15513-1	CG
Dixie Valley OS15513-2	CG
EagleLake BB18	CC.
-	CG
Pyramid Lake BB25	CG
Pyramid Lake BB28	
"pectinifer" BB48	CG
"pectinifer" BB49	CG
"snyderi" OS15747-1	CG
Fish Lake Valley OS15520-1	CG
Fish Lake Valley OS15520-2	CG
Bull Creek OS15514-1	CG
Kate Spr OS15515-2	CG
Blue Eagle Spr. OS15517-2	CG
Green Spring OS15519-2	CG
Little Fish Lake BB1	CG
Big Smoky Valley OS15522-1	CG
Abert Lake OS16774-1	CG
Abert Lake OS16774-2	CG
Abert Lake OS16774-3	CC
Abert Lake OS16722-1	CG
Abert Lake OS16722-2	CG
Abert Lake PMH9503-1	CG
	CG
Abert Lake PMH9503-2	
Abert Lake PMH9503-3	CG
Hutton Spring PMH9320-1	CG
Hutton Spring PMH9320-3	CG
Rickert Spring OS15312-33	CG
County Road 417 OS15437-47	CG
County Road 417 OS15437-49	CG
Klippel Spring OS15438-16	CG
Klippel Spring OS15438-17	CG
Klippel Spring OS15438-18	CG
Ana Reservoir OS15440-2	CG
Ana Reservoir OS15440-4	CG
S. isolatus OS15622-1	.GC
<i>S. isolatus</i> OS15622-3	.GC
S. newarkensis OS15743-1	.GCG
S. newarkensis OS15743-2	.GCG
D. HEWGINCHDID ODID/HJ Z	

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	newarkensis OS15744-1	.GCG	
s.	newarkensis OS15744-2	.GCG	
s.	eurysomas OS15572-1	GG	
s.	eurysomas OS15572-2	GG	
s.	eurysomas OS15572-3	GG	
s.	eurysomas OS15635-1	GG	
s.	eurysomas OS15635-2	GG	
s.	eurysomas OS15635-3	GG	
s.	eurysomas PMH9504-3	G	
s.	eurysomas PMH9505-1	G	
s.	eurysomas PMH9505-2	GG	
s.	eurysomas PMH9505-3	G	
s.	eurysomas OS15574-4	G	
s.	columbianus OS15577-4	CG	
s.	columbianus OS15577-6	CG	
S.	columbianus OS15577-8	CG	
Sip	phateles sp. PMH9401-1	CG	
Sip	phateles sp. PMH9401-2	CG	
	bicolor OS16665-1	CG	
	bicolor OS16665-2	CG	
s.	thalassinus BB60	CG	
s.	thalassinus BB62	CG	
s.	thalassinus OS15312-32	CG	
s.	thalassinus OS15312-31	CG	
s.	thalassinus OS15437-48	CG	
s.	thalassinus BBW1	CG	
s.	thalassinus BBW2	CG	
s.	thalassinus DFM979-1	CG	
R.	solitarus OS15745-1		
Η.	symmetricus OS15746-1		
Ε.	acros LVT1537	TT.	
G.	orcutti OS15748-1	.GT	

8 8 0 4 n 1 S. alvordensis TATCCAGCCCGAATGATATTTCTTATTTGCCTACGCCATC S. boraxobius S. mohavensis S. obesus Walker Lake OS15111-1 Walker Lake OS15111-2 Humboldt River OS15637-1 Humboldt River OS15637-3 Pleasant Valley OS15512-2 Dixie Valley OS15513-1 Dixie Valley OS15513-2 EagleLake BB18 ....Т..... Pyramid Lake BB25 Pyramid Lake BB28 "pectinifer" **BB48** "pectinifer" BB49 "snyderi" OS15747-1 Fish Lake Valley OS15520-1 Fish Lake Valley OS15520-2 .....Т...... .....T.....T..... Bull Creek OS15514-1 Kate Spr OS15515-2 Blue Eagle Spr. OS15517-2 Green Spring OS15519-2 ....Т..... Little Fish Lake BB1 Big Smoky Valley OS15522-1 .....Т..... Abert Lake OS16774-1 Abert Lake OS16774-2 Abert Lake OS16774-3 Abert Lake OS16722-1 Abert Lake OS16722-2 Abert Lake PMH9503-1 Abert Lake PMH9503-2 Abert Lake PMH9503-3 Hutton Spring PMH9320-1 Hutton Spring PMH9320-3 Rickert Spring OS15312-33 County Road 417 OS15437-47 County Road 417 OS15437-49 Klippel Spring OS15438-16 Klippel Spring OS15438-17 Klippel Spring OS15438-18 Ana Reservoir OS15440-2 .....T.... .....T.... Ana Reservoir OS15440-4 S. isolatus OS15622-1 S. isolatus OS15622-3 S. newarkensis OS15743-1 С....Т...Т... С....Т...Т... S. newarkensis OS15743-2

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S. newarkensis OS15744-1	CT	••
S. newarkensis OS15744-2	CT	
S. eurysomas OS15572-1	GCTTT.	
S. eurysomas OS15572-2	GCTTT.	
S. eurysomas OS15572-3	GCT	
S. eurysomas OS15635-1	GCTTT.	
S. eurysomas OS15635-2	GCTTT.	
S. eurysomas OS15635-3	GCTTT.	
S. eurysomas PMH9504-3	GCT	
S. eurysomas PMH9505-1	GCTTT.	
S. eurysomas PMH9505-2	GCTTT.	
S. eurysomas PMH9505-3	GCT	
S. eurysomas OS15574-4	GCT	
S. columbianus OS15577-4	GCT	
S. columbianus OS15577-6	GCT	
S. columbianus OS15577-8	GCTTT.	
Siphateles sp. PMH9401-1	GCTTT.	
Siphateles sp. PMH9401-2	GCT	
S. bicolor OS16665-1	GCTTT.	
S. bicolor OS16665-2		
S. thalassinus BB60	GCT	
S. thalassinus BB62	GCT	••
S. thalassinus OS15312-32		••
S. thalassinus OS15312-31	GCT	
S. thalassinus OS15437-48	GCT	
S. thalassinus BBW1	GCT	
S. thalassinus BBW2	GCTTT.	
S. thalassinus DFM979-1	GCTTT.	
R. solitarus OS15745-1	GGGTT.	
H. symmetricus OS15746-1	GGGTT.	
E. acros LVT1537	T	
G. orcutti OS15748-1	T	••

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	4 8
	1 0
S. alvordensis	CTACGATCTATTCCCAATAAACTAGGAGGGGGTCCTAGCAC
S. boraxobius	•••••
S. mohavensis	CG
S. obesus	
Walker Lake OS15111-1	CAC
Walker Lake OS15111-2	CAC
Humboldt River OS15637-1	CA
Humboldt River OS15637-3	CAC
Pleasant Valley OS15512-2	CA
Dixie Valley OS15513-1	CA
Dixie Valley OS15513-2	CA
EagleLake BB18	CA
Pyramid Lake BB25	C
Pyramid Lake BB28	CA
"pectinifer" BB48	AC
"pectinifer" BB49	CA
"snyderi" OS15747-1	CA
Fish Lake Valley OS15520-1	CA
Fish Lake Valley OS15520-2	CAC
-	AC
Bull Creek OS15514-1	
Kate Spr 0S15515-2	C
Blue Eagle Spr. 0S15517-2	C
Green Spring OS15519-2	C
Little Fish Lake BB1	C
Big Smoky Valley OS15522-1	CA
Abert Lake OS16774-1	CAC
Abert Lake OS16774-2	CAC
Abert Lake OS16774-3	CA
Abert Lake OS16722-1	CA
Abert Lake OS16722-2	CA
Abert Lake PMH9503-1	CA
Abert Lake PMH9503-2	CA
Abert Lake PMH9503-3	CACC
Hutton Spring PMH9320-1	CA
Hutton Spring PMH9320-3	CA
Rickert Spring OS15312-33	CA
County Road 417 OS15437-47	CA
County Road 417 OS15437-49	CACCC.
Klippel Spring OS15438-16	CA
Klippel Spring OS15438-17	CA
Klippel Spring OS15438-18	CAC
Ana Reservoir OS15440-2	CAC
Ana Reservoir OS15440-4	CAC
S. isolatus OS15622-1	CA
S. isolatus OS15622-3	CAC
S. newarkensis OS15743-1	CA
S. newarkensis OS15743-2	CA
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	newarkensis OS15744-1	CA	
	newarkensis OS15744-2	CAC	
s.	eurysomas OS15572-1	GCAAC	
s.	eurysomas OS15572-2	ACAAC	
s.	eurysomas OS15572-3	A	
s.	eurysomas OS15635-1	AAACC.	
s.	eurysomas OS15635-2	A	
s.	eurysomas OS15635-3	ACAAC	
s.	eurysomas PMH9504-3	ACAAC	
s.	eurysomas PMH9505-1	CA	
s.	eurysomas PMH9505-2	ACAAA	
s.	eurysomas PMH9505-3	ACAAC	
s.	eurysomas OS15574-4	CACC	
	columbianus OS15577-4	CA	
	<i>columbianus</i> OS15577-6	CAC	
	columbianus OS15577-8	CA	
	ohateles sp. PMH9401-1	GCA	
	phateles sp. PMH9401-2	GCA	
s.	bicolor OS16665-1	CAC	••
s.	<i>bicolor</i> OS16665-2	CACC	
s.	thalassinus BB60	CACC	
s.	thalassinus BB62	CA	
s.	thalassinus OS15312-32	CA	
s.	thalassinus OS15312-31	CA	
s.	thalassinus OS15437-48	CAC	
s.	thalassinus BBW1	CA	
s.	thalassinus BBW2	CA	
s.	thalassinus DFM979-1	CA	
R.	solitarus OS15745-1	CA	
H.	symmetricus OS15746-1	C	••
Ε.	acros LVT1537	G	•••
G.	orcutti OS15748-1		

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S. alvordensis	TACTGTTTAGTATTCTTGTATTACTAGTCGTGCCAGTTCT
S. boraxobius	
S. mohavensis	ACAC.GGGAA.C
S. obesus	
Walker Lake OS15111-1	ACAC.GGAA.C
Walker Lake OS15111-2	ACAC.GGAA.C
Humboldt River OS15637-1	ACAC.GGAA.C
Humboldt River OS15637-3	ACAC.GGAA.C
Pleasant Valley OS15512-2	ACAC.GGAA.C
Dixie Valley OS15513-1	ACAC.GGAA.C
Dixie Valley OS15513-2	ACAC.GGAA.C
EagleLake BB18	ACAC.GGAA.C
Pyramid Lake BB25	ACAC.GGAA.C
Pyramid Lake BB28	ACAC.GGAA.C
"pectinifer" BB48	ACAC.GGAA.C
"pectinifer" BB49	ACAC.GGAA.C
"snyderi" OS15747-1	ACAC.GGAA.C
Fish Lake Valley OS15520-1	ACAC.GGAA.C
Fish Lake Valley OS15520-2	ACAC.GGAA.C
Bull Creek OS15514-1	ACAC.GGAA.C
Kate Spr OS15515-2	ACAC.GGAA.C
Blue Eagle Spr. OS15517-2	ACAC.GGAA.C
Green Spring OS15519-2	ACAC.GGAA.C
Little Fish Lake BB1	ACAC.GGAA.C
Big Smoky Valley OS15522-1	ACAC.GGAA.C
Abert Lake OS16774-1	ACAC.GGAA.C
Abert Lake OS16774-2	ACAC.GGAA.C
Abert Lake OS16774-3	ACAC.GGAA.C
Abert Lake OS16722-1	ACAC.GGAA.C
Abert Lake OS16722-2	ACAC.GGAA.C
Abert Lake PMH9503-1	ACAC.GA.GAA.C
Abert Lake PMH9503-2	ACAC.GGAA.C
Abert Lake PMH9503-3	ACAC.GA.GAA.C
Hutton Spring PMH9320-1	ACAC.GGAA.C
Hutton Spring PMH9320-3	ACAC.GGAA.C
Rickert Spring OS15312-33	ACAC.GGAA.C
County Road 417 OS15437-47	ACAC.GGAA.C
County Road 417 OS15437-49	ACAC.GGGAA.C.
Klippel Spring OS15438-16	ACAC.GGAA.C
Klippel Spring OS15438-17	ACAC.GGAA.C
Klippel Spring OS15438-18	ACAC.GG.AA.C.
Ana Reservoir OS15440-2	ACAC.GGAA.C
Ana Reservoir OS15440-4	A
S. isolatus OS15622-1	AC
S. isolatus OS15622-3	ACGC.GGAC.
<i>S. newarkensis</i> 0515743-1	ACAC.GGGAA.C
S. newarkensis 0315743-1 S. newarkensis 0515743-2	AC.AC.GGGAA.C.
C. HEWAINCHDID ODIJIJJ Z	······································

