AN ABSTRACT OF THE DISSERTATION OF

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presented on December 19, 2008

Title: The Oregon Coastal Subprovince, a New Biogeographic Subprovince for Primary Freshwater Fishes in Oregon.

Abstract approved:

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The Pacific Northwest has a relatively low diversity of primary freshwater fishes with most of the endemism and diversity in the Columbia River and Klamath River. However, the Oregon Coastal Subprovince defined as the coastal rivers from Miami River in the north to Sixes River in the south, has a relatively diverse primary freshwater fish fauna, and, potentially unrecognized endemism. The species diversity and endemism of these systems is not clear because their taxa are allopatric members of more wide ranging taxa with some recognized as distinct species while others are not. The presence of primary freshwater fishes in the Oregon Coastal Subprovince has been explained as either due to their origin in the coastal river systems or dispersal to coastal rivers from the Willamette River.

The goals of this study were: 1) to describe fishes in the genera *Catostomus* and *Ptychocheilus* in the Oregon Coastal Subprovince using morphological data and mitochondrial cytochrome *b* sequences; 2) to investigate the relationships of fishes in the Oregon Coastal Subprovince to nearby provinces; and 3) to address competing

distribution theories.

In this study, I re-described *C. tsiltcoosensis*, *C. macrocheilus*, *C. rimiculus*, *P. umpquae* and *P. oregonensis* and recognized *C. sp A* (Coquille River) and *C. sp. B* (Rogue River). *Catostomus tsiltcoosensis* and *C. sp. A* tend to have higher counts of infraorbital pores and fewer dorsal fin rays than *C. macrocheilus*. *Catostomus tsiltcoosensis* had six fixed base pair differences from *C. macrocheilus*. *Catostomus sp. A* had 14 fixed base pair differences in cytochrome b from *C. macrocheilus* and *C. tsiltcoosensis* and had a narrower body width at base of the pectoral fin than *C. tsiltcoosensis*. In the cytochrome b phylogeny, *C. macrocheilus* (Columbia) was sister to *C. tsiltcoosensis* (Siuslaw River, Umpqua River and Coos River) and *C. sp. A* (Coquille River) was sister to *C. macrocheilu*s and *C. tsiltcoosensis* plus *C. columbianus* and *C. tahoensis*. The Rogue River *C. sp B* was recognized as a separate species from *C. rimiculus* because it had higher counts of vertebrae anterior to the dorsal fin and had nine fixed base pair differences from *C. rimiculus*. Although *Catostomus sp. B* was previously placed in *C. rimiculus*, phylogenetic analysis showed *C. rimiculus* was more closely related to other catostomids in the Klamath Basin than to *C. sp. B*. This was likely caused by hybridization among four different species of suckers in the Klamath system.

Ptychocheilus oregonensis tended to have fewer scales around the caudal peduncle, fewer scales above the lateral line, fewer transverse scales, deeper body depth at the origin of the dorsal fin, and shallower caudal peduncle than *P. umpquae*. *Ptychocheilus umpquae* had 15 fixed base pair differences from *P. oregonensis*. Based on phylogenetic analysis, *P. oregonensis* (Columbia - Willamette River) was sister to the *P. umpquae* (Siuslaw and Umpqua) and *P. grandis* (Sacramento) was sister to both.

If *C. tsiltcoosensis* and *C sp. A* are considered separate species, the Oregon Coastal Sub-Province has 62.5% endemism. This suggests that it is another important area of endemism in the Pacific Northwest.

Based on the suckers and pikeminnow phylogenies, two common nodes (Siuslaw-Umpqua vs. Willamette-Columbia and Sacramento vs. Willamette-Columbia-Siuslaw-Umpqua) were found in sucker and pikeminnow phylogenies. If pikeminnow and suckers shared a common history, two vicariant events (Sacramento from Willamette – Columbia – Umpqua - Siuslaw and Willamette - Columbia from Siuslaw - Umpqua) were responsible for such pattern. On the other hand, if the two groups had separate histories, the phylogeny of the suckers also suggested two additional vicariant events (Coquille from Willamette – Columbia – Umpqua - Siuslaw and Willamette - Columbia from Siuslaw - Umpqua). Similar to the sucker phylogeny, the phylogeny of the pike minnow suggested a vicariance pattern. The estimated divergence times among taxa were supported by geological evidence.

The Oregon Coastal Subprovince, a New Biogeographic Subprovince for Primary Freshwater Fishes in Oregon

by
Jes Kettratad

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirement for the
degree of

Doctor of Philosophy

Presented December 19, 2008
Commencement June 2009

Doctor of Philosophy dissertation of Jes Kettratad presented on December 19, 2008

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

Jes Kettratad, Author

ACKNOWLEDGEMENTS

I would like to thank my family for all the moral support and financial support they have provided throughout the years. I thank Dr. Douglas Markle for editing my manuscript and for being my mentor and my friend. I've learned a lot from Dr. Markle and I'm grateful for that. I thank Dr. Andrew Brower, Dr. Darlene Judd and Dr. Joseph Spatafora for letting me use the genetic lab, moral support and their friendships. I thank Drs. Aaron Liston, Joseph Spatafora and David Noakes for helping me edit my manuscript and serving on my committee. I thank Dr. Barbara Shields for all the moral support and recommendations that she provided throughout my years at OSU and for helping me edit my manuscript. I thank Dr. Dan Arp for serving as graduate representative on my committee. I specially thank Dr. Kentaro Hosaka and Dr. Gi Ho Sung for helping me with my analysis and their friendship. I thank Dr. Dan Edge for all the moral support. I thank Dr. Art Boucot for his friendship and for helping me understanding the world of paleobiology. I thank Dr. Eric Taylor from the University of British Columbia for *Catostomus catostomus* tissues. I thank Fred Fryrer for the *Ptychocheilus grandis* specimens. I thank Chuck McAda for *P. lucius* tissues. I thank Michael Grey, Jason Adams, Shari Anderson, Patrick Luke, Brent Crowe, William Tenniswood, Michael Cunningham, Kaley Clauson, and Kelvin Stertz for helping me collecting specimens. I thank Stewart Reid for his friendship and his moral support. I would like to thank Dr. Ronald Fritsche and Dr. Suraphol Sudara for their encouragement and moral support. Without their help and encouragement, I would not have pursued my Ph.D. degree. I am grateful to Dr. Suraphol Sudara (who recently pass away), my undergraduate degree adviser, for introducing me to the world of marine science and

ichthyology. I thank Dr. Nitharatana for all the moral support she has given since my undergraduate degree. I would like to thank Stacy Remple, Tod Miller and Able Brumo for their wonderful friendship. I thank Dr. Conrad Schoch and Dr. Barbara Schoch for their friendship and for playing tennis with me. Lastly, I would like to thank all of my colleagues and my friends for all the moral support they have provided though out the year.

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Chapter 1

General Introduction

The diversity of primary freshwater fishes in Oregon coastal river systems has an intriguing pattern. Most Oregon coastal rivers are relatively small, with one to three species of primary freshwater fishes. Three areas have relatively high diversity: the Columbia River (23 species), the Siuslaw-Umpqua-Coos (7 species) and the Klamath (10 species) (McPhail and Lindsey, 1986; Minckley et al., 1986; Snyder, 1908). The high diversity in the Columbia and Klamath can be explained by the size of the systems and past connections with the proto-Snake River (Smith, 1975; Wheeler and Cook, 1954; Taylor and Smith, 1981; Repenning et al., 1995). What remains puzzling is the diversity of primary freshwater fishes in the Siuslaw-Umpqua-Coos systems.

The Siuslaw-Umpqua-Coos systems share two genera of primary freshwater fishes with both the Columbia and Klamath systems: *Catostomus* and *Rhinichthys* (Snyder, 1908; Evermann and Meek, 1898), and uniquely share three genera with the Columbia system: *Oregonichthys, Ptychocheilus,* and *Richardsonius* (Minckley et al., 1986; McPhail and Lindsey, 1986; Markle et al., 1991). The similarity of primary freshwater fishes in the Columbia-Willamette system with the Siuslaw-Umpqua-Coos systems suggests a similar history.

Two competing theories differ in proposed direction of dispersal. Minckley et al. (1986) proposed that primary freshwater fishes in the Siuslaw-Umpqua-Coos systems were the result of peripheral isolation from the Willamette River. McPhail and Lindsey (1986) suggested that primary freshwater fishes in the Siuslaw-Umpqua-Coos systems

were ancient and had recently invaded the Willamette River. The Long Tom River was formerly a part of the Siuslaw River system but was captured by the Willamette River in the Late Pleistocene (Baldwin and Howell, 1949). This geological evidence suggests fauna transfer could have occurred from the Siuslaw River to the Willamette River, thus supporting McPhail and Lindsey (1986). Other geological evidence suggests the Umpqua River was a tributary to the Willamette River, but in the Plio-Pliestocene was captured by a westward flowing stream and became the current Umpqua River (Diller, 1915; Baldwin, 1959). This suggests faunal transfer from the Willamette to the Siuslaw-Umpqua-Coos systems, thus supporting Minckley et al. (1986).

Currently there are two areas of endemism in two biogeographic provinces in Oregon: Columbia (Cascadia province) and Klamath (Great Basin- Baja- Klamath-Sacramento province) (Miller, 1958; Burr and Mayden, 1992). There are conflicting ideas about whether the Siuslaw-Umpqua-Coos systems belong to the Great Basin- Baja-Klamath-Sacramento province or the Cascadia province (Miller, 1958; Burr and Mayden, 1992). Miller (1958) included the Siuslaw-Umpqua-Coos systems in the same area of endemism with the Columbia system, while Burr and Mayden (1992) included the Siuslaw-Umpqua-Coos systems in the Great Basin- Baja- Klamath-Sacramento province in their biogeographic province map of freshwater fishes.

An important criterion for establishing biogeographic provinces and subprovinces is high level of endemism (Brown and Lomolino, 1998). Using this criterion, it might be possible to recognize a new subprovince: the Oregon Coastal Subprovince. Based on the distinctiveness of its primary freshwater fishes, the Oregon Coastal Subprovince is defined in this study as the Oregon coastal river systems ranging from the Miami River in