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S. newarkensis OS15744-1       A. A. C. A. C. G. G. G. A. A.C.         S. newarkensis OS15744-2       A. C. A. C. G. G. G. A. A.C.         S. eurysomas OS15572-1       A. C. A. C. G. G. G. A. A.C.         S. eurysomas OS15572-2       A. C. A. C. G. G. G. A. A.C.         S. eurysomas OS15572-3       A. C. A. C.G. G. G. A. A.C.         S. eurysomas OS15635-1       A. C. A. C.G. G. G. A. A.C.         S. eurysomas OS15635-2       A. C. A. C.G. G. G. A. A.C.         S. eurysomas OS15635-3       A. C. A. C.G. G. G. A. A.C.         S. eurysomas OS15635-3       A. C. A. C.G. G. G. A. A.C.         S. eurysomas OS15635-3       A. C. A. C.G. G. G. A. A.C.         S. eurysomas PMH9505-1       A. C. A. C.G. G. G. A. A.C.         S. eurysomas PMH9505-2       A. C. A. C.G. G. G. A. A.C.         S. eurysomas OS15577-4       A. C. A. C.G. G. G. A. A.C.         S. eurysomas OS15577-4       A. C. A. C.G. G. G. A. A.C.         S. columbianus OS15577-6       A. C. A. C.G. G. G. A. A.C.         S. columbianus OS15577-8       A. C. A. C.G. G. G. A. A.C.         S. bicolor OS16665-1       A. C. A. C.G. G. G. A. A.C.         S. bicolor OS16665-2       A. C. A. C.G. G. G. A. A.C.         S. thalassinus BB60       A. C. A. C.G. G. G. A. A.C.         S. thalassinus OS15312-31       A. C. A. C.G.T.G. G. A. A.C.         S.		8 2
S. newarkensis OS15744-2      AC.AC.GGG.A.A.C         S. eurysomas OS15572-1      AC.AC.GGG.A.A.C         S. eurysomas OS15572-2      AC.AC.GGG.A.A.C         S. eurysomas OS15572-3      AC.AC.GGG.A.A.C         S. eurysomas OS15635-1      AC.AC.GGG.A.A.C         S. eurysomas OS15635-2      AC.AC.GGG.A.A.C         S. eurysomas OS15635-3      AC.AC.GGG.A.A.C         S. eurysomas OS15635-3      AC.AC.GGG.A.A.C.         S. eurysomas PMH9504-3      AC.AC.GGG.A.A.C.         S. eurysomas PMH9505-1      AC.AC.GGG.A.A.A.C.         S. eurysomas PMH9505-2      AC.AC.GGG.A.A.C.         S. eurysomas PMH9505-3      AC.AC.GGG.A.A.C.         S. eurysomas OS15571-4      AC.AC.GGG.A.A.A.C.         S. eurysomas OS15577-4      AC.AC.GGG.A.A.A.C.         S. columbianus OS15577-6      AC.AC.GGG.A.A.A.C.         S. columbianus OS15577-7      AC.AC.GGG.A.A.A.C.         S. bicolor OS16665-1      AC.AC.GGG.A.A.C.         S. bicolor OS16665-1      AC.AC.GGG.A.A.A.C.         S. thalassinus BB62      AC.AC.GT.GG.A.A.A.C.         S. thalassinus OS15312-31      AC.AC.GT.GG.	S powerkongig OS15744-1	1
S. eurysomas OS15572-1       AC.AC.GGG.AA.C.         S. eurysomas OS15572-2       AC.AC.GGG.AA.C.         S. eurysomas OS15635-1       AC.AC.GGG.AA.C.         S. eurysomas OS15635-1       AC.AC.AC.GGG.AA.C.         S. eurysomas OS15635-2       AC.AC.GGG.AA.C.         S. eurysomas OS15635-3       AC.AC.GGG.AA.C.         S. eurysomas OS15635-3       AC.AC.GGG.AA.C.         S. eurysomas PMH9504-3       AC.AC.GGG.AA.C.         S. eurysomas PMH9505-1       AC.AC.GGG.AA.C.         S. eurysomas PMH9505-2       AC.AC.GGG.AA.C.         S. eurysomas PMH9505-3       AC.AC.GGG.AA.C.         S. eurysomas OS15577-4       AC.AC.AC.GGG.AA.C.         S. eurysomas OS15577-4       AC.AC.GGG.AA.C.         S. columbianus OS15577-6       AC.AC.GGG.AA.C.         S. columbianus OS15577-8       AC.AC.GGG.AA.C.         S. columbianus OS15577-8       AC.AC.GGG.AA.C.         S. bicolor OS16665-1       AC.AC.GGG.AA.C.         S. bicolor OS16665-2       AC.AC.GGG.AA.C.         S. thalassinus BB62       AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-31       AC.AC.GT.G.G.AA.C. <td></td> <td></td>		
S. eurysomas OS15572-2       AC.AC.GGGAA.C.         S. eurysomas OS15635-1       AC.AC.GGGAA.C.         S. eurysomas OS15635-2       AC.AC.GGGAA.C.         S. eurysomas OS15635-3       AC.AC.G.G.GAA.C.         S. eurysomas OS15635-3       AC.AC.G.G.G.AA.C.         S. eurysomas OS15635-3       AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-1       AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-2       AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-3       AC.AC.G.G.G.AA.C.         S. eurysomas OS15574-4       AC.AC.G.G.G.AA.C.         S. eurysomas OS15577-4       AC.AC.G.G.G.AA.C.         S. eurysomas OS15577-4       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-6       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-8       AC.AC.G.G.G.AA.C.         S. phateles sp. PMH9401-1       AC.AC.G.G.G.AA.C.         S. bicolor OS16665-1       AC.AC.G.G.G.AA.C.         S. thalassinus BB60       AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-32       AC.AC.GT.G.G.AA.C.         S. thalassinus OS15437-48       AC.AC.GT.GG.AA.C.         S. thalassinus OS15412-41       AC.AC.GT.GG.AA.C. <tr< td=""><td></td><td></td></tr<>		
S. eurysomas OS15572-3       AC.AC.GGGAA.C.         S. eurysomas OS15635-1       AC.AC.GGGAA.C.         S. eurysomas OS15635-2       AC.AC.GGGAA.C.         S. eurysomas OS15635-3       AC.AC.G.GGAA.C.         S. eurysomas OS15635-3       AC.AC.G.G.G.AA.C.         S. eurysomas OS15635-3       AC.AC.G.G.G.AA.C.         S. eurysomas PMH9504-3       AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-1       AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-2       AC.AC.G.G.G.AA.C.         S. eurysomas OS15574-4       AC.AC.G.G.G.AA.C.         S. eurysomas OS15577-4       AC.AC.G.G.G.AA.C.         S. eurysomas OS15577-4       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-6       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-8       AC.AC.G.G.G.AA.C.         S. phateles sp. PMH9401-1       AC.AC.G.G.G.AA.C.         S. bicolor OS16665-1       AC.AC.G.G.G.AA.C.         S. thalassinus BB60       AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-31       AC.AC.GT.G.G.AA.C.         S. thalassinus OS15437-48       AC.AC.GT.G.G.AA.C.         S. thalassinus BB62       AC.AC.GT.GG.AA.C.         S. thalassinus		
S. eurysomas OS15635-1       AC.AC.GGG.AA.C         S. eurysomas OS15635-2       AC.AC.GGG.AA.C         S. eurysomas OS15635-3       AC.AC.GGG.AA.C         S. eurysomas PMH9504-3       AC.AC.GGG.AA.C         S. eurysomas PMH9505-1       AC.AC.GGG.AA.C         S. eurysomas PMH9505-2       AC.AC.GGG.AA.C         S. eurysomas PMH9505-3       AC.AC.GGG.AA.C         S. eurysomas OS15574-4       AC.AC.GGG.AA.C         S. eurysomas OS15574-4       AC.AC.GGG.AA.C         S. eurysomas OS15577-4       AC.AC.GGG.AA.C         S. eurysomas OS15577-4       AC.AC.GGG.AA.C         S. columbianus OS15577-6       AAC.AC.GGG.AA.C         S. columbianus OS15577-8       AC.AC.GGG.AA.C         S. columbianus OS15577-8       AC.AC.GGG.AA.C         S. bicolor OS16665-1       AC.AC.GGG.AA.C         S. bicolor OS16665-2       AC.AC.GGG.AA.C         S. thalassinus BB60       AC.AC.G.GG.AA.C         S. thalassinus OS15312-32       AC.AC.G.GG.AA.C         S. thalassinus OS15312-31       AC.AC.GT.GG.AA.C         S. thalassinus BB62 <t< td=""><td>-</td><td></td></t<>	-	
S. eurysomas OS15635-2      AC.AC.G.G.G.AA.C.         S. eurysomas PMH9504-3      AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-1      AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-2      AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-3      AC.AC.G.G.G.AA.C.         S. eurysomas PMH9505-2      AC.AC.G.G.G.AA.C.         S. eurysomas OS15574-4      AC.AC.G.G.G.AA.C.         S. eurysomas OS15577-4      AC.AC.G.G.G.AA.C.         S. eurysomas OS15577-4      AC.AC.G.G.G.AA.C.         S. eurysomas OS15577-4      AC.AC.G.G.G.AA.C.         S. columbianus OS15577-6      AC.AC.G.G.G.AA.C.         S. columbianus OS15577-8      AC.AC.G.G.G.AA.C.         S. columbianus OS15577-8      AC.AC.G.G.G.AA.C.         S. bicolor OS16665-1      AC.AC.G.G.G.AA.C.         S. bicolor OS16665-2      AC.AC.G.G.G.AA.C.         S. thalassinus BB62      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-32      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C.         S. thalassinus BBV1      AC.A		
S. eurysomas OS15635-3      AC.AC.GGG.AA.C.         S. eurysomas PMH9504-3      AC.AC.GGG.AA.C.         S. eurysomas PMH9505-1      AC.AC.GGG.AA.C.         S. eurysomas PMH9505-2      AC.AC.GGG.AA.C.         S. eurysomas PMH9505-3      AC.AC.GGG.AA.C.         S. eurysomas OS15574-4      AC.AC.GGG.AA.C.         S. eurysomas OS15577-4      AC.AC.GGG.AA.C.         S. columbianus OS15577-6      AC.AC.GGG.AA.C.         S. columbianus OS15577-6      AC.AC.GGG.AA.C.         S. columbianus OS15577-6      AC.AC.GGG.AA.C.         S. columbianus OS15577-8      AC.AC.GGG.AA.C.         S. columbianus OS15577-8      AC.AC.GGG.AA.C.         S. bicolor OS16665-1      AC.AC.GGG.AA.C.         S. bicolor OS16665-2      AC.AC.G.GGAA.C.         S. thalassinus BB60      AC.AC.G.GGAA.C.         S. thalassinus OS15312-32      AC.AC.GT.GGAA.C.         S. thalassinus OS15312-31      AC.AC.GT.GGAA.C.         S. thalassinus OS15437-48      AC.AC.GT.GGAA.C.         S. thalassinus OS15312-31      AC.AC.GT.GGAA.C.         S. t		
S. eurysomas PMH9504-3      AC.AC.GGGAA.C         S. eurysomas PMH9505-1      AC.AC.GGGAA.C         S. eurysomas PMH9505-2      AC.AC.GGGAA.C         S. eurysomas PMH9505-3      AC.AC.GGGAA.C         S. eurysomas OS15574-4      AC.AC.GGGAA.C         S. eurysomas OS15577-4      AC.AC.GGGAA.C         S. columbianus OS15577-4      AC.AC.GGGAA.C         S. columbianus OS15577-6      AC.AC.GGGAA.C         S. columbianus OS15577-8      AC.AC.GGGAA.C         S. columbianus OS15577-8      AC.AC.GGGAA.C         S. bicolor OS16665-1      AC.AC.GGGAA.C         S. bicolor OS16665-2      AC.AC.GGGAA.C         S. thalassinus BB60      AC.AC.GT.GG.AA.C         S. thalassinus OS15312-31      AC.AC.GT.GG.AA.C         S. thalassinus OS15437-48      AC.AC.GT.GG.AA.C         S. thalassinus BB62      AC.AC.GT.GG.AA.C         S. thalassinus DS15437-48      AC.AC.GT.GG.AA.C         S. thalassinus BB92      AC.AC.GT.GG.AA.C         S. thalassinus BB92      AC.AC.GT.GG.AA.C	-	
S. eurysomas PMH9505-2       AC.AC.GGG.AA.C.         S. eurysomas OS15574-4       AC.AC.GGG.AA.C.         S. eurysomas OS15574-4       AC.AC.GGG.AA.C.         S. columbianus OS15577-4       AC.AC.GGG.AA.C.         S. columbianus OS15577-6       AC.AC.GGG.AA.C.         S. columbianus OS15577-6       AC.AC.GGG.AA.C.         S. columbianus OS15577-8       AC.AC.GGG.AA.C.         S. phateles sp. PMH9401-1       AC.AC.GGG.AA.C.         S. bicolor OS16665-1       AC.AC.GGG.AA.C.         S. bicolor OS16665-2       AC.AC.GGG.AA.C.         S. thalassinus BB60       AC.AC.GT.GG.AA.C.         S. thalassinus OS15312-32       AC.AC.GT.GG.AA.C.         S. thalassinus OS15312-31       AC.AC.GT.GG.AA.C.         S. thalassinus OS15437-48       AC.AC.GT.GG.AA.C.         S. thalassinus OS15437-48       AC.AC.GT.GG.AA.C.         S. thalassinus BBW1       AC.AC.GT.GG.AA.C.         S. thalassinus BBW2       AC.AC.GT.GG.AA.C.         S. thalassinus DFM979-1       AC.AC.GT.GG.AA.C.         S. thalassinus DFM979-1       AC.AC.GT.GG.AA.C.         S. thalassinus DFM979-1       AC.AC.GT.GG.AA.C.	S. eurysomas PMH9504-3	
S. eurysomas PMH9505-3      AC.AC.GGGAA.C         S. eurysomas OS15574-4      AC.AC.GGGAA.C         S. columbianus OS15577-4      AC.AC.GGGAA.C         S. columbianus OS15577-6      AC.AC.GGGAA.C         S. columbianus OS15577-6      AC.AC.GGGAA.C         S. columbianus OS15577-6      AC.AC.GGGAA.C         S. columbianus OS15577-6      AC.AC.GGGAA.C         S. columbianus OS15577-8      AC.AC.GGGAA.C         S. phateles sp. PMH9401-1      AC.AC.GGGAA.C         S. bicolor OS16665-1      AC.AC.GGGAA.C         S. bicolor OS16665-2      AC.AC.G.GGAA.C         S. thalassinus BB60      AC.AC.GT.GGAA.C         S. thalassinus OS15312-32      AC.AC.GT.GGAA.C         S. thalassinus OS15312-31      AC.AC.GT.GGAA.C         S. thalassinus BBW1	S. eurysomas PMH9505-1	ACAC.GGGAA.C
S. eurysomas OS15574-4       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-4       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-6       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-6       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-6       AC.AC.G.G.G.AA.C.         S. columbianus OS15577-8       AC.AC.G.G.G.G.AA.C.         S. columbianus OS15577-8       AC.AC.G.G.G.G.AA.C.         S. phateles sp. PMH9401-1       AC.AC.G.G.G.G.AA.C.         S. bicolor OS16665-1       AC.AC.G.G.G.G.AA.C.         S. bicolor OS16665-2       AC.AC.G.G.G.G.AA.C.         S. thalassinus BB60       AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-32       AC.AC.GT.G.G.AA.C.         S. thalassinus OS15437-48       AC.AC.GT.G.G.AA.C.         S. thalassinus BBW1       AC.AC.GT.G.G.AA.C.         S. thalassinus BBW2       AC.AC.GT.G.G.AA.C.         S. thalassinus DFM979-1       AC.AC.GT.G.G.AA.C.         R. solitarus OS15745-1       AC.AC.GT.G.G.AA.C.	S. eurysomas PMH9505-2	ACAC.GGGAA.C
S. columbianus OS15577-4      AC.AC.GGGAA.C         S. columbianus OS15577-6      AC.AC.GGGAA.C         S. columbianus OS15577-8      AC.AC.GGGAA.C         Siphateles sp. PMH9401-1      AC.AC.GGGAA.C         Siphateles sp. PMH9401-2      AC.AC.GGAA.C         S. bicolor OS16665-1      AC.AC.GGAA.C         S. bicolor OS16665-2      AC.AC.GGGAA.C         S. thalassinus BB60      AC.AC.GT.GGAA.C         S. thalassinus OS15312-32      AC.AC.GT.GGAA.C         S. thalassinus OS15312-31      AC.AC.GT.GGAA.C         S. thalassinus OS15437-48      AC.AC.GT.GGAA.C         S. thalassinus BBW1      AC.AC.GT.GGAA.C         S. thalassinus BBW2      AC.AC.GT.GGAA.C         S. thalassinus DFM979-1      AC.AC.GT.GGAA.C         R. solitarus OS15745-1      AGCCAC.GT.GGAA.C	S. eurysomas PMH9505-3	
S. columbianus OS15577-6      AC.AC.G.G.G.AA.C.         Siphateles sp. PMH9401-1      AC.AC.G.G.G.AA.C.         Siphateles sp. PMH9401-2      AC.AC.G.G.G.AA.C.         S. bicolor OS16665-1      AC.AC.G.G.G.AA.C.         S. bicolor OS16665-2      AC.AC.G.G.G.AA.C.         S. thalassinus BB60      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-32      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15437-48      AC.AC.GT.G.G.AA.C.         S. thalassinus BBW1      AC.AC.GT.G.G.AA.C.         S. thalassinus DFM979-1      AC.AC.GT.G.G.AA.C.         S. thalassinus DFM979-1      AC.AC.GT.G.G.AA.C.         R. solitarus OS15745-1      AGC.AC.GT.G.G.AA.C.	-	
S. columbianus OS15577-8      AC.AC.GGG.AA.C.         Siphateles sp. PMH9401-1      AC.AC.GG.AA.C.         Siphateles sp. PMH9401-2      AC.AC.GG.AA.C.         S. bicolor OS16665-1      AC.AC.G.GG.AA.C.         S. bicolor OS16665-2      AC.AC.G.G.G.AA.C.         S. thalassinus BB60      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-32      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15437-48      AC.AC.GT.G.G.AA.C.         S. thalassinus BBW1      AC.AC.GT.G.G.AA.C.         S. thalassinus BBW2      AC.AC.GT.G.G.AA.C.         S. thalassinus DFM979-1      AC.AC.GT.G.G.AA.C.         R. solitarus OS15745-1      AGC.AC.GT.G.G.AA.C.		
Siphateles sp. PMH9401-1      ACAC.GGAA.C         Siphateles sp. PMH9401-2      ACAC.GGAA.C         S. bicolor OS16665-1      ACAC.GGGAA.C         S. bicolor OS16665-2      ACAC.GGGAA.C         S. thalassinus BB60      ACAC.GT.GGAA.C         S. thalassinus BB62      ACAC.GT.GGAA.C         S. thalassinus OS15312-32      ACAC.GT.GGAA.C         S. thalassinus OS15312-31      ACAC.GT.GGAA.C         S. thalassinus OS15312-31      ACAC.GT.GGAA.C         S. thalassinus OS15437-48      ACAC.GT.GGAA.C         S. thalassinus BBW1      ACAC.GT.GGAA.C         S. thalassinus BBW2      ACAC.GT.GGAA.C         S. thalassinus DFM979-1      ACAC.GT.GGAA.C         R. solitarus OS15745-1      A		
Siphateles sp. PMH9401-2      AC.AC.GG.AA.C.         S. bicolor OS16665-1      AC.AC.GGG.AA.C.         S. bicolor OS16665-2      AC.AC.G.GG.AA.C.         S. thalassinus BB60      AC.AC.GT.GG.AA.C.         S. thalassinus BB62      AC.AC.GT.GG.AA.C.         S. thalassinus OS15312-32      AC.AC.GT.GG.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.GG.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.GG.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.GG.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.GG.AA.C.         S. thalassinus OS15437-48      AC.AC.GT.GG.AA.C.         S. thalassinus BBW1      AC.AC.GT.GG.AA.C.         S. thalassinus DFM979-1      AC.AC.GT.GG.AA.C.         R. solitarus OS15745-1      AGCCAC.GT.GG.AA.C.		
S. bicolor OS16665-1      ACAC.GGGAA.C         S. bicolor OS16665-2      ACAC.GGGAA.C         S. thalassinus BB60      ACAC.GT.GGAA.C         S. thalassinus BB62      ACAC.GT.GGAA.C         S. thalassinus OS15312-32      ACAC.GT.GGAA.C         S. thalassinus OS15312-31      ACAC.GT.GGAA.C         S. thalassinus OS15312-31      ACAC.GT.GGAA.C         S. thalassinus OS15437-48      ACAC.GT.GGAA.C         S. thalassinus BBW1      ACAC.GT.GGAA.C         S. thalassinus BBW2      ACAC.GT.GGAA.C         S. thalassinus DFM979-1      ACAC.GT.GGAA.C         R. solitarus OS15745-1      A		
S. bicolor OS16665-2      AC.AC.G.G.G.AA.C.         S. thalassinus BB60      AC.AC.GT.G.G.AA.C.         S. thalassinus BB62      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-32      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C.         S. thalassinus OS15437-48      AC.AC.GT.G.G.AA.C.         S. thalassinus BBW1      AC.AC.GT.G.G.AA.C.         S. thalassinus BBW2      AC.AC.GT.G.G.AA.C.         S. thalassinus DFM979-1      AC.AC.GT.G.G.AA.C.         R. solitarus OS15745-1      AGCGCCA		
S. thalassinus BB60      AC.AC.GT.G.G.AA.C         S. thalassinus OS15312-32      AC.AC.GT.G.G.AA.C         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C         S. thalassinus OS15312-31      AC.AC.GT.G.G.AA.C         S. thalassinus OS15437-48      AC.AC.GT.G.G.AA.C         S. thalassinus BBW1      AC.AC.GT.G.G.AA.C         S. thalassinus BBW2      AC.AC.GT.G.G.AA.C         S. thalassinus DFM979-1      AC.AC.GT.G.G.AA.C         R. solitarus OS15745-1      AGCCAC.GT.GG.AA.C		
S. thalassinus BB62      AC.AC.GT.GGAA.C         S. thalassinus OS15312-32      AC.AC.GT.GGAA.C         S. thalassinus OS15312-31      AC.AC.GT.GGAA.C         S. thalassinus OS15437-48      AC.AC.GT.GGAA.C         S. thalassinus BBW1      AC.AC.GT.GGAA.C         S. thalassinus BBW2      AC.AC.GT.GGAA.C         S. thalassinus DFM979-1      AC.AC.GT.GGAA.C         R. solitarus OS15745-1      AGCGC		
S. thalassinus OS15312-32      AC.AC.GT.GG.AA.C         S. thalassinus OS15312-31      AC.AC.GT.GG.AA.C         S. thalassinus OS15437-48      AC.AC.GT.GG.AA.C         S. thalassinus BBW1      AC.AC.GT.GG.AA.C         S. thalassinus BBW2      AC.AC.GT.GG.AA.C         S. thalassinus DFM979-1      AC.AC.GT.GG.AA.C         R. solitarus OS15745-1      AC.AC.GT.GG.AA.C		
S. thalassinus OS15312-31      AC.AC.GT.GG.AA.C         S. thalassinus OS15437-48      AC.AC.GT.GG.AA.C         S. thalassinus BBW1      AC.AC.GT.GG.AA.C         S. thalassinus BBW2      AC.AC.GT.GG.AA.C         S. thalassinus DFM979-1      AC.AC.GT.GG.AA.C         R. solitarus OS15745-1      AC.AC.GT.GG.AA.C		
S. thalassinus OS15437-48      AC.AC.GT.G.G.AA.C.         S. thalassinus BBW1      AC.AC.GT.G.G.AA.C.         S. thalassinus BBW2      AC.AC.GT.G.G.AA.C.         S. thalassinus DFM979-1      AC.AC.GT.G.G.AA.C.         R. solitarus OS15745-1      AGCCAC.GT.G.C.AA.C.		
S. thalassinus BBW1      AC.AC.GT.GGAA.C         S. thalassinus BBW2      AC.AC.GT.GGAA.C         S. thalassinus DFM979-1      AC.AC.GT.GGAA.C         R. solitarus OS15745-1      AGCGCCA		
S. thalassinus BBW2      AC.AC.GT.GGAA.C         S. thalassinus DFM979-1      AC.AC.GT.GGAA.C         R. solitarus OS15745-1      AGCCAC.A		
S. thalassinus DFM979-1      ACAC.GT.GGAA.C         R. solitarus OS15745-1      AGCGCCA		
<i>R. solitarus</i> OS15745-1AGCCA		
	H. symmetricus OS15746-1	
<i>E. acros</i> LVT1537 .G.ACCACA	-	
G. orcutti OS15748-1 .T.AGCACA		