the north to the Sixes River in the south. The subprovince has seven species of primary freshwater fishes: *Catostomus tsiltcoosensis*, *Oregonichthys kalawatseti, Ptychocheilus umpquae*, *Richardsonius siuslawi*, *Rhinichthys cataractae*, *Rh. evermanni* and *Rh. osculus*. All have been described as, or may be, unique endemics, though most are currently not recognized. *Catostomus tsiltcoosensis* (recognized as *C. macrocheilus)* ranges from the Siuslaw River in the north to the Umpqua River in the south (Evermann and Meek, 1898.). *Oregonichthys kalawatseti* (Markle et al., 1991) and *Rhinichthys evermanni* (Snyder, 1908) are recognized Umpqua River endemics. *Ptychocheilus umpquae* (Snyder, 1908) is found in the Siuslaw and Umpqua rivers and intervening freshwater lakes. *Richardsonius siuslawi* (recognized as *R. balteatus)* is found in the Siuslaw River, the Umpqua River and Tsiltcoos Lake (Evermann and Meek, 1898.). *Rhinichthys cataractae* in the Coos River is morphologically different from other populations and, though unnamed, has been suggested as possibly a distinct species (Bisson and Reimers, 1977). *Rhinichthys osculus* is a widely distributed, poorly understood polytypic fish (Oakey et al., 2004; Pfrender et al., 2004). In the Oregon coastal systems, *Rh. osculus nubilis* (Girard, 1856) tends to have a much narrower body than the Columbia population (Zirges, 1973), again suggesting differences.

Recent studies suggest further reasons to support the distinctiveness of these fishes (Mayden et al., 1991; Gold and Li, 1994; Zirges, 1973). Mayden et al (1991) reported that *Ptychocheilus umpquae* in the Siuslaw River differed morphologically from *P. umpquae* in the Umpqua River. Gold and Li (1994) also found differences between the genome size of the two populations were more than would be expected from two randomly picked cyprinid species.

If *C. tsiltcoosensis*, Siuslaw *P. umpquae*, *Richardsonius siuslawi*, Coastal *R. osculus*, and Coos *R. cataractae* are valid species, the potential endemism of the Oregon Coastal Subprovince would be 100%. This level of endemism is much higher than other places in the Pacific Northwest and would strongly support recognition of the Oregon Coastal Subprovince. In Chapter 2, I describe allopatric taxa for *Catostomus* and *Ptychocheilus* in the Oregon Coastal Subprovince and neighboring subprovinces. Because relationships among the Oregon Coastal Subprovince and other neighbor biogeographic regions are unknown, the phylogeographic relationships of *Catostomus* and *Ptychocheilus* are investigated in Chapter 3.

Chapter 2

Taxonomy of *Ptychocheilus* (Cyprinidae) and *Catostomus* (Catostomidae) in the Oregon Coastal Subprovince

Abstract

Most Oregon coastal rivers between the Columbia River and the Klamath River have low diversity except the Siuslaw, Umpqua and Coos rivers which may have as many as eight endemic primary freshwater fishes. The actual species diversity of these systems is not clear because most of their taxa are allopatric members of more wide -ranging taxa with some recognized as distinct species while others are not. The purpose of this study is to describe coastal taxa in two representative genera *Catostomus* and *Ptychocheilus* using morphological data and mitochondrial cytochrome b sequences. Principal component analysis and discriminant function analysis were used to analyze morphological data. Maximum parsimony algorithm was used to analyze cytochrome b sequences.

In this study, I recognized *C. tsiltcoosensis*, *C. macrocheilus*, *C. rimiculus*, *C. sp. A*, *C. sp. B*, *P. umpquae* and *P. oregonensis* based on morphological and genetic characters. *Catostomus sp. A* had 14 base pair differences from *C. macrocheilus* and *C. tsiltcoosensis* and was the basal member of the macrocheilus clade. *Catostomus sp B* (Rogue River) was recognized as a separate species from *C. rimiculus*, which was more closely related to other species of catostomids in the Klamath system than to *C. sp. B*. This supports the finding of previous studies that hybridization occurred among four different species of suckers in the Klamath system.

Ptychocheilus oregonensis and *P. umpquae* differed by 15 base pairs and several morphometric and meristic characters. I did not find morphological differences between Siuslaw *P. umpquae* and Umpqua *P. umpquae*. In the phylogenetic analysis, Siuslaw *P. umpquae* was embedded within Umpqua *P. umpquae*. When *C. tsiltcoosensis* and *C sp. A* were taken into account, the Oregon Coastal Subprovince had 62.5% level of endemism. This suggests that it is another important area of endemism in the Pacific Northwest.

Introduction

Allopatric taxa are a problem for the biological species concept because there is no possibility to disprove reproductive isolation (Mayr, 1988). Consequently, species taxonomy has to be based on total morphological or genetic distinctiveness. When taking this approach, some have advocated congruence of both data sets (Brower, 1999), but single data approaches, especially of morphology, are still widespread in fish taxonomy. This problem is more severe in species-deprived areas with recent complex geological changes. The geological changes could have resulted in isolation among different populations of allopatric freshwater fishes. As a result of the isolation, primary freshwater fishes form demes ranging from slight differences to major differences among demes. The level of divergence between these demes depends on five effects: founder effect, population size, the intensity of local selection, the amount of gene exchange among the adjacent populations, and time (McPhail, 2007). The result of the isolation could be more obvious in genetic data but not as clear in morphological data because genetic differentiation does not necessarily result in morphological differentiation.

The Pacific Northwest has a relatively low diversity of primary freshwater fishes when compared to the eastern United States (MacAllister et al., 1986). In Oregon there are two large rivers, the Columbia on the northern border and the Klamath, which enters the Pacific Ocean in California, in the south. Most of the fish diversity in Oregon is found in these two drainages (McPhail and Lindsey, 1986; Minckley et al., 1986). Between them are a series of smaller rivers, the Oregon Coastal Subprovince, with the Miami River on the northern boundary and the Sixes River on the southern boundary. Most have

relatively few primary freshwater fishes, but the Siuslaw, Umpqua, and Coos rivers are relatively diverse. Their actual species diversity is not clear since most of their taxa are allopatric members of more wide ranging taxa with some recognized as distinct species while others are not. These primary freshwater fishes in the Oregon Coastal Subprovince tend to have allopatric sister taxa in either the Columbia River or, less commonly, in the Klamath River.

In the Oregon Coastal Subprovince, there are five Ostariophysan taxa with presumed sister taxa in the Columbia River and one found in all three areas. The generally recognized sister species of the Columbia taxa are *Oregonichthys kalawatseti* (Markle et al., 1991), *Ptychocheilus umpquae* (Mayden et al., 1991; Carney and Page, 1990; Markle et al., 1991), and *Rh. evermanni*. In addition, Mayden el al (1991) found that Umpqua *P. umpquae* differed morphologically from Siuslaw *P. umpquae* and their genome sizes also differed (Gold and Li, 1994). *Rhinichthys cataractae* is a wide-ranging species known in the Oregon Coastal Subprovince only from the Coos River. It is morphologically different from other *Rh. cataractae* and, though unnamed, has been suggested to be a distinct species (Bisson and Reimers, 1977). The Columbia taxa that are not currently recognized as distinct species are assigned to *Catostomus macrocheilus,* and *Richardsonius balteatus,* however both were previously described: *C. tsiltcoosensis* Evermann and Meek, 1898, (synonymized with *C. macrocheilus* by Snyder, 1908) and *Richardsonius siuslawi* Evermann and Meek, 1898, but not recognized by subsequent authors. *Rh. osculus* is the only ostariophysan found in all three areas. A wide ranging, but poorly described form, *Rh. osculus nubilis*, has been described as the coastal form. Thus, the Oregon Coastal Subprovince may contain as many as eight endemic

ostariophysan taxa.

South of the Oregon coastal province, *Catostomus rimiculus* (Gilbert, 1897) is found in the Rogue River and the Klamath River (Snyder, 1908; Lee et al., 1980; Moyle, 2002). Snyder (1908) found that Rogue populations differed in the placement of some fins but concluded that the differences were not important. Recently, both morphological and genetic differences have been described (Tranah et al., 2001; Markle et al., 2005). It is possible that Rogue *C. rimiculus* is an allopatric sister taxon of Klamath *C. rimiculus*.

The purpose of this study is to describe the taxonomy of two genera of Oregon coastal river ostariophysans, one with presumed connections to the Columbia, *Ptychocheilus*, and one with presumed connections to both the Columbia and the Klamath, *Catostomus*. Fishes in *Ptychocheilus* belong to subfamily Leuciscinae, family Cyprinidae, and order Cypriniformes. There are four species of *Ptychocheilus*: *P. lucius*, *P. grandis*, *P. oregonensis,* and *P. umpquae*. *Ptychocheilus lucius* is found in the Colorado River drainage. *Ptychocheilus grandis* is found in the Sacramento drainage. *Ptychocheilus oregonensis* is found in the Columbia drainage and *P. umpquae* is found in the Siuslaw River, the Umpqua River and the freshwater lakes between the two river systems. All *Ptychocheilus* are large cyprinids. The smallest species is *P. umpquae* (440 mm) and the largest species is *P. lucius* (1800 mm). All of the *Ptychocheilus* are predators. Their food items include insects, crustaceans, and fishes (Moyle, 2002 and Naughton and Bennett, 2003).

Fishes in the genus *Catostomus* belong to the tribe Catostomini, subfamily Catostominae, family Catostomidae and order Cypriniformes (Nelson, 1994). There are about 23 currently recognized species of recognized *Catostomus*. Most are restricted to

the Western United States with the exception to *Catostomus catostomus* and *C. commersoni*, which have extended distribution in the North and the East (Nelson 1994 and Smith, 1992). All *Catostomus* are benthic fishes. Catostomids have the second highest diversity for primary freshwater fishes in the United States of America (Mayden et al 1992). Furthermore, catostomids display a great degree of endemism (Lee et al, 1981), gene evolution (Ferris and White, 1978), and life history traits (Fuiman, 1985). Catostomids are allotetraploids, which shows gene diploidization in their evolution (Buth, 1979; Buth, 1982; Buth, 1992 and Ferris and White, 1978). All of these characters make them a very interesting subject for the study of evolution.

This study is based on examination of both morphological and mitochondrial cytochrome b data. To facilitate presentation of currently unrecognized taxa, the following are recognized within *C. macrocheilus* (*sensu lato*): *C. macrocheilus* (*sensu stricto*) from the Columbia Basin), *C. tsiltcoosensis* (Oregon Coastal Subprovince) and *C. sp. A*. (from the Coquille River), and within *C. rimiculus* (*sensu lato*): *C. rimiculus* (*sensu stricto* from Klamath Basin) and *C. sp. B* (from the Rogue River).

Study Area

The Oregon Coastal Subprovince is composed of two geological provinces: the Oregon coastal province and a portion of the Klamath province (Sixes River) (Dicken, 1955; Baldwin, 1959 and Orr and Orr, 2002). The Oregon Coastal Subprovince has a complicated geological history. Located on top of a subduction zone, the Coast Range, which runs in a North-South direction, may have been created in one of two ways. One theory suggests that in the late Cretaceous (64 million years ago), a volcanic island chain collided with the North American plate and was incorporated into the western coast of the

plate. The volcanic rock of this island formed the foundation of the current day Coast Range (Orr and Orr, 2000). The alternative theory suggests that a volcanic mountain chain evolved over a series of crustal rips along the coastal margin. Large-scale differential movement of the plate could release lava to the surface, which later formed the base of the coast range (Orr and Orr, 2000).