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Abert Lake PMH9503-3	GAT
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S. newarkensis 0S15743-1	GTA
S. newarkensis OS15743-2	GTA

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S. newarkensis OS15744-1	GTA	Α
S. newarkensis OS15744-2	GT	Α
S. eurysomas OS15572-1	GT	Α
S. eurysomas OS15572-2	GT	A
S. eurysomas OS15572-3	GTA	A
S. eurysomas OS15635-1	GTA	
S. eurysomas OS15635-2	GTA	
S. eurysomas OS15635-3	GTA	A
S. eurysomas PMH9504-3	GTA	
S. eurysomas PMH9505-1	GTA	
S. eurysomas PMH9505-2	GTA	
S. eurysomas PMH9505-3	GTA	
S. eurysomas OS15574-4	GTA	
S. columbianus OS15577-4		
S. columbianus OS15577-6		
S. columbianus OS15577-8	GGTA	
Siphateles sp. PMH9401-1	GTA	Α
Siphateles sp. PMH9401-2	GTA	A
S. bicolor OS16665-1	GTA	A
S. bicolor OS16665-2	GTA	A
S. thalassinus BB60	GTA	
S. thalassinus BB62	GTA	A.A
S. thalassinus OS15312-32	GTA	A.A
S. thalassinus OS15312-31	GTA	A.A
S. thalassinus OS15437-48	GTA	A.A
S. thalassinus BBW1	GTA	A.A
S. thalassinus BBW2		A.A
S. thalassinus DFM979-1		A.A
R. solitarus OS15745-1	T2	A.A
H. symmetricus OS15746-1		A.A
E. acros LVT1537		A.A
G or cutti OS15748-1	Ψ	

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S. alvordensis	ACCCAATTCCTATTCTGAACTCTAGTGGCAGATATACTAA
S. boraxobius	
S. mohavensis	TT
S. obesus	
Walker Lake OS15111-1	T
Walker Lake OS15111-2	TCGC
Humboldt River OS15637-1	T
Humboldt River OS15637-3	T
Pleasant Valley OS15512-2	T
Dixie Valley OS15513-1	T
Dixie Valley OS15513-2	T
EagleLake BB18	TCGC
Pyramid Lake BB25	TCGC
Pyramid Lake BB28	T
"pectinifer" BB48	TCGCT.
"pectinifer" BB49	T
"snyderi" OS15747-1	TTCGCT.
Fish Lake Valley OS15520-1	T
Fish Lake Valley OS15520-2	T
Bull Creek OS15514-1	T
Kate Spr OS15515-2	T
Blue Eagle Spr. OS15517-2	TCGCT.
Green Spring OS15519-2	TT.
Little Fish Lake BB1	TCGT.
Big Smoky Valley OS15522-1	TCGCT.
Abert Lake OS16774-1	TCGCT.
Abert Lake OS16774-2	TCGCT.
Abert Lake OS16774-3	TCGCT.
Abert Lake OS16722-1	TCGCT.
Abert Lake OS16722-2	TT.
Abert Lake PMH9503-1	TCGCT.
Abert Lake PMH9503-2	TCGCT.
Abert Lake PMH9503-3	TCG
Hutton Spring PMH9320-1	TCGCT.
Hutton Spring PMH9320-3	T
Rickert Spring OS15312-33	
County Road 417 0S15437-47	T
County Road 417 OS15437-49	TCGCT.
Klippel Spring OS15438-16	T
Klippel Spring OS15438-17	T
Klippel Spring OS15438-18	T
Ana Reservoir OS15440-2	TCGCT. TCGCT.
Ana Reservoir OS15440-4	GTCGCT.
S. isolatus OS15622-1 S. isolatus OS15622-3	GT
<i>S. Isolatus</i> OSI5622-3 <i>S. newarkensis</i> OS15743-1	GT
J. HEWAIKEHSIS USIST43-1	· · · · · · · · · · · · · · · · · · ·

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S. newarkensis OS15743-2	G
S. newarkensis OS15744-1	G
S. newarkensis OS15744-2	GTCG
S. eurysomas OS15572-1	T
S. eurysomas OS15572-2	TT
S. eurysomas OS15572-3	T
S. eurysomas OS15635-1	T
S. eurysomas OS15635-2	T
S. eurysomas OS15635-3	T
S. eurysomas PMH9504-3	T
S. eurysomas PMH9505-1	T
S. eurysomas PMH9505-2	T
S. eurysomas PMH9505-3	TT.
S. eurysomas OS15574-4	TT.
S. columbianus OS15577-4	T
S. columbianus OS15577-6	T
S. columbianus OS15577-8	T
Siphateles sp. PMH9401-1	
Siphateles sp. PMH9401-2	
S. bicolor OS16665-1	T
S. bicolor OS16665-2	
S. thalassinus BB60	GTT.
S. thalassinus BB62	GTCG,CT.
S. thalassinus OS15312-32	GTCGCT.
S. thalassinus OS15312-31	CT.
S. thalassinus OS15437-48	GTCGCT.
S. thalassinus BBW1 S. thalassinus BBW2	GTCCT. GTCC
	GTCCC
S. thalassinus DFM979-1 R. solitarus OS15745-1	GTCCT.
	GTCCT.
H. symmetricus OS15746-1 E. acros LVT1537	CCCC
<i>G. orcutti</i> 0S15748-1	T
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S. alvordensis	TTTTTAACATGAATTGGGGGGCATACCTGTAGAACACCCCATA
S. boraxobius	
S. mohavensis	.CAGCT
	.CGGC
S. obesus	
Walker Lake OS15111-1	.CAGC
Walker Lake OS15111-2	.CAGC
Humboldt River OS15637-1	.CAGC
Humboldt River OS15637-3	.CAGC
Pleasant Valley OS15512-2	.CAGC
Dixie Valley OS15513-1	.CT
Dixie Valley OS15513-2	.CT
EagleLake BB18	.C
Pyramid Lake BB25	.CAGC
Pyramid Lake BB28	.CAGC
"pectinifer" BB48	.CAGC
"pectinifer" BB49	.CTAGCT
"snyderi" OS15747-1	.CGAGC
Fish Lake Valley OS15520-1	.CAGC
Fish Lake Valley OS15520-2	.CAGC
Bull Creek OS15514-1	.CAGC
Kate Spr OS15515-2	.CAGC
Blue Eagle Spr. OS15517-2	.CAGC
Green Spring OS15519-2	.CAGC
Little Fish Lake BB1	.CCAGC
Big Smoky Valley OS15522-1	.CGAGC
Abert Lake OS16774-1	.CAGC
Abert Lake OS16774-2	.CAGC
Abert Lake OS16774-3	.CAGC
Abert Lake OS16722-1	.CAGC.
Abert Lake OS16722-2	.CAGC
Abert Lake PMH9503-1	.CAGC
Abert Lake PMH9503-2	.CAGC
Abert Lake PMH9503-3	.CAGC.
Hutton Spring PMH9320-1	.CAGC
Hutton Spring PMH9320-3	.CAGC
Rickert Spring OS15312-33	.CAGC
County Road 417 OS15437-47	.CAGC
County Road 417 OS15437-49	.CAGC
Klippel Spring OS15438-16	.CAGC
Klippel Spring OS15438-17	.CAGC
Klippel Spring OS15438-18	.CAGC
Ana Reservoir OS15440-2	.CAGC
Ana Reservoir OS15440-4	.C
S. isolatus OS15622-1	.CAGCT
S. isolatus OS15622-3	.CAGCT
S. newarkensis OS15743-1	.CGAGCT

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S. newarkensis OS15743-2	.CGAGCT	
S. newarkensis OS15744-1	.CGAGCT	
S. newarkensis OS15744-2	.CGAGCT	
S. eurysomas OS15572-1	.CAGC	
S. eurysomas OS15572-2	.CAGC	
S. eurysomas OS15572-3	.CAGC	
S. eurysomas OS15635-1	.CAGC	
S. eurysomas OS15635-2	.CAGC	
S. eurysomas OS15635-3 S. eurysomas PMH9504-3	.CAGC	
S. eurysomas PMH9504-3 S. eurysomas PMH9505-1	.CAGC	
S. eurysomas PMH9505-1 S. eurysomas PMH9505-2	.CAGC	
S. eurysomas PMH9505-3	.CAGC.	
S. eurysomas OS15574-4	.CAGC	
S. columbianus OS15577-4	.CAGC	
S. columbianus OS15577-6	.CAGC	
S. columbianus OS15577-8	.C	
Siphateles sp. PMH9401-1	.CT	
Siphateles sp. PMH9401-2	.CAGCT	••
S. bicolor OS16665-1	.CT	
S. bicolor OS16665-2	.CTAGCT	
S. thalassinus BB60	.CTAGCT	
S. thalassinus BB62	.C	
S. thalassinus OS15312-32	.CAGCT	
S. thalassinus OS15312-31	.C	
S. thalassinus OS15437-48	.C	
S. thalassinus BBW1	.CAGCT	
S. thalassinus BBW2	.CAGCT	
S. thalassinus DFM979-1	.CAGCT	
R. solitarus OS15745-1	.cAc	
H. symmetricus OS15746-1	.C	
E. acros LVT1537 G. orcutti OS15748-1	.CGCACGTG	
G. 0100001 0515740-1	G	

1 1 0 Ω 8 4 1 0 TATTGTTATTGGCCAAGCAGCATCAATTCTATACTTTGCA S. alvordensis S. boraxobius S. mohavensis S. obesus Walker Lake OS15111-1 Walker Lake OS15111-2 ...C......C.....G.C...... Humboldt River OS15637-1 ...C......C.....G.C...... Humboldt River OS15637-3 Pleasant Valley OS15512-2 ...C......C.....G.C...... Dixie Valley OS15513-1 Dixie Valley OS15513-2 ...C......C.....G.C...... EagleLake BB18 Pyramid Lake BB25 ...C......C.....G.C...... Pvramid Lake BB28 "pectinifer" BB48 "pectinifer" BB49 "snyderi" OS15747-1 ...C......C.....G.C..... ...C......C.....GG.C..... Fish Lake Vallev OS15520-1 Fish Lake Valley OS15520-2 Bull Creek OS15514-1 Kate Spr OS15515-2 ...C......C.....G.C...... Blue Eagle Spr. OS15517-2 Green Spring OS15519-2 Little Fish Lake BB1 ...C......C.....G.C...... ...C.....C.....G.C..... Big Smoky Valley OS15522-1 ...C.....C.....C.....C..... Abert Lake OS16774-1 Abert Lake OS16774-2 Abert Lake OS16774-3 Abert Lake OS16722-1 Abert Lake OS16722-2 Abert Lake PMH9503-1 Abert Lake PMH9503-2 ...C......C.....G.C..... Abert Lake PMH9503-3 Hutton Spring PMH9320-1 ...C.....C.....C.....G.C..... Hutton Spring PMH9320-3 Rickert Spring OS15312-33 County Road 417 OS15437-47 County Road 417 OS15437-49 ...C......C.....G.C...... Klippel Spring OS15438-16 Klippel Spring OS15438-17 ...C......C.....G.C...... Klippel Spring OS15438-18 Ana Reservoir OS15440-2 ...C......C.....G.C..... Ana Reservoir OS15440-4 ...C.....C....C.....G.C.....C... S. isolatus OS15622-1 S. isolatus OS15622-3 ...C.....C....C.....G.C.....C... S. newarkensis OS15743-1