In the Early Eocene, there were well-developed river systems that transferred sediments from the Klamath Mountains to the Pacific Ocean (Baldwin, 1959 and Orr and Orr, 2000). In the Late Eocene, the source of the sediment changed from the Klamath Mountains to the Idaho area. These sediments were later covered by ashes and pyroclastics from the newly formed ancestral Cascade volcanoes (Orr and Orr, 2000). At the northeastern boundary of the Oregon Coastal Subprovince, there was a basaltic lava flow from northeastern Oregon through the Columbia Gorge into the Willamette Valley. This created a flat landscape with only several tops of the hills projecting above the basaltic layer. Due to the tremendous hydrostatic pressure of the basaltic lava flow, this flow spread into portions of the northeastern part and central part of the Oregon Coastal subprovince (Baldwin, 1959 and Orr and Orr, 2000). In the Oligocene, the Coast Range started to uplift. The Nehalem Basin and the Willamette Basin were under a shallow sea. Volcanic activity was high. This resulted in more ash and volcanic material being transferred to the Coastal region. During the Miocene, sea level retreated to the current level.

The coast range block continued to uplift into the Pleistocene epoch. The area between Cape Blanco and Cape Arago had the highest rate of the uplifting. Sea level rose as a consequence of the melting glacial ice during the late Pleistocene, resulting in

several bays and coves along the shoreline. Several coastal streams were dammed by sand creating several freshwater dune lakes. The structure of these lakes is highly unstable due the fact that they were created above very saturated sandstone, which is highly susceptible to landslides (Orr and Orr, 2000).

Western Oregon experiences more rain than Eastern Oregon as a result of the Cascade Mountains that act like a barrier to the moisture from the Pacific Ocean. The Coastal system experiences above average rainfall for Western Oregon because the Coast Range traps part of the moisture from the Pacific Ocean and it precipitates in the coastal area. The highest precipitation normally occurs in January to early March. The driest time is normally in July (Loy et al., 2001). All of the coastal drainages flow into the Pacific Ocean. Because of the rainfall and the susceptibility of the sandstone, this area could have experienced significant changes in the topography of the area since the Pleistocene.

In this study I examined specimens from six rivers systems in the Oregon Coastal Subprovince: the Nehalem River, the Siuslaw River, the Umpqua River, the Coos River, the Coquille River and the Sixes River. I also examined specimens from Woahink Lake which is a coastal freshwater lake located between the Siuslaw River and the Umpqua River. I examined four additional drainages outside of the Oregon Coastal Subprovince: the Columbia-Willamette River, the Rogue River, the Klamath River and the Smith River (Figure 2.1).

Materials and Methods

Morphological data

Institutional abbreviations follow Leviton et al. (1985). Meristic and morphometric description follow Hubbs and Lagler (1964). Twenty-three meristic characters and 31 morphometric characters were used in *Ptychocheilus* morphological study. Twenty-five meristic characters and 31 morphometric characters were used in *Catostomus* morphological study. Twenty-four meristic characters and 31 morphometric characters were used in the morphological study of *C. rimiculus* and *C. sp. B*. The list of these characters and acronyms is provided in Appendix 2.1.

Precaudal vertebrae were distinguished by the absence of a well-developed haemal spine, which determined the first caudal vertebra. Morphometric measurements were used as a ratio to standard length (SL) in univariate analysis. In addition, two morphometric characters were standardized by two additional characters: body width at the pectoral fin base was standardized as a ratio to the body depth at the pectoral fin base and caudal peduncle depth was standardized as a ratio to the body depth at the origin of the dorsal fin for the univariate analysis.

Statistical software Statgraphics Centurion (StatPoint, 2005) and SPSS V11.0.4 (SPSS, 2005) were used. Non-normal univariate data were analyzed using Mann-Whitney test for two samples comparison and Kruskal-Wallis one-way ANOVA with Bonferoni correction for multiple comparisons. All comparisons were tested at alpha=0.05. The relative contributions of species identification, sex, and their interaction were evaluated with multifactor ANOVA to test for significant effects after removing the effect of the

other (type III sums of squares). Linear regression ANOA was used to determine relationship between diagnostic morphometric character and standard length. Multivariate data were analyzed by using Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA). PCA was used to reduce data and justify the grouping of the taxon in ordination space. Only character that showed significant differences in the univariate comparison were subsequently used in the principal component analysis. Males were analyzed separately from females in multivariate analysis. Morphometric measurements were regressed with standard length in order to separate size and shape variation. The residues from the regression were subsequently used in the multivariate analyses. PCA with no axis rotation was used to analyze correlation matrix. Only data that showed initial separation between each taxon in the PCA were subsequently analyzed by DFA. Discriminant Function Analysis was based on variables with absolute loading > 0.3 in PCA.

Molecular Data

Sequences were obtained from GenBank and from specimens in the OSU Fish Collection. Voucher specimens for some OSU fish tissues are deposited at the OSU fish collections and none of the GenBank samples have vouchers. The sequences obtained from GenBank were *Catostomus tahoensis* (AF454874), *C. occidentalis* (AF454873), *Deltistes luxatus* (AF454870), *Moxostoma anisurum* (AF454881), *Mylocheilus caurinus* (AF117169) and *Cyprinus carpio* (AY347295). The entire mitochondrial cytochrome b gene was sequenced for 143 specimens. I extracted DNA from 11 tissue samples of Willamette *Catostomus macrocheilus*, 2 Columbia *C. macrocheilus*, 4 Millicoma *C. tsiltcoosensis,* 6 Coos *C. tsiltcoosensis*, 12 Umpqua *C. tsiltcoosensis*, 9 Siuslaw *C.*

tsiltcoosensis, 2 Woahink *C. tsiltcoosensis*, 10 Coquille *Catostomus macrocheilus*, 1 *C. columbianus*, 1 *C. catostomus*, 1 *C. occidentalis*, 16 *C. sp. B*, 16 *C. rimiculus*, 1 *C. snyderi,* 1 *Chasmistes brevirostris*, 19 Umpqua *Ptychocheilus umpquae*, 17 Siuslaw *P. umpquae*, 13 Willamette *Ptychocheilus oregonensis*, 1 *P. grandis*, and 1 *P. lucius*. DNA was extracted from ethanol preserved specimens by using a Qiagen DNeasy Tissue Kit (Catalog No. 69504). The mitochondrial DNA cytochrome b gene was amplified from genomic DNA using primers L14724 (5′-GTGACTTGAAAAACCACCGTTG-3′;(Schmidt and Gold, 1993) and H15915 (5′-AACTGCAGTCATCTCCGGTTTACAAG AC-3′;(Irwin et al., 1991).

PCR reactions used 0.5 µg genomic DNA; 5 µL 10x buffer (0.1 M tris-HCL pH 8.5, 0.015 M MgCl2, 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, and deionized water added for a final volume of 50 µL. 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. The annealing temperature used in this study for *Ptychocheilus* analysis was 45° C. Double strand DNA was purified with Qiagen QIAquick PCR purification kit (Catalog number 28106).

The purified double stranded DNA was sent to Macrogen Inc (Korea) for sequencing. The double strands DNA of the catostomid samples were sequenced with primers trimL14724 (5′-GTGACTTGAAAAACCAC-3′; modified from Schmidt and Gold, 1993), trimH15919 (5′-AACTGCAGTCATCTCCGGTTTAC-3′; modified from Irwin et al., 1991), L15424 (5′- ATTTCTTTCCACCCATACTTTTC -3′; Edwards et al., 1991) and H15149 (5′-AAACTGCAGCCCCTCAGAAATATTTGTC CTCA -3′; Kocher

et al., 1989). Cyprinids double stranded DNA were sequenced with primers trimL14724, trimH15919, L479496 (5′-TTGTYCAATGAATCTGAG-3′), H600615 (5′-TCGATCCGGTTTCGTG -3′), L531546 (5′- ATTCTTCGCCTTCCAC -3′) and H636652 (5′- TTTTATCCGCATCAGAG -3′).

DNA sequences were edited and assembled in SeqEd v1.0.3 (Applied Biosystems, Inc., Forest City, USA) and aligned by eye in PAUP*(Swofford, 1998). Phylogenetic relationships were estimated using maximum parsimony (MP) and Maximum likelihood (ML) in PAUP* (Swofford, 1998). The heuristic method (1000 random additional sequences with tree bisection reconstruction for MP) was used to generate the tree. Nonparametric bootstrap analysis with 1000 pseudoreplicates and 100 random additional sequences were conducted for MP. If there was more than one parsimonious tree, the strict consensus topology of the most parsimonious tree was used.

Sequence divergence was calculated using PAUP* (Swofford, 1998) based on the DNA substitution model selected by Modeltest 3.7 (Posada and Crandall, 1998) under the likelihood criteria. The selected model for the catostomid data set was general time reversible with some sites assumed to be invariant. Variable sites follow gamma distribution (i.e. GTR+I+G). Maximum likelihood settings were as follows: nucleotide frequencies, A = 0.2925, C = 0.3018, G = 0.1213 and T = 0.2844; rate matrix, A-C= 0.8754, A-G = 68.2602, A-T = 0.4872, C-G = 2.4331, C-T = 11.0539, G-T = 1.0000; Proportion of invariant sites (I) = 0.5694; Gamma distribution shape parameter = 1.1263.

The selected model for cyprinid data was Tamura-Nei with some site assumed to be invariant (TrN+I). Maximum likelihood settings were as follows: nucleotide frequencies, A = 0.2918, C = 0.3058, G = 0.1281 and T = 0.2743; rate matrix, A-C=

1.0000, A-G = 23.3055, A-T = 1.0000, C-G = 1.0000, C-T = 10.5666, G-T = 1.0000; Proportion of invariant sites (I) = 0.6628; Gamma distribution shape parameter = equal rates for all sites.

The ingroup for *Catostomus* analysis consisted of *Catostomus macrocheilus*, *C. tsiltcoosensis*, *C. catostomus, C. occidentalis, C. tahoensis, C. snyderi, C. rimiculus, C. sp. B, Chasmistes brevirostris* and *Deltistes luxatus.* Outgroups were *Moxostoma anisurum* and *Cyprinus carpio*. *Moxostoma* is a sister group to *Catostomus* (Smith, 1992), an appropriate outgroup. *Cyprinus carpio* is more distantly related to *Catostomus* than *Moxostoma.* Including *C. carpio* in the outgroup made the outgroup more robust. The ingroup for the *Ptychocheilus* analysis was *Ptychocheilus oregonensis*, *P. umpquae*, *P. lucius* and *P. grandis*. Outgroups for *Ptychocheilus* were *Mylocheilus caurinus* and *Cyprinus carpio*. Based on morphological data, *Mylocheilus caurinus* is a sister group to *Ptychocheilus* (Smith et al. 2002). Based on Mitochondrial DNA, *Mylocheilus caurinus* also belong to the *Rhinichthys-Mylocheilus-Pogonichthys–Lotichthys-Richardsonius* clade which is sister to the *Phoxinus-Mylopharodon-Gila-Klamathella-Acrocheilus-Siphateles-Hesperoleucus-Erimichthys-Relictus-Orthodon-Ptychocheilus* clade (Smith et al. 2002). Rooting has been a problem with the study of *Ptychocheilus*'s phylogeny. To make the outgroup more robust, *Cyprinus carpio*, which is a more distantly related group to *Ptychocheilus* than *Mylocheilus* is to *Ptychocheilus*, was include in the outgroup.