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	warkensis OS15743-2	
	warkensis OS15744-1	TGGG
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S. eui	rysomas OS15572-3	TGGG
S. eu	ry <i>somas</i> OS15635-1	
S. eu	rysomas OS15635-2	GGG
S. eu	r <i>ysomas</i> OS15635-3	TGGG
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	rysomas PMH9505-2	
	rysomas PMH9505-3	
	rysomas OS15574-4	T
	lumbianus OS15577-4	
	lumbianus OS15577-6	T
	lumbianus OS15577-8	T
	<i>teles</i> sp. PMH9401-1	T
-	<i>teles</i> sp. PMH9401-2	
	color OS16665-1	TGGG
	color OS16665-2	TGGG
	alassinus BB60	GG
	alassinus BB60	GG
	alassinus OS15312-32	GG
	alassinus OS15312-31	GG
	alassinus OS15512-51 alassinus OS15437-48	
	alassinus BBW1	GG
	alassinus BBW2	GG
	alassinus DFM979-1	GG
	litarus OS15745-1	TGCGTA.G
	nmetricus OS15746-1	TGCGA.G
	ros LVT1537	G.GCTA.G
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S. alvordensis	ACAAAGCACTGAAATGAGCT
S. boraxobius	
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S. obesus	
Walker Lake OS15111-1	. T
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Walker Lake OS15111-2	
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Humboldt River OS15637-3	. T
Pleasant Valley OS15512-2	. T
Dixie Valley OS15513-1	. Т
Dixie Valley OS15513-2	. T
EagleLake BB18	.T
Pyramid Lake BB25	.T
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"snyderi" OS15747-1	. T
Fish Lake Valley OS15520-1	.T
Fish Lake Valley OS15520-2	.T
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Bull Creek OS15514-1	. T
Kate Spr OS15515-2	.T
Blue Eagle Spr. OS15517-2	. T
Green Spring OS15519-2	. T
Little Fish Lake BB1	. T
Big Smoky Valley OS15522-1	.Т
Abert Lake OS16774-1	.T
Abert Lake OS16774-2	.T
Abert Lake OS16774-3	.T
Abert Lake OS16722-1	.T
Abert Lake OS16722-2	.T
Abert Lake PMH9503-1	. T
Abert Lake PMH9503-2	. Т
Abert Lake PMH9503-3	. Т
Hutton Spring PMH9320-1	.T
Hutton Spring PMH9320-3	.T
Rickert Spring OS15312-33	.T
County Road 417 OS15437-47	.T
County Road 417 OS15437-49	.T
Klippel Spring OS15438-16	.T
Klippel Spring OS15438-17	. T
Klippel Spring OS15438-18	.T
Ana Reservoir OS15440-2	.T
Ana Reservoir OS15440-4	.T
S. isolatus OS15622-1	.T
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S. eurysomas OS15572-1	.T
S. eurysomas OS15572-2	.T
S. eurysomas OS15572-3	.T
S. eurysomas OS15635-1	.T
S. eurysomas OS15635-2	. T
S. eurysomas OS15635-3	. T
S. eurysomas PMH9504-3	. T
S. eurysomas PMH9505-1	.Т
S. eurysomas PMH9505-2	.T
S. eurysomas PMH9505-3	.T
S. eurysomas OS15574-4	.T
S. columbianus OS15577-4	. T T
S. columbianus OS15577-6	.TT
S. columbianus OS15577-8	.T
Siphateles sp. PMH9401-1	. T
Siphateles sp. PMH9401-2	.T
S. bicolor OS16665-1	.T
S. bicolor OS16665-2	.T
S. thalassinus BB60	.Т .Т
S. thalassinus BB62 S. thalassinus OS15312-32	. T
S. thalassinus OS15312-32 S. thalassinus OS15312-31	.T
S. thalassinus OS15512-51 S. thalassinus OS15437-48	. I
S. thalassinus BBW1	.T
S. thalassinus BBW1 S. thalassinus BBW2	.T
S. thalassinus DFM979-1	.T
R. solitarus OS15745-1	.TGA
H. symmetricus OS15746-1	.TGA
E. acros LVT1537	.TT.A
G. orcutti 0S15748-1	.T
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#### CHAPTER 3

# MERISTIC AND MORPHOMETRIC VARIATION IN TUI CHUBS (SIPHATELES BICOLOR CLADE) FROM OREGON

# ABSTRACT

Tui chubs (Siphateles bicolor complex) have been long recognized as a widespread, polytypic species inhabiting the endorheic basins of the Great Basin and surrounding areas of western North America. A recent phylogenetic analysis of this putative polytypic species based on mitochondrial DNA cytochrome b gene sequences supported the recognition of six allopatrically distributed species of tui chubs in Oregon. Five species (S. bicolor, S. columbianus, S. eurysomas, S. thalasinnus and Siphateles sp. (Silver Lake)) formed the S. bicolor species group; populations from Summer Basin, Abert Basin and Hutton Springs were assigned to S. obesus. Little information is available on meristic and morphometric variation in the S. bicolor species group, beyond the original subspecies descriptions and an unpublished Masters thesis on geographic variation in S. bicolor oregonensis (= S. obesus this study). Univariate and multivariate analyses of meristic and morphometric characters in the S. bicolor species group and Oregon populations of S. obesus support recognition of the species, and for S. obesus relationships among populations, proposed by the molecular phylogenetic hypothesis. Conservation status and recommendations for these species are also presented.

# INTRODUCTION

Tui chubs (*Siphateles bicolor* species complex; Chapter 2) have been long recognized as a widespread, polytypic species inhabiting the endorheic basins of the Great Basin and surrounding areas of western North America (Bills, 1978; Hubbs and Miller, 1948; Hubbs et al., 1974; Williams et al., 1989; Williams et al., 1985). Given the propensity of tui chubs to exhibit both intra- and inter-basin variation in trophic morphology and body shape (Bills, 1978; Hubbs and Miller, 1948; Hubbs et al., 1974), and the allopatric distribution of the constituent populations, it is not surprising that thirteen subspecies of tui chub were described and at least 16 other populations await taxonomic examination (Williams et al., 1985; Williams et al., 1989). Until recently, there has never been a thorough phylogenetic analysis of either morphological or molecular variation in this species in which to intrepret such patterns of geographic, morphological variation.

Harris (Chapter 2) examined phylogenetic relationships within *Siphateles* based on mitochondrial DNA cytochrome *b* gene sequences. Parsimony analysis of these gene sequences revealed two major evolutionary lineages within the *S. bicolor* species complex: *S. mohavensis* and the *S. bicolor* clade. The *S. bicolor* clade is composed of *S. obesus*, consisting of a large polytomy of populations from California, Nevada and southcentral Oregon; *S. isolatus* species group, consisting of *S. isolatus* and *S. newarkensis* from eastern Nevada; and the *S. bicolor* species group from southern and southeastern Oregon, consisting of (*S. thalasinnus* ((*S.* 

*columbianus* + *S. eurysomas*)(*S. bicolor* + *Siphateles* sp. (Silver Lake))))). Interpretation of patterns of geographic variation within *S. obesus* remains open without a resolved phylogeny on which to base such comparisons. Hubbs et al. (1974) provided comparisons of meristic, morphometric, coloration and tubercle characters between *S. isolatus* and *S. newarkensis*. Little information is available on meristic and morphometric variation in the *S. bicolor* species group, with the exception of the limited comparisons and diagnoses provided in the original subspecies descriptions, and an unpublished Masters thesis on geographic variation in *S. bicolor oregonensis* (= *S. obesus* this study) (Bills, 1978). Herein, meristic and morphometric variation in the *S. bicolor* species group and *S. obesus* is examined in the context of the molecular phylogeny proposed by Harris (Chapter 2). In addition, a discussion is also presented on the conservation status of some species within the *S. bicolor* group and populations within *S. obesus*.

# METHODS

Variation among Oregon populations of tui chub was explored using standard meristic and morphometric characters following Hubbs and Lagler (1958, Table 3.1), except as follows. Prepelvic length was measured from the tip of the

Character	Abbreviation
Standard length	SL
Predorsal length	PREDL
Postdorsal length	POSTDL
Preanal length	PANAL
Prepelvic length	PREP2L
Postpelvic length	POSTP2L
Pelvic-anal length	P2ANAL
Anal-caudal peduncle length	ACAUDL
Head length	HL
Postorbital head length	POSTHL
Head depth	HD
Eye diameter	EYE
Snout length	SNL
Upper jaw length	JAWL
Maximum body depth	BD
Dorsal origin to anal origin length	DAO
Least depth of caudal peduncle	LDCP
Length of caudal peduncle	CPL
Dorsal fin base length	DBASE
Anal fin base length	ABASE
Dorsal fin height	DHT
Anal fin height	AHT
Pectoral fin length	P1L
Pelvic fin length	P2L
Dorsal fin rays	DRAYS
Anal fin rays	ARAYS
Pectoral fin rays	PIRAYS
Pelvic fin rays	P2RAYS
Lateral line scales	LL
Scale rows above lateral line	ALL
Scale rows below lateral line	BLL
Scale rows around caudal peduncle	ACP
Predorsal scales	PDS

Table 3.1. Meristic and morphometric characters and their associated abbreviations.

origin to anal origin was a diagonal measurement between the fins (Chernoff et al., 1982). Post-dorsal fin and post pelvic fin lengths were from the respective origins of the fins to the caudal base. Body measurements were generated using either electronic calipers (nearest 0.01 mm) or a video imaging system; all morphometric data was input directly into an electronic database.

In order to reduce subjectivity in analyzing species composition, one-way analysis of variance (ANOVA) was performed on nine meristic variables among populations within species identified by Harris (Chapter 2). When no differences were found, populations were combined. However, populations were treated as separate entities whenever a majority ( $\geq$ 5) of the meristic variables were significantly different among populations within a species. The level of significance for all ANOVAs was P < 0.01.

Meristic and morphometric data were analyzed separately following Chernoff (1982), Chernoff et al. (1982) and Warren (1992). Meristic analyses were based on 790 individuals from all species in the *S. bicolor* species group plus Oregon populations of *S. obesus* (see Material Examined). Univariate and multivariate statistical analysis of the meristic data was performed using Statistica for Windows, version 4.0 (Statsoft, Inc., Tulsa, OK). Principal components analysis (PCA) of meristic variation was computed from the correlation matrix of nine meristic characters.

Multivariate analysis of morphometric characters was based on 589 individuals. Sheared PCA (Humphries et al., 1981; Bookstein et al., 1985) of morphometric characters was used to eliminate overall size effects and summarize changes in body shape among species and populations within species. Sheared PCA were computed from the covariance matrix of 24 morphometric characters (SAS code for running sheared PCA provided by D.L. Swofford).

### **RESULTS AND DISCUSSION**

# Meristic Variation

One-way ANOVA of the nine meristic characters found no significant intraspecific variation, except within *S. thalassinus*. This species is found in two basins, Goose Lake and Warner Basin; within the Warner Basin are two allopatric populations, Warner Valley and Cowhead Lake. Within *S. thalassinus*, significant differences (P < 0.01) were found in seven of nine meristic characters (DRAYS, ARAYS, P1RAYS, P2RAYS, LL, ALL, BLL, ACP, PDS) among Goose Lake, Warner Valley and Cowhead Lake populations. Variation in the nine meristic characters are summarized as frequency distributions in Tables 3.2–3.11.

Principal components analysis for the nine meristic characters indicates that principal components axes one (PC 1) and two (PC 2) account for 51% (36% and 15%, respectively) of the total variance among 790 individuals (Table 3.12). Loadings show PC 1 is associated with scale counts and PC 2 with fin ray counts.

			D	orsal Fin Ra	ys		
Species	7	8	9	10	N	$\overline{x}$	SD
Siphateles bicolor		9	25		34	8.79	0.41
Siphateles sp.	1	66	65		132	8.48	0.52
Siphateles columbianus	1	9	25		35	8.75	0.65
Siphateles eurysomas							
Catlow Basin		28	2		30	8.17	0.40
Guano Basin		25	5		30	8.07	0.25
Siphateles thalassinus							
Goose Lake Basin		4	26		30	8.87	0.35
Warner Valley		16	30		46	8.65	0.48
Cowhead Lake	1	26	3		30	8.07	0.37
Siphateles obesus							
Summer Basin	1	158	23		182	8.12	0.34
Abert Basin	2	109	16		127	8.11	0.36
Hutton Springs		.97	13	3	113	8.16	0.44

Table 3.2. Frequency distributions of dorsal fin rays in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

			1	Anal Fin Ray	'S		
Species	7	8	9	10	N	$\overline{x}$	SD
Siphateles bicolor	1	30	3	·	34	8.05	0.34
Siphateles sp.	23	105	4		132	7.86	0.43
Siphateles columbianus	1	34	1		36	8.00	0.24
Siphateles eurysomas							
Catlow Basin	a	30			30	8.00	0.00
Guano Basin		26	3	1	30	8.17	0.46
Siphateles thalassinus							
Goose Lake Basin		28	2		30	8.06	0.25
Warner Valley	2	43	1		46	7.98	0.26
Cowhead Lake		27	3		30	8.10	0.31
Siphateles obesus							
Summer Basin	82	97	3		182	7.57	0.53
Abert Basin	26	97	4		127	7.83	0.46
Hutton Springs	11	95	6		113	7.98	0.48

Table 3.3. Frequency distributions of anal fin rays in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

				Pec	toral Fin R	lays			
Species	13	14	15	16	17	18	N	$\overline{x}$	SD
Siphateles bicolor			26	7			33	15.21	0.42
Siphateles sp.	2	30	83	17			132	14.87	0.63
Siphateles columbianus	1	2	33				36	14.89	0.40
Siphateles eurysomas									
Catlow Basin		1	28	1			30	15.00	0.46
Guano Basin		6	23	1			30	14.83	0.26
Siphateles thalassinus									
Goose Lake Basin		1	18	10	1		30	15.37	1.61
Warner Valley		2	3	25	13	3	46	16.26	0.85
Cowhead Lake		16	14				30	14.47	0.51
Siphateles obesus									
Summer Basin	4	48	108	21	1		182	14.82	0.68
Abert Basin		5	34	66	19	3	127	15.85	0.81
Hutton Springs	1	14	72	21	3	2	113	15.15	0.72

Table 3.4. Frequency distributions of pectoral fin rays in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

· · · · · · · · · · · · · · · · · · ·	-	Pelvic Fin Rays										
Species	7	8	9	10	11	N	$\overline{x}$	SD				
Siphateles bicolor		1 .	30	3		34	9.06	0.34				
Siphateles sp.	1	12	103	15		132	9.00	0.49				
Siphateles columbianus		3	33			36	8.92	0.28				
Siphateles eurysomas												
Catlow Basin			30			30	9.00	0.00				
Guano Basin		4	22	4		30	9.00	0.53				
Siphateles thalassinus												
Goose Lake Basin		2	27	1		30	8.97	0.32				
Warner Valley		2	35	9		46	9.15	0.47				
Cowhead Lake		13	17			30	8.57	0.50				
Siphateles obesus												
Summer Basin	1	20	139	22		182	9.00	0.50				
Abert Basin		4	63	59	1	127	9.45	0.57				
Hutton Springs		1	89	23		113	9.19	0.42				

Table 3.5. Frequency distributions of pelvic fin rays in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

Species	Lateral Line Scales													
	42	43	44	45	46	47	48	49	50	51	52	53	54	55
Siphateles bicolor		2		4	2	5	5	4	5	5	1			
Siphateles sp.		1	4	4	4	6	14	10	15	18	13	7	1	1
Siphateles columbianus	2		4	2	4	6	3	5	5	1	2			
Siphateles eurysomas														
Catlow Basin									1		1 -	2	3	4
Guano Basin										1		2	2	2
Siphateles thalassinus														
Goose Lake Basin		1				1	5	5	4	4	4	2	2	2
Warner Valley						1		5	6	4	8	3	5	6
Cowhead Lake											4	1	2	2
Siphateles obesus														
Summer Basin			2	4	6	8	17	14	18	26	22	18	14	11
Abert Basin						1	1		9	10	15	21	18	19
Hutton Springs			1	2		5	3	7	9	15	18	16	8	10

Table 3.6. Frequency distributions of lateral line scales in species of the Siphateles bicolor group and Oregon populations of S. obesus.

Tab	le 3.7.	Continued.