Results

Catostomus sp. A

Material examined

Asterisk (*) indicates both DNA sequences and morphological data were collected. Delta (∂) indicates that only DNA sequences were collected. Solid circle (●) indicates that only DNA sequences were collected from OSU frozen tissue collection with no carcass deposited. The absence of a symbol indicates that only morphological data were taken from the samples. Numbers in parentheses indicate sample size.

Holotype

352 mm, South Fork Coquille River, Oregon, 24 October 2003, M. Grey

Paratype

Coquille River

USNM 58353 (10), 90.1-172.3 mm, 1879, J. Snyder; USNM 62253 (10), 90.7-183.5 mm, 1899, J. Snyder; *OS17864 (2), 345-362 mm, 15 October 2003, M. Grey; *OS17866 (1), 352 mm, 24 October 2003, M. Grey; OS17867 (3), 350-442 mm, 23 January 2004, M. Grey; *OS17868 (5), 298-345 mm, 6 June 2003, D. Markle.

Sixes River

USNM 62250 (2), 330-350 mm, 1879, J. Snyder; USNM 58153 (2), 86.8-114 mm, 1879, J. Snyder.

Diagnosis

There were two lineages (Northern clade: *C. macrocheilus*, *C. tsiltcoosensis*, *C. sp. A*, *C. tahoensis* and *C. columbianus* and Southern Clade: *C. sp. B*, *C. rimiculus*, *C. snyderi* and *C. occidentalis*, *Chasmistes brevirostris*, *Deltistes luxatus*) of catostomids found in this study. *Catostomus sp. A* belonged to the Northern clade which had 33 fix differences (66(C), 69(A), 75(T), 111(G), 153(G), 198(C), 225(C), 312(C), 360(T), 361(T), 378(T), 381(T), 465(C), 591(C), 603(C), 642(C), 657(C), 666(T), 687(T), 726(T), 741(C), 756(T), 789(G), 795(T), 798(C), 801(C), 804(T), 852(C), 933(G), 997(G), 998(C), 1023(A) and 1038(C)) from the Southern clade. All positions except positions 66, 69, 361, 591, 997, 998 and 1038 were third codon transitions. Positions 66, 69, 591 and 1038 were third codon transversions. Positions 361 and 997 were second codon transitions and position 998 was a second codon transition. *Catostomus sp. A* differs from *C. columbianus*, *C. catostomus* and *C. tahoensis* in the number of lateral line scales. *Catostomus columbianus*, *C. catostomus* and *C. tahoensis* have more than 80 lateral line scales (Bond, 1994; La Rivers, 1994 and Wydoski and Whitney, 2003). *Catostomus sp. A* had less than 80 lateral line scales. *Catostomus platyrhynchus* differs from *C. sp. A* in having distinct notches at the corner of the mouth between the upper lip and the lower lip (Bond, 1994 and Wydoski and Whitney, 2003) while *C. sp. A* does not (Figure 2.2).

Catostomus sp. A tended to have fewer dorsal fin rays (*C. sp. A*: 10-13, = 11.9 and *C. macrocheilus*: 12-15, = 13.6) and infraorbital pores (*C. sp. A*: 19-38, = 30.6 and *C. macrocheilus*: 33-50, = 41.4) than *C macrocheilus.* Most (87.1%; N=31) of *C. sp. A* had dorsal fins with fewer than 13 rays while 97.6% of *C. macrocheilus* had dorsal fins with

more than 12 rays. Most (96.8%; N=31) of *C. sp. A* had fewer than 37 infraorbital pores while 88.1% of *C. macrocheilus* had more than 36 infraorbital pores.

Catostomus sp. A had a relatively deeper caudal peduncle depth (CPD/DDO = 0.33-0.45, μ =0.38) than *C. macrocheilus* (*C. macrocheilus*: 0.25-0.39, μ = 0.33). Most (93.5%; N=31) of *C. sp. A* had the ratio of caudal peduncle depth to body depth at the origin of the dorsal fin higher than 0.350 while 80.5% (N=41) of *C. macrocheilus* had the ratio caudal peduncle depth to body depth at the origin of the dorsal fin lower than 0.351.

Catostomus sp. A tended to have narrower caudal peduncle depth (CPD/DDO = 0.33-0.45, μ = 0.38) and lower ratio of body width at pectoral fin bases to body depth at anterior margin of pectoral fin bases (WP1/DP1 = 0.70-0.89, μ = 0.81) than *C. tsiltcoosensis* (CPD/DDO= 0.29-0.49, μ = 0.43; WP1/DP1= 0.80-1.09, μ = 0.93). Most (80.6%; N=31) of *C. sp. A* had the ratio of caudal peduncle depth to body depth at the origin of the dorsal fin lower than 0.411 while 78.1% (N=64) of *C. tsiltcoosensis* had the ratio higher than 0.410. Most (96.8%; N=31) of *C. sp. A* had the ratio of body width at pectoral fin bases to body depth at anterior margin of pectoral fin bases lower than 0.866 while 96.7% (N=60) of *C. tsiltcoosensis* had the ratio body width at pectoral fin bases to body depth at anterior margin of pectoral fin more than 0.865.