Species	Lateral Line Scales											
	56	57	58	59	60	61	62	63	N	$\overline{x}$	SD	
Siphateles bicolor									33	48.06	2.98	
Siphateles sp.									123	50.43	2.40	
Siphateles columbianus									34	47.35	2.68	
Siphateles eurysomas												
Catlow Basin	5	7	1	2	2				30	56.23	2.60	
Guano Basin	7	5	9			1	1	1	30	56.63	2.40	
Siphateles thalassinus												
Goose Lake Basin									30	50.37	3.14	
Warner Valley	5	2	1						46	52.67	2.67	
Cowhead Lake	4	2	7	1	3				30	56.23	2.58	
Siphateles obesus												
Summer Basin	7	4	3	1					175	51.20	3.09	
Abert Basin	9	12	6	3	1	2	1		127	54.02	2.62	
Hutton Springs	8	8	3						113	52.34	3.00	

	Scale Rows Above Lateral Line										
Species	9	10	11	12	13	14	15	N	$\overline{x}$	SD	
Siphateles bicolor	12	18	4					34	9.76	0.71	
Siphateles sp.		23	69	36	2			130	11.13	0.60	
Siphateles columbianus		5	23	8				36	11.08	0.65	
Siphateles eurysomas											
Catlow Basin				7	13	10		30	13.10	0.76	
Guano Basin			1	13	12	4		30	12.63	0.76	
Siphateles thalassinus											
Goose Lake Basin	1	10	16	3				30	10.70	0.70	
Warner Valley		1	13	22	9		1	46	11.93	0.88	
Cowhead Lake			1	5	14	10		30	13.10	0.80	
Siphateles obesus											
Summer Basin	3	44	78	49	5			179	11.05	0.84	
Abert Basin			9	61	46	11		127	12.46	0.75	
Hutton Springs			23	66	22	2		113	12.03	0.69	

Table 3.8. Frequency distributions of scale rows above lateral line in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

	Scale Rows Above Below Line										
Species	5	6	7	8	9	10	N	$\overline{x}$	SD		
Siphateles bicolor	7	26	1				34	5.82	0.46		
Siphateles sp.		29	79	21	1		130	6.95	0.65		
Siphateles columbianus	2	15	17	2			36	6.52	0.70		
Siphateles eurysomas											
Catlow Basin		1	16	13			30	7.40	0.56		
Guano Basin			5	21	4		30	7.97	0.56		
Siphateles thalassinus											
Goose Lake Basin	4	16	10				30	6.20	0.66		
Warner Valley		3	29	14			46	7.24	0.57		
Cowhead Lake			13	12	5		30	7.73	0.74		
Siphateles obesus											
Summer Basin		27	97	44	11		179	7.22	0.77		
Abert Basin		2	38	72	13	2	127	7.80	0.70		
Hutton Springs		16	50	46		1	113	7.29	0.74		

Table 3.9. Frequency distributions of scale rows below lateral line in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

	Scale Rows Around Caudal Peduncle																
Species	20	21	22	23	24	25	26	27	28	29	30	31	32	33	N	$\overline{x}$	SD
Siphateles bicolor		4	4	11	8	4	1								32	23.22	1.29
Siphateles sp.			2	10	13	19	31	18	24	5	5	2	1		130	26.25	1.99
Siphateles columbianus				1	3	5	7	8	4	1					29	26.17	1.44
Siphateles eurysomas																	
Catlow Basin						4	3	8	6	7	1	1			30	27.53	1.40
Guano Basin					5	11	4	6	3	1					30	25.80	1.55
Siphateles thalassinus																	
Goose Lake Basin		2		2	5	8	9	4							30	25.00	1.55
Warner Valley	1		1	2	3	6	10	9	7	4	2			1	46	26.48	2.27
Cowhead Lake							2	4	6	6	5	1			24	28.46	1.35
Siphateles obesus																	
Summer Basin		1	2	2	20	25	22	30	28	19	18	6	5		178	27.07	2.62
Abert Basin						1	7	10	23	20	24	20	17	4	126	29.50	1.86
Hutton Springs				1	3	10	14	17	31	16	11	6	4		113	27.82	1.91

Table 3.10. Frequency distributions of scale rows around caudal peduncle in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

	Predorsal Scales																			
Species	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	N	$\overline{x}$	SD
Siphateles bicolor	1	8	7	7	5	6									_			34	21.74	1.50
Siphateles sp.				2	7	13	18	30	17	15	4	6	1					113	26.13	1.88
Siphateles columbianus				1	1	4	7	5	9	6	2							35	26.14	1.70
Siphateles eurysomas																				
Catlow Basin										1		8	5	6	5	4		29	28.59	1.88
Guano Basin							1	3	5	6	4	5	3	2				29	31.59	1.57
Siphateles thalassinus																				
Goose Lake Basin	1	1		2	3	16	4	2	1									30	23.83	1.58
Warner Valley						1	4	10	16	9	2	3	1					46	26.48	2.27
Cowhead Lake							1	3	4	5	2	4	4	1	3	2		30	29.38	2.58
Siphateles obesus																				
Summer Basin				4	12	16	33	30	18	13	7	11	5	1	3	3		156	26.44	2.60
Abert Basin							7	18	34	17	20	13	9	4			1	125	28.01	1.86
Hutton Springs					1	3	13	19	24	26	10	9	3	1				109	27.29	1.73

Table 3.11. Frequency distributions of predorsal scales in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

	Principal Component					
Variable	PC 1	PC 2				
Dorsal fin rays	-0.434	-0.444				
Anal fin rays	0.032	-0.585				
Pectoral fin rays	0.239	-0.691				
Pelvic fin rays	0.209	-0.557				
Lateral line scales	0.747	-0.036				
Scale rows above lateral line	0.821	-0.065				
Scale rows below lateral line	0.767	0.066				
Scale rows around caudal peduncle	0.732	0.058				
Predorsal scales	0.783	0.121				
% Total Variance	36.2	15.1				

Table 3.12. Variance loadings for principal components analysis of meristic variables in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

While there is considerable overlap in patterns of meristic variation among species (Fig. 3.1a), there are patterns that are concordant with sister-species relationships proposed by the molecular phylogeny. *S. bicolor* and *Siphateles* sp. (Silver Lake) demonstrate minimal overlap in multivariate space, with *S. bicolor* having lower mean scale counts than *Siphateles* sp. (Silver Lake). Similarly, *S. columbianus* and *S. eurysomas* display minimal overlap in meristic variation, with *S. columbianus* having lower mean scale counts. The greatest amount of intraspecific variation in multivariate space was exhibited by *S. obesus* and *S. thalassinus*. No distinct pattern of population separation was observed in PCA scores among populations comprising *S. obesus*. PCA scores of specimens within *S. thalassinus*, however, demonstrated marked separation of Goose Lake and Cowhead Lake specimens,

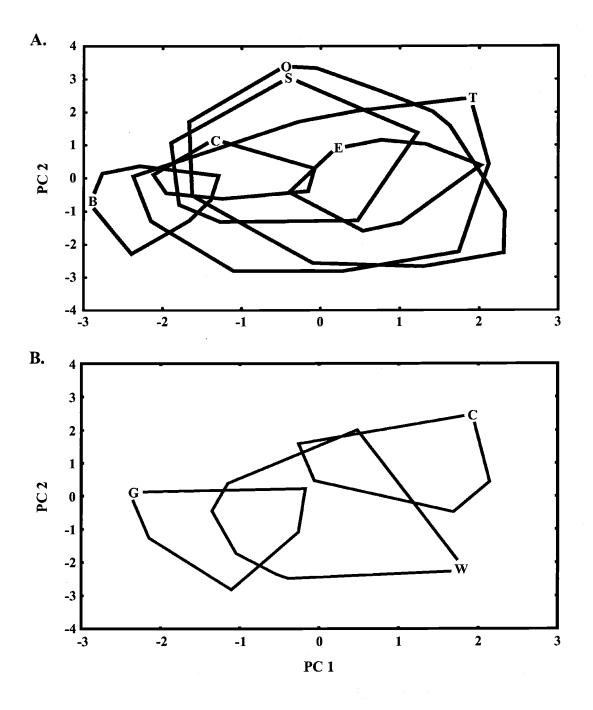


Figure 3.1. Principal component analysis of nine meristic variables. A) Analysis of 790 individuals from the *S. bicolor* species group and Oregon populations of *S. obesus*. B = *S. bicolor*; C = *S. columbianus*; E = *S. eurysomas*; O = *S. obesus*; S = *Siphateles* sp. (Silver Lake); T = *S. thalassinus*. B) Plot of principal component scores for the three populations comprising *S. thalassinus*. C = Cowhead Lake; G = Goose Lake; W = Warner Valley.

with Warner Valley specimens occupying an intermediate position between the aforementioned populations (Fig. 3.1b).

# Morphometric Variation

General patterns of body shape variation are summarized by the sheared principal components analysis (Fig. 3.2a; Table 3.13), discriminant functions analysis (Fig. 3.3; Table 3.14) and bivariate plots of mensural characters (Figs. 3.4-3.5). While broad overlap exists in the variation of mensural characters among species of tui chubs in Oregon, there is some concordance between mensural variation and evolutionary lineages identified by the molecular phylogeny. For example, S. obesus is separated from the S. bicolor species group by having a comparably longer head (Fig. 3.4). The hypothesis of homogeneity of slopes between the two taxa is rejected (P < 0.01). In morphometric space, S. bicolor and Siphateles sp. (Silver Lake) minimally overlap along sheared PC2 (Fig. 3.2A). Mensural characters loading heavily (i.e., eigenvector > 0.3) on sheared PC2 include snout length and upper jaw length; along sheared PC3 dorsal fin and anal fin base length loaded most heavily. A plot of snout length against SL indicates that Siphateles sp. (Silver Lake) has a comparatively longer snout than its sister species, S. bicolor (Fig. 3.5a). The hypothesis of homogeneity of slopes between the two species is rejected (P < 0.01).

Discriminant functions analysis of the five species comprising the *S. bicolor* species group yielded an 86.1% correct classification rate (Fig. 3.3; Table 3.14).

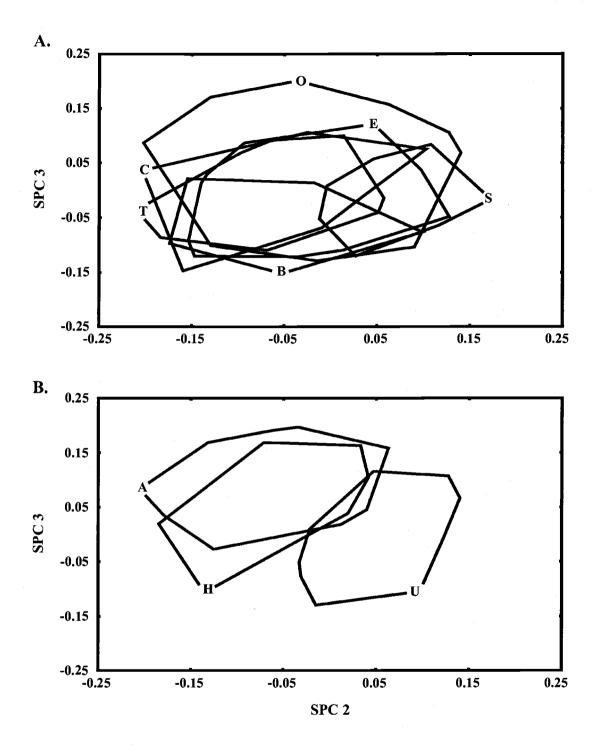


Figure 3.2. Principal components analysis of 24 sheared morphometric variables. A) Analysis of 589 individuals from the *S. bicolor* species group and Oregon populations of *S. obesus*. B = *S. bicolor*; C = *S. columbianus*; E = *S. eurysomas*; O = *S. obesus*; S = *Siphateles* sp. (Silver Lake); T = *S. thalassinus*. B) Plot of sheared principal component scores for populations from the three basins comprising *S. obesus*. A = Abert Basin; H = Hutton Springs; U = Summer Basin.

	Sheared Principal Components						
Measurement	SPC 2	SPC 3					
Standard length	-0.042	0.087					
Predorsal length	-0.008	0.151					
Postdorsal length	-0.065	0.009					
Preanal length	-0.043	0.177					
Prepelvic length	-0.010	0.168					
Postpelvic length	-0.048	0.007					
Pelvic-anal length	-0.096	0.226					
Anal-caudal peduncle length	-0.025	-0.125					
Head length	0.096	0.119					
Postorbital head length	-0.146	0.251					
Head depth	-0.044	0.204					
Eye diameter	0.194	0.096					
Snout length	0.692	-0.026					
Upper jaw length	0.476	0.024					
Maximum body depth	-0.096	0.155					
Dorsal origin to anal origin length	-0.139	0.159					
Least depth of caudal peduncle	-0.154	0.107					
Length of caudal peduncle	0.167	-0.047					
Dorsal fin base length	-0.009	-0.409					
Anal fin base length	0.044	-0.565					
Dorsal fin height	-0.162	-0.102					
Anal fin height	-0.174	-0.214					
Pectoral fin length	-0.190	-0.263					
Pelvic fin length	-0.172	-0.198					

Table 3.13. Variance loadings for sheared principal components analysis of morphometric variables in species of the *Siphateles bicolor* group and Oregon populations of *S. obesus*.

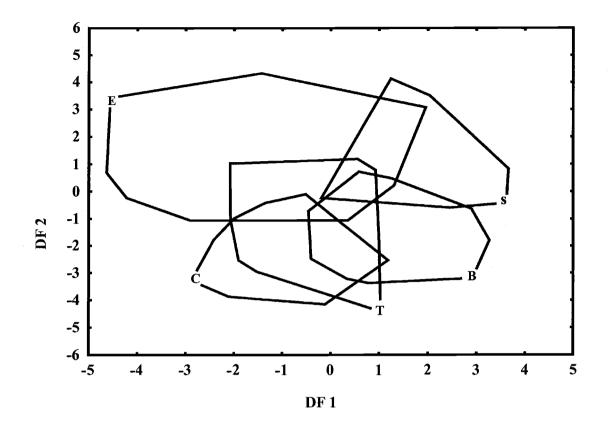


Figure 3.3. Discriminant functions of 18 morphometric variables for 340 individuals from the *S. bicolor* species group. B = S. bicolor; C = S. columbianus; E = S. eurysomas; S = Siphateles sp. (Silver Lake); T = S. thalassinus.

	Standardized Coefficients						
Measurement	CV 1	CV 2					
Standard length	2.549	0.523					
Predorsal length	-1.838	-1.326					
Postdorsal length	0.946	-3.535					
Postpelvic length	-0.598	1.110					
Pelvic-anal length	-0.132	-1.173					
Anal-caudal peduncle length	-0.626	1.718					
Postorbital head length	-2.346	-0.100					
Head depth	1.325	0.522					
Snout length	0.994	-1.326					
Maximum body depth	0.371	0.649					
Dorsal origin to anal origin length	0.829	3.392					
Least depth of caudal peduncle	-2.396	-0.381					
Length of caudal peduncle	0.617	-1.375					
Dorsal fin base length	0.272	0.793					
Anal fin base length	-0.488	-0.326					
Dorsal fin height	1.429	0.859					
Anal fin height	-0.249	1.189					
Pectoral fin length	-1.000	-1.500					

Table 3.14. Standardized coefficients for canonical variables in the discriminant functions analysis of 18 morphometric characters in species of the *Siphateles bicolor* group.

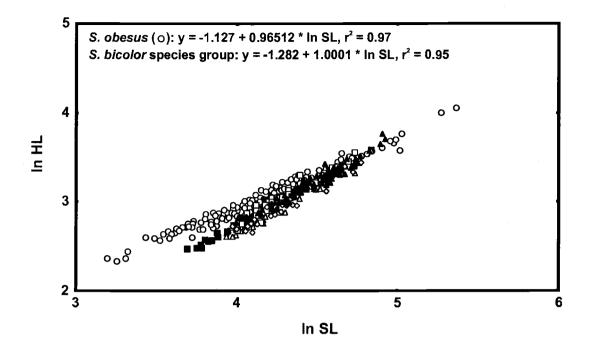


Figure 3.4. Relationship between head length and standard length between Oregon populations of *S. obesus* and the *S. bicolor* species group. *S. obesus* = open circles; *S. bicolor* species group = all other symbols.

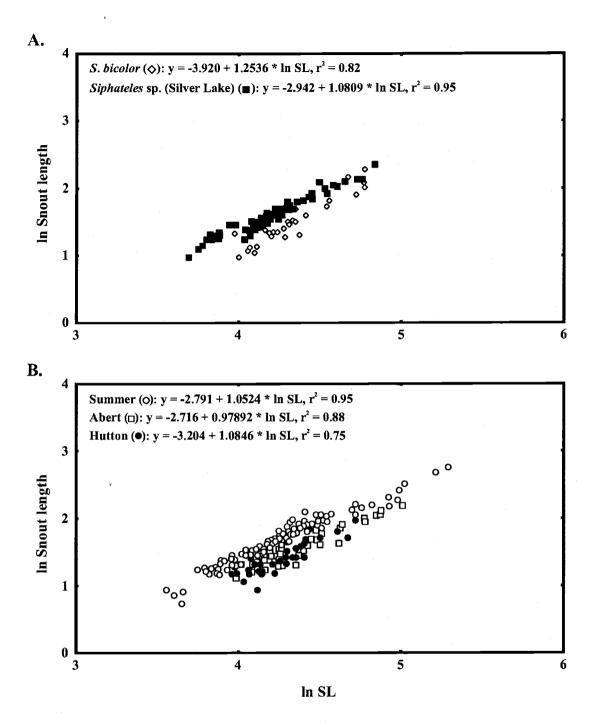


Figure 3.5. Linear regression relationship between snout length and standard length in A) *S. bicolor* (open diamonds) and *Siphateles* sp. (Silver Lake) (filled squares); and B) Oregon populations of *S. obesus*, Summer Basin (open circles), Abert Basin (open squares) and Hutton Springs (filled circles).

The first two canonical axes accounted for approximately 79% of total variation (eigenvalue for canonical variate 1 = 1.79 [42% of total variation]; eigenvalue for canonical variate 2 = 1.58 [37% of total variation]); PANAL, PREP2L, HL, EYE, JAWL and P2L did not significantly contribute to discrimination and were excluded from this analysis. This classification was calculated to be 83% better than by chance alone (kappa = 0.83, Z = 830.00, P << 0.001). A plot of individual scores on the first two canonical axes demonstrates minimal to moderate overlap in scores among four of five species in the *S. bicolor* group; only *S. thalassinus* exhibits almost complete overlap of canonical scores with the other species. For individual species, the classification results were as follows: *S. bicolor*, 94% (32 of 34); *S. columbianus*, 86% (31 of 36); *S. eurysomas*, 84% (100 of 119); *Siphateles* sp. (Silver Lake), 99% (85 of 86); and *S. thalassinus*, 72% (43 of 60). *S. thalassinus* was most often classified as either *S. eurysomas* (12%) or *S. columbianus* (10%).

Siphateles obesus exhibits the greatest amount of intraspecific morphometric variation among Oregon populations of tui chubs (Fig. 3.2b); Lahontan tui chubs from Summer Basin populations are almost entirely separated from Abert Basin and Hutton Springs populations. Within *S. obesus*, Summer Basin tui chubs have comparatively longer snouts than either Abert Basin or Hutton Springs tui chubs (Fig. 3.5b); Abert Basin tui chubs have slightly longer snouts than Hutton Springs tui chubs. The hypothesis of homogeneity of slopes among the three basins is rejected (P < 0.01). Patterns of inter-basin variation for upper jaw length against SL are comparable to those depicted for snout length.

### Comparisons

While patterns of meristic and morphometric variation among Oregon species of tui chubs are similar, there are meristic and morphometric characters that support the species identified by the molecular phylogenetic hypothesis (Chapter 2). *S. bicolor* has lower mean scale counts (Tables 3.6-3.11; Fig. 3.1a), shorter snout and upper jaw lengths and longer dorsal fin and anal fin base lengths than it sister-species *Siphateles* sp. (Silver Lake) (Fig. 3.2a). A similar pattern in scale count characters is found between *S. columbianus* and *S. eurysomas* (Table 3.6-3.11; Fig. 3.1a), although these two species broadly overlap in morphometric space (Fig. 3.2A).

Siphateles thalassinus exhibits the greatest range of intraspecific meristic variation in the *S. bicolor* species group; no meristic or morphometric characters separate this species from other members of the species group. Examination of the three populations comprising *S. thalassinus*, however, indicates that Cowhead Lake tui chubs have higher scale and fin ray counts than either Goose Lake or Warner Valley tui chubs (Fig. 3.1b). Comparison of PC scores for Cowhead Lake tui chubs (Fig. 3.1b) with those of the other species in meristic space (Fig. 3.1a) indicates that this population would minimally overlap *S. obesus*, *S. eurysomas* and *Siphateles* sp. (Silver Lake), and would not overlap either *S. bicolor* or *S. columbianus*. This pattern is consistent with the molecular phylogenetic

hypothesis, which found that while S. thalassinus was monophyletic, the species is composed of two reciprocally monophyletic lineages, Goose Lake + Warner Basin (Warner Valley + Cowhead Lake Valley) tui chubs. The relationship between Warner and Cowhead Lake tui chubs is expected, given that Cowhead Lake is a sub-basin within Warner Basin. Character and bootstrap support for this relationship in the molecular phylogeny is minimal, however, and when coupled with the pattern of meristic variation suggests that further taxonomic study, with additional morphological characters and a faster evolving molecular marker, is needed. Some could argue that the distinctness of the Cowhead Lake tui chub in meristic space would warrant its recognition as a subspecies. As discussed in Chapter 2, however, application of the Evolutionary Species Concept (Simpson, 1961: Wiley, 1978) as the primary species concept in the recognition of evolutionary lineages, with the Phylogenetic Species Concept (Eldridge and Cracraft, 1980; Cracraft, 1983), as modified by McKitrick and Zink (1988) to include the criterion of monophyly of species, as the operational definition used in the recognition of monophyletic groups of populations as species makes no distinction between species or subspecies in a polytypic species (Cracraft, 1983; Mayden, 1997, 1999; Warren, 1992). Thus, subspecies have no ontological status under this concept and Cowhead Lake tui chubs are not recognized as such. Should further evidence substantiate this taxon as a distinct species, vaccaceps remains available as the specific epithet (Chapter 2).

**Conservation Status** 

Based on the known distributions of the six species of tui chubs in Oregon, two species are in need of special status by state and federal resource management agencies. *Siphateles* sp. (Silver Lake) occurs in Buck, Bridge and Silver Creeks in Silver Lake Valley. All three creeks drain into Paulina Marsh, which has been substantially drained for livestock grazing. To date, no thorough status survey has been conducted for this species.

*Siphateles eurysomas* occurs in the Guano and Catlow Valleys of northwestern Nevada and southeastern Oregon. The six populations of *S. eurysomas* analyzed in this study are allopatrically distributed in small, isolated springs and intermittant streams in these two valleys without the possibility of migration or transport among populations. The greatest threat to this species is loss of habitat due to diversion of water supplies for agricultural purposes. While part of the distribution of this species is on the Sheldon National Antelope Refuge (Guano Valley) and Hart Mountain Wildlife Refuge (Catlow Valley), which actively manage their aquatic ecosystems, three of four populations in Catlow Valley are on private land. Conservation agreements between management agencies and land owners will help to ensure the survival of these populations.

There are four populations within *S. obesus* that also warrant conservation attention. Tui chubs have been extirpated from the Chewaucan River in Abert Lake Basin due to the introduction of gamefish, and now exist only in upper Crooked Creek, a tributary of the Chewaucan River. Conservation efforts should include a thorough status survey of this population, as well as preventing further migration of gamefish into upper Crooked Creek. XL, Brittain and Hutton Springs occur on Bureau of Land Management property. XL and Brittain Springs drain into alkaline Abert Lake; Hutton Spring is one of two springs found in Alkali Basin. In addition to a status survey, conservation efforts should focus on limiting livestock intrusions into these springs and prevent groundwater pumping in adjacent areas.

The general predilection of tui chubs is to occupy low elevation, no gradient aquatic habitats that are reminants of larger Plio-Pleistocene aquatic ecosystems. The fragmented distribution of populations within the ranges of several of these species, together with threats to the aquatic and surrounding terrestrial ecosystems, warrant concern for their survival. Cooperative management efforts between resource agencies and private landowners will be necessary to ensure the continued existence of these unique, high desert fish species.

## MATERIAL EXAMINED

Taxon, locality information, size and catalog number for specimens examined in this study. Institutional abbreviations follow Leviton et al. (1985); PMH numbers are my field collection numbers. Parenthetical numbers following catalog number refer to samples sizes used in meristic and morphometric analyses, respectively.

Siphateles bicolor. Klamath Lake, Klamath Co., OR: UMMZ 130613 (30, 30); UMMZ 130624 (4, 4).

Siphateles columbianus. Warm Springs, Harney Co., OR: UMMZ 112934 (30, 30); UMMZ 139030 (6, 6).

*Siphateles eurysomas.* Catlow Basin. Roaring Springs, Harney Co., OR: UMMZ 132257 (0, 9); UMMZ 136699 (30, 30). Rock Creek, Harney Co., OR: UMMZ 189288 (0, 30). Guano Basin. Badger Creek, Washoe Co., NV: UMMZ 141586 (30, 30). Fish Creek, Washoe Co., NV: UMMZ 136702 (0, 30).

Siphateles obesus. Summer Lake Basin. Ana River and Reservoir, Lake Co., OR: CAS 109813 (30, 30); OS 10230 (3, 3); OS 15311 (7, 7); OS 5126 (10, 10); OS 5154 (10, 10); OS 5317 (10, 0); OS 5510 (1, 1); UMMZ 136669 (16, 16); UMMZ 139024 (4, 4). County Road 4-17 Springs, Lake Co., OR: OS 15309 (20, 20). Klippel Springs, Lake Co., OR: OS 15310 (20, 20). Rickert Springs (formerly called Foster Springs), Lake Co., OR: OS 15312 (15, 15); UMMZ 13667 (30, 0). Collahan Springs, Lake Co., OR: OS 15418 (4, 4); OS 15419 (12, 12). Abert Lake Basin. Brittain Springs, Lake Co., OR: UMMZ 136690 (0, 30). Chewaucan River, Lake Co., OR: OS 5004 (6, 0); OS 5229 (34, 0). Crooked Creek, Lake Co., OR: PMH 9503 (12, 0). XL Springs, Lake Co., OR: OS 5001 (2, 0); OS 5135 (18, 0); OS 5157 (9, 0); OS 5315 (10, 0); PMH 9318 (20, 0); UMMZ 139021 (4, 0); UMMZ 136692 (0, 30); USNM 125663 (2, 0); USNM 55596 (1, 0); USNM 58357 (4, 0); USNM 59840 (2, 0). Alkali Basin. Hutton Springs, Lake Co., OR: OS 4208 (11, 0); OS 4209 (14, 0); OS 5005 (4, 0); OS 5136 (8, 0); OS 5158 (30, 0); PMH 9320 (9, 0); UMMZ 130506 (1, 1); UMMZ 130507 (10, 30); UMMZ 139028 (0, 6); USNM 58364 (20, 0).

Siphateles thalassinus. Goose Lake Basin. Goose Lake, Lake Co., OR: UMMZ 138587 (30, 30). Warner Basin. Warner Lake system, including Hart Lake, Honey Creek and Warner Creek, Lake Co., OR: OS 5002 (10, 0); OS 5128 (14, 0); OS 5159 (16, 0); UMMZ 139027 (6, 0). Cowhead Lake slough, Modoc Co., CA: UMMZ 196190 (30, 30).

*Siphateles* sp. Silver Lake, Lake Co., OR: CAS 109812 (30, 30); USNM 58212 (10, 0); USNM 88557 (1, 0). Buck Creek, Lake Co., OR: UMMZ 136672 (1, 1); USNM 58197 (9, 0). Bridge Creek, Lake Co., OR: UMMZ 139023 (4, 4). Guyer Creek, Lake Co., OR: PMH 9502 (25, 0). Silver Creek, Lake Co., OR: OS 5003 (4, 4); OS 5153 (15, 15); OS 10217 (15, 15); UMMZ 136677 (18, 18).

### CHAPTER 4

# TEMPORAL VARIATION IN MERISTIC CHARACTERS IN TUI CHUB (SIPHATELES BICOLOR CLADE) POPULATIONS FROM SOUTHEASTERN OREGON

## ABSTRACT

Temporal variation in five scale-count characters was examined in *Siphateles obesus* and *Siphateles* sp. (Silver Lake) from southcentral Oregon. Temporal variation occurred in all populations; comparisons among basins and three temporal groups (pre1958, 1975-1985 and 1993 collections) indicated approximately equal magnitudes of meristic variation in these two factors. There was no consistent trend to patterns of temporal variation, except within Summer Lake in which all mean scale counts were significantly different between pre1958 collections and 1975-1985 and 1993 collections. In addition, there was no correlation between mean meristic counts and average daily summer air temperatures. The consistent meristic differences in Summer Lake tui chubs may be due to the presence of Goose Lake tui chubs (*S. thalassinus*) in this basin, either through hybridization or by biasing population characterization. Regardless, temporal variation in meristic characters is a widespread phenomena among tui chubs, and presumably among other species, in southcentral Oregon.

### INTRODUCTION

Understanding patterns of character variation is central to the sciences of taxonomy and systematics, and has important implications in evolutionary (e.g., Brooks and McLennan, 1991) and conservation biology (Stiassny, 1992; e.g., Barrowclough, 1992; Mayden and Wood, 1995). Analyses of such patterns within and among populations of a species often reveal geographic and/or temporal components to character variation (Chernoff, 1982; Endler, 1977; Warren, 1992; Gould and Johnston, 1972). Many studies have documented the influence of exogenous factors (e.g. temperature, food source) on character variation in fishes (Bosley and Conner, 1984; Barlow, 1961; Fahy, 1972; Dentry and Lindsey, 1978; Martin, 1949; Chernoff, 1982; Lindsey, 1981; Currens et al., 1989; Hubbs, 1922; Neff and Smith, 1979). In addition, sampling strategy (e.g., number of individuals/population, geographic coverage and periodicity) can also influence the characterization of morphological patterns in populations (Chernoff, 1982; Blouw et al., 1988). Finally, phylogenetic analyses have documented historic influences on character variation, with much of the geographic variation in polytypic species being attributable to inter-, rather than intra-, specific differences (Wiens et al., 1999; Wood and Mayden, 1993; Burr and Mayden, 1999; Warren, 1992).

Given the evidence for both exogenous and historic factors influencing geographic and temporal variation in morphological characters, it is surprising that in taxonomic studies of cyprinids (Bills, 1978) and salmonids (Behnke, 1992, and references therein) from southern and southeastern Oregon hybridization has been the sole mechanism invoked to explain temporal variation in morphological characters. These studies are limited by relatively small sample sizes, infrequent temporal sampling and the absence of a phylogeny in which to provide a historical framework in which to interpret patterns of geographic and temporal character variation. As such, the conclusion that hybridization is the sole underlying mechanism behind morphological temporal variation in these taxa is, at best, tentative. In addition, such a conclusion has the potential to hamper conservation efforts for these species, given recent changes in the Endangered Species Act denying protection to hybrid individuals (although there are caveats regarding protection of introgressed individuals; United States Fish and Wildlife Service, 1996).

The purpose of this paper is to examine potential causes behind temporal patterns of meristic variation in *S. obesus* populations from Abert Lake, Alkali and Summer Lake Basins of south-central Oregon. All populations exhibit some degree of temporal variation in meristic characters. Potential causes of this temporal variation, including a common exogenous influence (e.g., temperature), inadequate sampling leading to a bias in population characterization, or a combination of both factors, will be examined. A review of the taxonomic history of these populations is given below.

The Oregon Lakes tui chub, formerly recognized as *Gila bicolor oregonensis* (Snyder, 1908), was described from five endorheic basins in southeastern Oregon (Abert, Alkali, Silver Lake, Summer and Warner). In the first

taxonomic revision of the subspecies, Bills (1978) restricted G. b. oregonensis to Abert Basin (includes populations from XL and Brittain Springs and the Chewaucan River) and suggested that Alkali Basin (Hutton Springs hereafter), Silver Lake and Warner tui chubs represented three additional, undescribed subspecies. Bills excluded Summer Lake tui chubs from his revision because of concern about the "uncertain nature" of these chubs after analyzing data from collections made before and after three "rough fish" eradication programs conducted in 1958, 1961 and 1970 in Ana Reservoir and Ana River. Bills (1978) found significant differences in the mean number of scales around the caudal peduncle and predorsal scales among fish collected prior to 1958 (1908 and 1943) and fish collected in 1976. Based on these differences, he speculated that remnant Summer Lake tui chubs had hybridized with either introduced tui chubs from surrounding basins or California roach (Hesperoleucus symmetricus), which had caused the shift in the two meristic characters, and that original Summer Lake tui chubs probably no longer existed.

In 1985, four nominally "pure" Summer Lake tui chubs were collected from a culvert below the property of Raymond Collahan between mileposts 85 and 86 on Route 31. A preliminary, unpublished taxonomic evaluation by J.E. Williams (pers. comm. to Carl Bond, 13 Feb. 1986) indicated that these four specimens (OS 15418) were more similar to fish from 1908 and 1943 than to fish recently collected from Ana Reservoir and River; an additional 16 specimens (OS 15419) were not examined. Recent attempts to gain access to the Collahan property to sample the spring leading to the culvert have been unsuccessful and subsequent collections from the culvert have not produced any tui chubs. The Oregon Dept. of Fish and Wildlife and other agencies have followed the proposed taxonomy of Bills (1978) and Williams (1986) in elevating the unstudied, unanalyzed Summer Lake tui chub from the Collahan property to the status of an undescribed subspecies (Oregon Dept. of Fish and Wildlife, 1995). Further, Summer Lake tui chubs were designated a Category 1 candidate species for listing under the federal Endangered Species Act (ESA) because of the small number of fish collected and their potentially restricted distribution on the Collahan property.

Subsequent to Bills' (1978) taxonomic revision two phylogenetic analyses based on morphology (Coburn and Cavender, 1992) and mtDNA 12S and 16S rRNA sequences (Simons and Mayden, 1998) found *Gila*, then consisting of the subgenera *Gila*, *Klamathella*, *Siphateles* and *Snyderichthys*, to be paraphyletic; *Siphateles* was found to be monophyletic in both analyses and raised to generic status by Simons and Mayden (1998). Harris (Chapter 2) examined phylogenetic relationships within *Siphateles* based on mtDNA cytochrome *b* gene sequences and found the subspecies *oregonensis* to be paraphyletic: Warner Basin tui chubs are *S. thalassinus*; Silver Lake tui chubs represent an undescribed species related to *S. bicolor* from Klamath Lake; and, Summer Lake and Abert Basin and Hutton Springs tui chubs are part of *S. obesus*, consisting of tui chub populations from southcentral Oregon, California and Nevada. Abert Basin + Hutton Springs tui chubs formed a monophyletic group sister to tui chubs from Railroad Valley,

Nevada; Summer Lake tui chubs were the most-basal clade within *S. obesus*. Thus, Bills' (1978) conclusions about the distinctness of Silver Lake and Warner Valley chubs were consistent with the molecular phylogeny. Of the 12 tui chubs examined from populations within Summer Lake, no specimen exhibited an *H. symmetricus* haplotype; however, three fish collected in Summer Lake (two from Rickert Springs and one from Co. Rd. 417 Springs) shared complete sequence identity with *S. thalassinus* from Goose Lake (which is the next basin to the south; Chapter 2). Whether *S. thalassinus* represents a recent introduction or *S. obesus* and *S. thalasinnus* are naturally sympatric in Summer Lake remains to be tested.

#### METHODS

Temporal variation in meristic characters among populations of *S. obesus* and *Siphateles* sp. (Silver Lake) was explored using the five meristic traits from Bills (1978): lateral line scales (LL), scale rows above lateral line (ALL), scale rows below lateral line (BLL), scales around caudal peduncle (ACP) and predorsal scales (PDS). I examined 415 specimens of *S. obesus* from Summer Lake, Abert Basin and Hutton Springs and 123 specimens from *Siphateles* sp. (Silver Lake) (see Materials Section).

One-way analysis of variance (ANOVA), multivariate analysis of variance (MANOVA), discriminant functions analysis and correlation coefficients were calculated using Statistica for Windows, version 4.0 (Statsoft, Inc., Tulsa, OK). Significance levels were set to  $P \le 0.05$ , as in Bills (1978). Meristic data were

partitioned into species, basins, populations and collection-date groups, which were organized as pre1958 (1897-1954), 1975-1985 and 1993 collections. Hubbs plots were constructed using SYSTAT (Wilkinson, 1990).

### **RESULTS AND DISCUSSION**

### Temporal Variation in Meristic Characters

To test the null hypothesis of temporal stability in the five meristic characters within Summer Lake, one-way ANOVA values were calculated for each character across collection-date groups for the two populations (Ana River and Reservoir and Rickert Springs) for which there are suitable temporal collections . Significant differences (P < 0.05) in mean number of scales were found for all five meristic characters in pre1958 and 1975-1985 collections from Ana River and Ana Reservoir; LL, ALL, ACP and PDS were found to exhibit significant differences in pre1958 and 1993 collections from Rickert Springs (Figs. 4.1-4.5; Table 4.1). These results differ from Bills (1978), who reported significant differences only in ACP and PDS.

To examine the nature of this temporal variation, means of each meristic character were plotted for each collection-date group for populations within Summer Lake. These plots show a general trend of decreasing mean number of scale counts over time in all meristic characters among populations of Lahontan tui chubs from Summer Lake (Figs. 4.1-4.5; Table 4.1). The general magnitude of

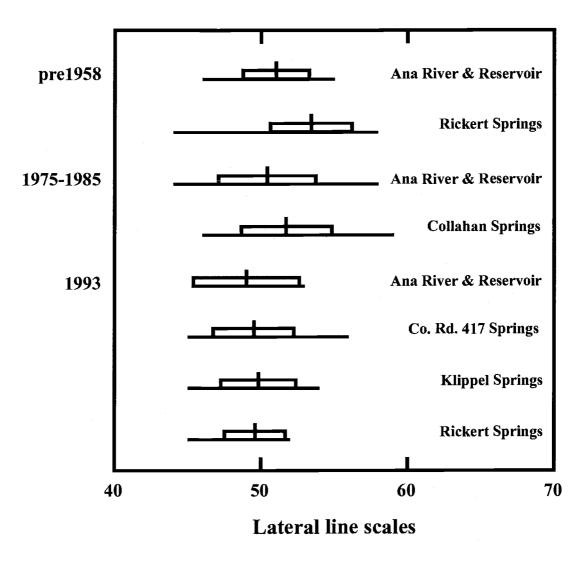


Figure 4.1. Variation in lateral line scales based on collection-date groups for populations within Summer Lake. Vertical line = mean; open box =  $\pm 1$  standard deviation.

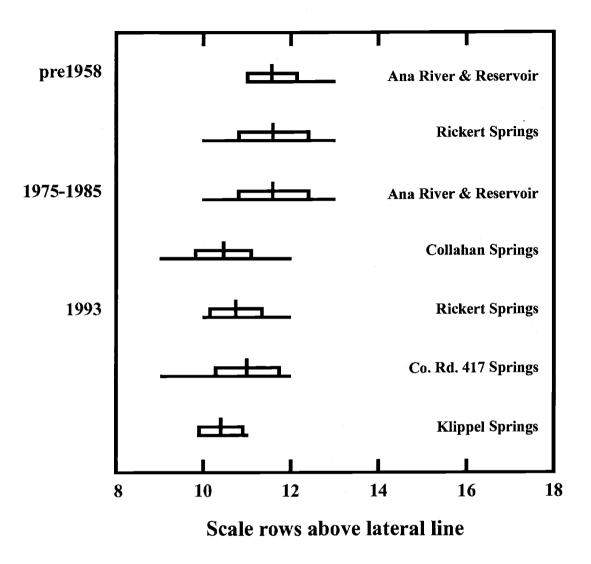


Figure 4.2. Variation in scale rows above lateral line based on collection-date groups for populations within Summer Lake. Vertical line = mean; open box =  $\pm 1$  standard deviation.

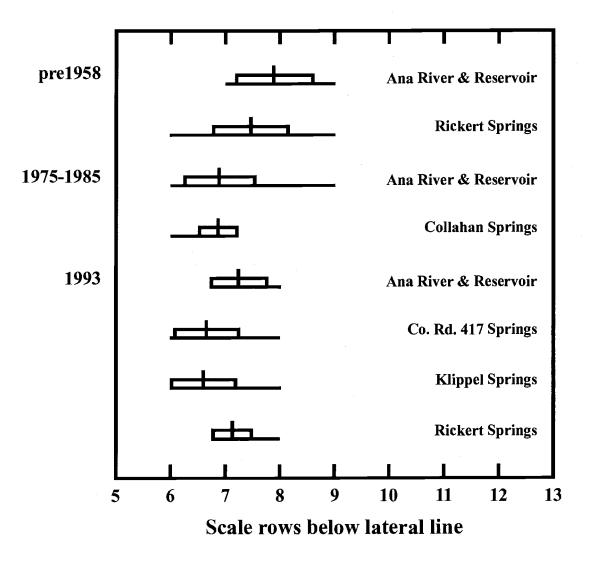


Figure 4.3. Variation in scale rows below lateral line based on collection-date groups for populations within Summer Lake. Vertical line = mean; open box =  $\pm 1$  standard deviation.

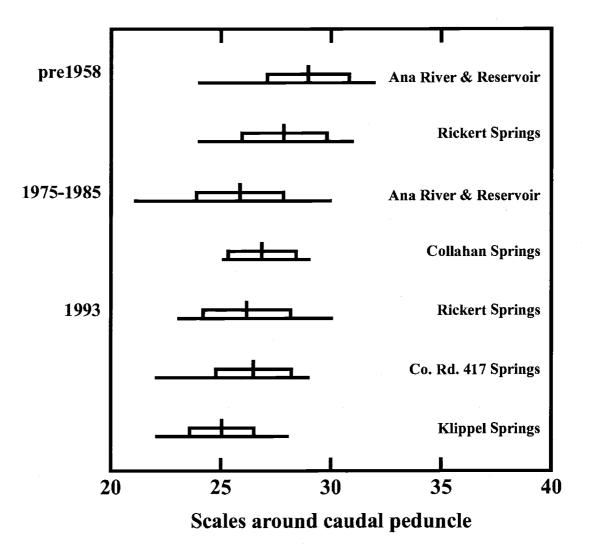


Figure 4.4. Variation in scales around caudal peduncle based on collection-date groups for populations within Summer Lake. Vertical line = mean; open box =  $\pm 1$  standard deviation.

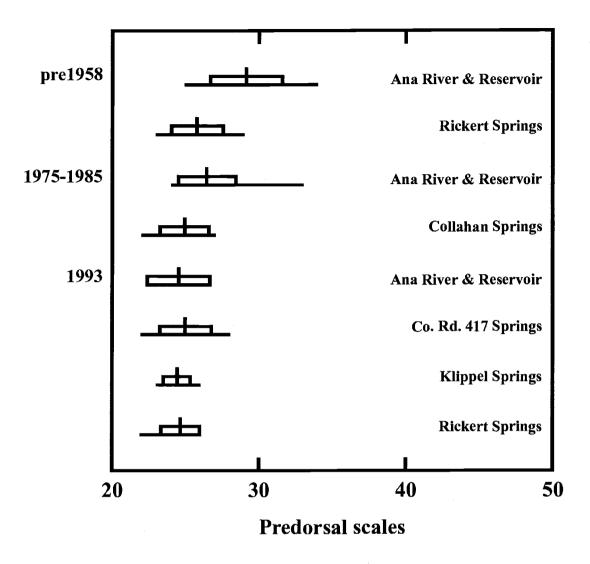


Figure 4.5. Variation in predorsal scales based on collection-date groups for populations within Summer Lake. Vertical line = mean; open box =  $\pm 1$  standard deviation.

Table 4.1. Sample size (N), mean and standard deviation of five meristic characters by population and collection-date group for *S. obesus* from Summer Lake, Oregon. Character abbreviations given in text. Bold values indicate significant differences ( $P \le 0.05$ ) between collection-date groups for Ana River and Reservoir and Rickert Springs populations.

Collection Date	Population	N	LL	N	ALL	N	BLL	N	ACP	N	PDS
Pre1958	Ana River and Reservoir	47	53.4 ± 2.78	47	11.6 ±0.80	47	7.9 ± 0.70	47	28.9 ± 1.85	44	<b>29.1</b> ± <b>2.4</b> 4
	Rickert Springs	30	51.0 ± 2.27	30	11.6 ±0.57	30	$7.5 \pm 0.68$	30	27.9 ± 1.94	28	25.8 ± 1.73
1975-1985	Ana River and Reservoir	26	50.4 ± 3.32	27	10.4 ±0.64	27	6.9 ± 0.64	27	25.8 ±1.96	24	26.4 ±1.95
	Collahan Springs	16	51.8 ±3.09	16	$11.0 \pm 0.63$	16	6.9 ± 0.34	16	$26.8 \pm 1.56$	13	24.9 ± 1.66
1993	Rickert Springs	15	49.6 ± 2.06	15	10.7 ±0.59	15	7.1 ± 0.35	15	26.1 ±2.00	14	24.6 ±1.28
	Co. Rd. 417 Springs	20	49.5 ± 2.74	20	$11.0 \pm 0.73$	20	6.7 ± 0.59	20	$26.5 \pm 1.70$	19	25.0 ± 1.73
	Klippel Springs	18	49.8 ± 2.55	20	$10.4 \pm 0.50$	20	6.6 ± 0.60	20	$25.0 \pm 1.45$	12	$24.4 \pm 0.90$

change was a decrease of 1-3 scales in populations among collection-date groups (Table 4.1).

This observed temporal variation may be due, in part, to small sample sizes for some Summer Lake populations. Because most Summer Lake populations lack historic collections, it is not possible to evaluate within-population sampling reliability. Sufficient sample sizes are available within collection-date groups, however, to examine patterns of temporal variation among basins. Populations within basins were pooled by collection-date group and one-way ANOVA was performed on these groups. Collections from Summer Lake prior to 1958 exhibited consistent differences from subsequent collections for all meristic characters; PDS was significantly different among 1975-1985 and 1993 collections (Figs. 4.6-4.10; Tables 4.2-4.3). Comparisons among collection-date groups for the other basins indicated that for most characters the greatest differences in meristic counts were found between pre1958 versus 1975-1985 collections, rather than in pre1958 versus 1993 or 1975-1985 versus 1993 collections (Figs. 4.6-4.10; Tables 4.2-4.3). There was no consistent downward trend in meristic counts among collection-date groups from the other basins, as found in Summer Lake collections (Figs. 4.6-4.10). Blouw et al. (1988) also found meristic traits among cohorts of Atlantic salmon (Salmo salar) tended to vary independently and concluded that such temporal variability is the normal condition for these traits.

In order to partition the variation attributable to either geography or time, a MANVOA was performed on the five scale count characters with basin and

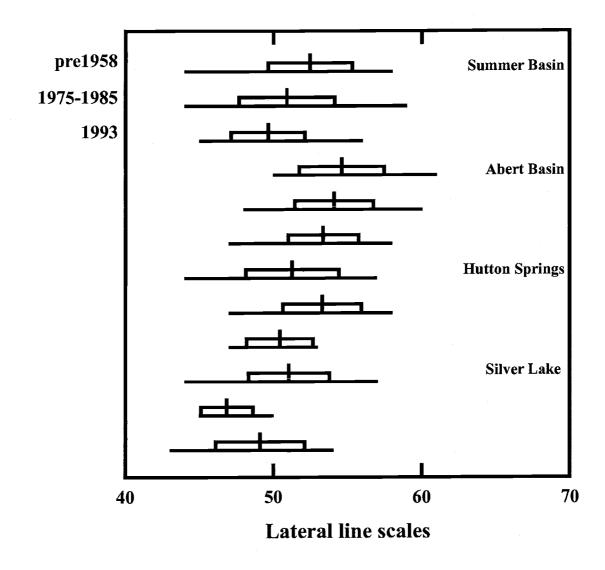


Figure 4.6. Variation in lateral line scales based on collection-date groups among populations of *S. obesus* and *Siphateles* sp. (Silver Lake). Vertical line = mean; open box =  $\pm 1$  standard deviation.

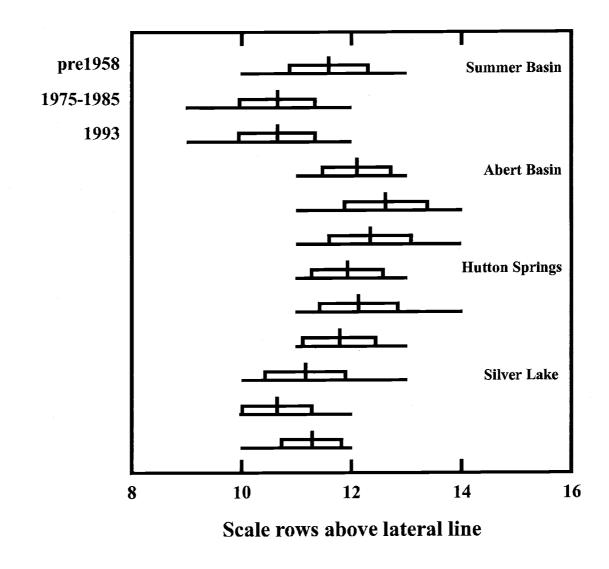


Figure 4.7. Variation in scale rows above lateral line based on collection-date groups among populations of *S. obesus* and *Siphateles* sp. (Silver Lake). Vertical line = mean; open box =  $\pm 1$  standard deviation.

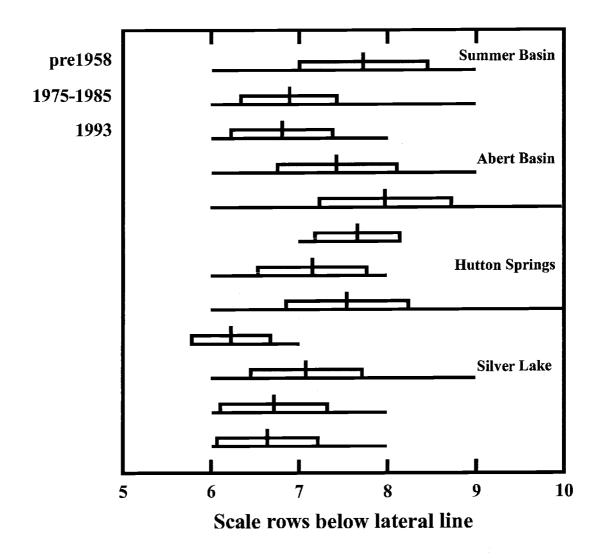


Figure 4.8. Variation in scale rows below lateral line based on collection-date groups among populations of *S. obesus* and *Siphateles* sp. (Silver Lake). Vertical line = mean; open box =  $\pm 1$  standard deviation.

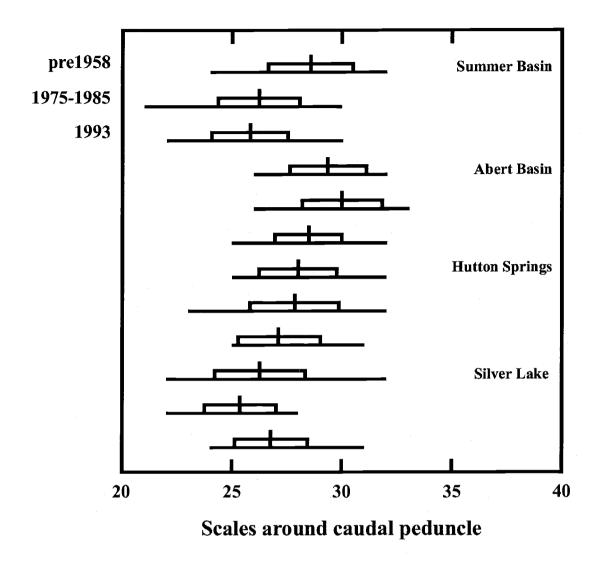


Figure 4.9. Variation in scales around caudal peduncle based on collection-date groups among populations of *S. obesus* and *Siphateles* sp. (Silver Lake). Vertical line = mean; open box =  $\pm 1$  standard deviation.

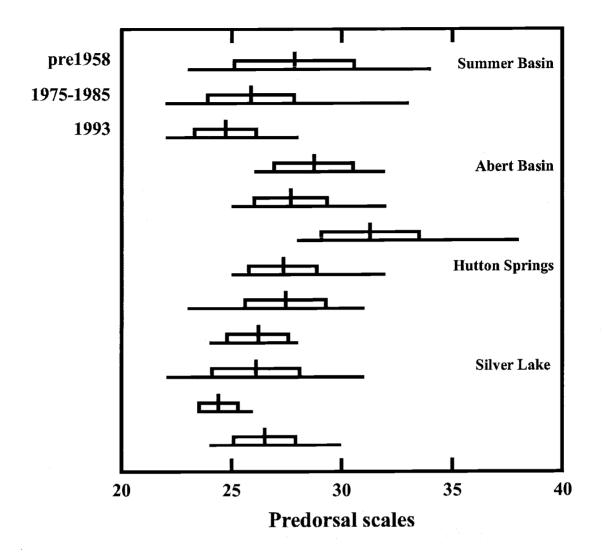


Figure 4.10. Variation in predorsal scales based on collection-date groups among populations of *S. obesus* and *Siphateles* sp. (Silver Lake). Vertical line = mean; open box =  $\pm 1$  standard deviation.

Species	Basin	Collection Date	N	LL	N	ALL	N	BLL	N	ACP	N	PDS
S. obesus	Summer Lake	Pre1958	77	$52.5 \pm 2.84$	77	$11.6 \pm 0.71$	77	$7.7 \pm 0.72$	77	$28.5 \pm 1.95$	72	$27.8 \pm 2.73$
		1975-1985	42	50.9 ± 3.26	43	$10.7 \pm 0.69$	43	6.9 ± 0.54	43	$26.2 \pm 1.87$	37	$25.9 \pm 1.97$
		1993	56	49.6 ± 2.49	59	$10.6 \pm 0.69$	59	$6.8 \pm 0.58$	58	$25.8 \pm 1.76$	47	$24.7 \pm 1.41$
	Abert	Pre1958	21	54.6 ± 2.91	21	$12.1 \pm 0.62$	21	$7.4 \pm 0.68$	21	29.3 ± 1.77	21	28.7 ±1.79
		1975-1985	74	54.1 ±2.63	74	$12.6 \pm 0.75$	74	$8.0 \pm 0.74$	. 74	$30.0 \pm 1.83$	74	$27.7 \pm 1.66$
		1993	32	53.4 ±2.35	32	$12.3 \pm 0.75$	32	$7.7 \pm 0.48$	31	$28.5 \pm 1.55$	30	$28.3 \pm 2.20$
	Hutton Springs	Pre1958	41	51.3 ±3.15	41	$11.9 \pm 0.65$	41	7.1 ±0.61	41	$28.0 \pm 1.75$	37	$27.3 \pm 1.56$
		1975-1985	63	53.3 ± 2.64	63	12.1 ±0.71	63	$7.5 \pm 0.69$	63	$27.8 \pm 2.02$	63	$27.4 \pm 1.84$
		1993	9	$50.4 \pm 2.24$	9	$11.8 \pm 0.67$	9	$6.2 \pm 0.44$	9	27.1 ± 1.90	9	$26.2 \pm 1.39$
Siphateles sp.	Silver Lake	Pre1958	91	51.1 ±2.74	91	$11.2 \pm 0.73$	91	$7.1 \pm 0.64$	91	$26.3 \pm 2.08$	83	$26.1 \pm 2.00$
		1975-1985	7	46.9 ± 1.77	14	$10.6 \pm 0.63$	14	6.7 ± 0.61	14	$25.4 \pm 1.65$	5	$24.4 \pm 0.89$
		1993	25	49.1 ±3.00	25	$11.3 \pm 0.54$	26	$6.6 \pm 0.57$	25	26.8 ±1.67	25	$26.5 \pm 1.42$

Table 4.2. Sample size (N), mean and standard deviation of five meristic characters by basin and collection-date group for *S. obesus* and *Siphateles* sp. (Silver Lake). Character abbreviations given in text.

Table 4.3. P-values for one-way ANOVA comparisons of mean scale counts for the five meristic characters among collectiondate groups. Bold values indicate significant ( $P \le 0.05$ ) differences. Abbreviations for collection-date groups as follows: 1 = pre1958; 2 = 1975-1985; 3 = 1993. Character abbreviations given in text.

Species	Basin	Collection Date Comparisons	LL	ALL	BLL	ACP	PDS
S. obesus	Summer Lake	1 - 2	< 0.01	< 0.01	< 0.01	< 0.01	< 0.02
		1 – 3	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
		2-3	< 0.60	< 0.07	< 0.55	< 0.63	< 0.01
	Abert	1 – 2	< 0.48	< 0.01	< 0.01	< 0.15	< 0.02
		1 – 3	< 0.09	< 0.21	< 0.15	< 0.06	< 0.48
		2-3	< 0.17	< 0.08	< 0.03	< 0.01	< 0.13
	Hutton Springs	1 – 2	< 0.01	< 0.15	< 0.01	< 0.70	< 0.77
		1 – 3	< 0.46	< 0.54	< 0.01	< 0.19	< 0.06
		2 – 3	< 0.01	< 0.17	< 0.01	< 0.32	< 0.06
<i>Siphateles</i> sp.	Silver Lake	1 – 2	< 0.01	< 0.01	< 0.05	< 0.12	< 0.06
		1 – 3	< 0.01	< 0.47	< 0.01	< 0.27	< 0.35
		2 - 3	< 0.07	< 0.01	< 0.70	< 0.02	< 0.01

collection-date group as independent variables. Geography accounted for 49% of observed variation in the meristic data ( $\eta^2 = 0.49$ , Wilks  $\Lambda = 0.51$ , P < 0.001), while the temporal component accounted for only 16% ( $\eta^2 = 0.16$ , Wilks  $\Lambda = 0.84$ , P < 0.001). This result is concordant with patterns of morphometric variation in which Summer Basin tui chubs were almost entirely separated from Abert Basin and Hutton Springs tui chubs in multivariate space; all three populations also exhibited differences in snout length relative to standard length (Chapter 3).

Another way to place the observed temporal variation in the context of geographic variation is to determine if it is possible to use temporal variation to discriminate among collection-date groups as though they were basins. I calculated discriminant functions among populations of *S. obesus* from the five meristic characters with either basin or collection-date group as the *a priori* assigned group. Discriminant functions within *S. obesus* were 63.9% among basins (PDS not significantly contributing) and 53.1% among collection-date groups. These classifications were calculated to be 45% and 23% better than by chance alone, respectively (kappa = 0.45, Z = 12.50, P < 0.001; kappa = 0.23, Z = 5.61, P < 0.001; Titus et al., 1984). Hence there is sufficient temporal variation to allow stastically significant discrimination among collection-date groups within *S. obesus*, but it is only 30% greater than by chance alone and at lower levels of accuracy than that achieved by discriminating among basins.

# Environmental Effects on Meristic Variation

In order to evaluate whether variation in meristic characters is related to environmental variation, correlation coefficients were calculated between the means of meristic characters and average daily summer air temperatures in June, July and August for the collection year and three years prior to collection (no comparable long term data set for water temperature was available). These years were chosen in order to ensure that correlations would include the year of hatching and development for most specimens. In addition, summer air temperature was deemed an acceptable surrogate for water temperature given that fish larvae tend to occupy the margins of shallow water where ambient temperatures would have the greatest influence on water temperature and, thus, potentially influence meristic characters during development (Hubbs, 1922; Fahy, 1972; Bosley and Conner, 1984).

None of the correlations between average daily summer air temperatures and mean scale counts were significant (Table 4.4). Although lack of correlation between air temperature and mean scale counts may not be indicative of actual environmental influences affecting meristic characters, this result is consistent with other correlative studies examining temporal variation across broad time scales (e.g., Blouw et al., 1988; Schmidt, 1921) and may be due to the inadequacy of relating broad-scale environmental factors to developmental events that are

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Table 4.4. Correlation coefficients between mean scale count and five-year running averages of daily mean air temperature for June, July and August from 1928-1993. Correlation coefficients given for year of collection and preceding three years. Numbers in parentheses are significance values.

	LL	ALL	BLL	ACP	PDS
Year	0.3779	0.1460	0.1175	0.0231	-0.0463
	(0.23)	(0.65)	(0.72)	(0.94)	(0.88)
Year - 1	0.2875	0.1184	0.0443	0.0115	-0.0505
	(0.36)	(0.71)	(0.89)	(0.97)	(0.87)
Year - 2	0.2893	0.2151	0.0420	0.0932	0.0737
	(0.36)	(0.50)	(0.90)	(0.77)	(0.82)
Year - 3	0.1338	0.1024	-0.0435	0.0247	-0.0148
	(0.68)	(0.75)	(0.89)	(0.94)	(0.96)

influenced by local, fine-scale factors of limited duration. Nevertheless, the predominant result is that meristic characters vary independently of air temperature.

# Conclusions

The results of this study indicate that temporal shifts in meristic characters are a common and widespread phenomenon among tui chub populations in southcentral Oregon. Not only do meristic traits vary over time, but they tend to vary independently (with the exception of Summer Lake tui chubs) and are not related to environmental variation. Instead, geography is the primary factor accounting for close to half of the observed variation in these characters. Given that most *S. obesus* populations in Oregon are allopatrically distributed, inter-basin and temporal meristic variation clearly has a genetic component; to elucidate the interaction of these factors would require long-term sampling of these populations.

It could be argued that the observed temporal variation is due, in part, to small sample sizes for some populations. This does not appear to be the case. For the two collection-date groups with the smallest sample sizes (Hutton Springs 1993 and Silver Lake 1975-1985), there is no consistent trend in significant differences among collection-date groups (Table 4.2).

The consistent decrease in mean scale counts observed in Summer Lake tui chubs suggests a localized phenomena is contributing to this pattern. The majority (47 of 77 specimens) of pre1958 collections from Summer Lake are from Ana River and Reservoir, and most were collected from 1897-1904. During this period, Ana River was a high volume, spring-fed river with a water temperature of 15.6° C (Snyder, 1908). Subsequent construction of a dam in the 1950's certainly changed the hydrology and thermal profile of this ecosystem. Unfortunately, there are no long-term water temperature records for Ana Reservoir; thus, it is not possible to correlate changes in water temperature with meristic changes.

An alternative explanation for the observed temporal variation is that recent collections (1975-1985 and 1993) from Summer Lake contain a mixture of two sympatric species, *S. obesus* and *S. thalassinus*. As discussed above, 3 of 12 fish examined from Summer Lake had *S. thalassinus* haplotypes; the magnitude of occurrence of *S. thalassinus* in Summer Lake is unknown (Chapter 2). Meristic characters in *S. thalassinus* average 1-3 scales greater than that of *S. obesus* 

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(Chapter 3). If *S. thalassinus* was common in recent collections from Summer Lake, or if hybridization was occurring, however, the higher scale counts would tend to raise, rather than decrease, the mean scale count of recent collections. The incidence of *S. thalassinus* in Summer Lake, and any potential impacts on meristic variation in *S. obesus*, requires further study.

A fundamental assumption of taxonomic studies is temporal stability of characters within a species. In the past, when this assumption has been violated, particularly in freshwater fish, temporal changes in characters often were ascribed to hybridization (e.g., Behnke, 1992; Bills, 1978). Behnke (1992) cites "hybrid influence" as the reason behind changes in meristic characters in populations of Oregon redband trout (O. mykiss). There is growing realization, however, that a variety of factors, including environmental variation, natural selection and competition, can also influence the temporal stability of characters (Blouw and hagen, 1987; Crowder, 1984; Jacobson et al., 1986). In addition, Blouw et al. (1988) found strong interannual meristic variation in a stock of Atlantic salmon (S. salar) that apparently was independent of environmental variables such as river discharge and water temperature, suggesting a possible genetic component to interannual meristic variation. Because of these alternative explanations, hybridization should be demonstrated with either traditional or molecular taxonomic methods rather than postulated as the sole mechanism behind temporal changes in meristic characters. The results of this study indicate that caution

should be exercised in conservation decisions which rely on taxonomic studies that do not fully address potential causes behind within-species morphological variation. Further taxonomic studies on freshwater fishes from southern and southeastern Oregon, particularly on redband trout (*O. mykiss*) and speckled dace (*Rhinichthys osculus*), that address within-species morphological variation are clearly warranted.

#### MATERIAL EXAMINED

Taxon, locality information, size and catalog number for specimens examined in this study. Institutional abbreviations follow (Leviton et al., 1985); PMH numbers are first authors field numbers. Parenthetical numbers following catalog number is sample size.

*Siphateles obesus.* Summer Lake Basin. Ana River and Reservoir, Lake Co., OR: CAS 109813 (30); OS 10230 (3); OS 15311 (7); OS 5126 (10); OS 5154 (10); OS 5317 (10); OS 5510 (1); UMMZ 136669 (16); UMMZ 139024 (4). County Road 417 Springs, Lake Co., OR: OS 15309 (20). Klippel Springs, Lake Co., OR: OS 15310 (20). Rickert Springs (formerly called Foster Springs), Lake Co., OR: OS 15312 (15); UMMZ 13667 (30). Collahan Springs, Lake Co., OR: OS 15418 (4); OS 15419 (12). Abert Lake Basin. Chewaucan River, Lake Co., OR: OS 5004 (6); OS 5229 (34). Crooked Creek, Lake Co., OR: PMH 9503 (12). XL Springs, Lake Co., OR: OS 5001 (2); OS 5135 (18); OS 5157 (9); OS 5315 (10); PMH 9318 (20); UMMZ 139021 (4); USNM 125663 (2); USNM 55596 (1);
USNM 58357 (4); USNM 59840 (2). Alkali Basin. Hutton Springs, Lake Co.,
OR: OS 4208 (11); OS 4209 (14); OS 5005 (4); OS 5136 (8); OS 5158 (30); PMH
9320 (9); UMMZ 130506 (1); UMMZ 130507 (10); USNM 58364 (20).

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*Siphateles* sp. Silver Lake, Lake Co., OR: CAS 109812 (30); USNM 58212 (10); USNM 88557 (1). Buck Creek, Lake Co., OR: UMMZ 136672 (1); USNM 58197 (9). Bridge Creek, Lake Co., OR: UMMZ 139023 (4). Guyer Creek, Lake Co., OR: PMH 9502 (25). Silver Creek, Lake Co., OR: OS 5003 (4); OS 5153 (15); OS 10217 (15); UMMZ 136677 (18).

### CHAPTER 5

# GENERAL CONCLUSIONS

Chapter 2 examined variation in mtDNA cytochrome b gene sequences in the S. bicolor complex from throughout its range. Patterns of molecular variation were intrepreted in concert with variation in outgroup species suggested by Coburn and Cavender(1992) and were used to document natural diversity within the species complex and determine phylogenetic relationships among its members. Sequence data supported recognition of nine allopatrically distributed species currently recognized under S. bicolor. These species included S. bicolor, S. columbianus, S. eurysomas, S. isolatus, S. mohavensis, S. newarkensis, S. obesus, S. thalasinnus and an unnamed species from Silver Lake, Oregon. In addition to elevating the above taxa to species status, it is recommend that tui chubs sensu lato be referred to as the Siphateles bicolor complex; excluding S. mohavensis, remaining tui chubs are referred to as the Siphateles bicolor clade. Within this clade are two species groups, S. bicolor and S. isolatus species groups, and S. obesus. Phylogenetic patterns elucidated in this chapter were interpreted in light of the hydrologic history of the Great Basin and surrounding areas to better understand the biogeographic history of the ichthyofauna in this region.

Based on the phylogenetic hypothesis and taxonomy proposed in Chapter 2, Chapter 3 examined meristic and morphometric variation in the *S. bicolor* species group and Oregon populations of *S. obesus*. The *S. bicolor* species group consists of *S. bicolor*, *S. columbianus*, *S. eurysomas*, *S. thalasinnus* and *Siphateles* sp. (Silver Lake). Little information was previously available on meristic and morphometric variation in the *S. bicolor* species group, beyond the original subspecies descriptions and an unpublished Masters thesis on geographic variation in *S. bicolor oregonensis* (= *S. obesus* this study). Univariate and multivariate analyses of meristic and morphometric characters in the *S. bicolor* species group and Oregon populations of *S. obesus* supported recognition of these species, and for *S. obesus* relationships among populations, proposed by the molecular phylogenetic hypothesis.

Chapter 4 examined potential causes behind temporal patterns of meristic variation in populations of *S. obesus* and *Siphateles* sp. (Silver Lake) from southcentral Oregon. Temporal variation in meristic characters led Bills (1978) to exclude Summer Lake tui chubs from his revision of the Oregon Lakes tui chub. Results of this study indicated that temporal variation in meristic characters is a common and widespread phenomenon among tui chub populations in southcentral Oregon. Not only do meristic traits vary over time, but they tend to vary independently, with the exception of Summer Lake tui chubs, and are not related to environmental variation. The implications of these results for taxonomic and conservation decisions on the icthyofauna of southern and southeastern Oregon were discussed.

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