Catostomus sp. A had 14 fixed cytochrome b positions ((228(A), 255(C), 450(G), 540(G), 573(G), 615(A), 675(C), 750(G), 867(G), 879(G), 906(G), 909(G), 960(G and A) and 1045 (A)) that differed from *Catostomus macrocheilus* and *C. tsiltcoosensis* (Appendix 2.2). All positions except positions 540, 615, 675 and 1045 were third codon transitions. Positions 540, 615 and 675 were third codon transversions. Position 1045 was a first codon transition.

Description

There was a sampling gap between specimens 185-298 mm. Therefore, morphological description only apply to specimens smaller than 186 mm and larger than 297 mm. Morphological description is based on up to 31 Coquille River specimens (SL = 90-186 mm and SL= 298-442 mm): elongate body, slightly laterally compressed; head is moderately long 21.3-25.3% SL; snout rounded and moderately long 9.0-11.6% SL; body depth greatest at the origin of dorsal fin 15.8-23.6 %SL; shallow caudal peduncle 6.4-8.8%SL; eye 2.7-5.7% SL (N=30); interorbital width 8.2-9.6 %SL; origin of dorsal fin in mid body; dorsal fin with 10-13 fin rays; pelvic fin origin posterior to dorsal fin origin; pelvic fin with 9-11 fin rays; infraorbital pores 19-38; mouth subterminal with large fleshy lips, covered with papillae; 1-2 rows of papillae across the symphysis of the lower lip; 2-4 rows of papillae on the upper lip; 2-8 rows of papillae on the lower lip; gill rakers on the lateral side of the first gill arch 26-34; gill rakers on medial side of the first gill arch 28-36; lateral line scales; 67-79; scales above the lateral line11-15; scales below the lateral line 8-13; post-Weberian vertebrae 42-45 (μ =43.6); peritoneum dark grey to black; dark body coloration on the dorsal side; light body coloration on the ventral side; light body coloration on lower half of the body on the lateral side (Figure 2.3).

Males tended to lower infraorbital pore counts (INFORBPOR, P=0.012) than females, narrower interorbital width (IW as %SL, P=0.009), shallower body depth at the pectoral fin (DP1 as %SL, P=0.029) and longer trunk length (LOP1_LOP2 as %SL, P=0.002) than females.

Catostomus macrocheilus (sensu lato) was not a monophyletic group because *C. sp. A* made the taxon paraphyletic (Figure 2.4). *Catostomus sp.* A had 5 unique

cytochrome b haplotypes (CO1, CO2, CO3, CO4 and CO5) and formed a monophyletic group sister to *C. tahoensis*, *C. columbianus*, *C. macrocheilus* and *C. tsiltcoosensis*. They were paraphyletic to *C. macrocheilus* and *C. tsiltcoosensis*.

Distribution

The distribution of *Catostomus sp A.* is in the Coquille River and Sixes River, south of, and adjacent to, *C. tsiltcoosensis*. The Sixes population has not been confirmed genetically so its assignment is tentative.

Catostomus tsiltcoosensis Evermann and Meek, 1898

Synonymy

Catostomus tsiltcoosensis Evermann and Meek, 1898:68-69, Fig.1. (Tsiltcoos Lake, Oregon)

Catostomus tsiltcoosensis, Snyder 1908:166; Gilbert 1998:202.

Material examined

Siuslaw River

*OS15461 (13), 251-401 mm, 7 June 1995, D. Markle; OS15465 (4), 87-129 mm, 7 June 1995, D. Markle; OS 16806 (4), 91-134 mm, 3 June 1998, M. Terwilliger; OS16813 (2), 85-119 mm, M. Terwilliger; OS16871 (2), 121-135 mm, 3 June 1998, C. Hill.

Woahink Lake

*OS13656 (3), 350-372 mm, 11 March 1992, G. Westfall.

Umpqua River

*OS15427 (5), 340-416 mm, 28 April 1995, D. Markle; OS16200 (2), 80.3-81.5 mm, 7 August 1997, C. Hill; OS17869 (2), 105.34-142.88 mm, 7 July 2004, S. Anderson; OS17870 (3), 99-113 mm, 8 July 2004, S. Anderson; *OS17871 (1), 343 mm, 9 July 2005, S. Anderson; *OS17872 (10), 303-367 mm, 19 August 2004, M. Blume and C. Baldwin.

Coos River

*OS17859 (3), 301-395 mm, 16 September 2004, S. Hurns; *OS17861 (3), 353-382 mm, 20 August 2004, S. Hurns.

Millicoma River

*OS15442 (8), 286-408 mm, 11-15 May 1995, P. Reimers; OS15879 (6), 99.01-138.37 mm, 25 June 1997, D. Plawman; OS15880 (4), 128.18-150.41 mm, 6 June 1997, D. Plawman; OS16351 (1), 152 mm, 19 August 1997, EPA; OS16320 (1), 100.8 mm, 2 September 1997, C. Hill.

Diagnosis

A northern clade member, *Catostomus tsiltcoosensis* differs from *C. columbianus, C. catostomus* and *C. tahoensis* in having fewer than 80 lateral line scales (Bond, 1994; La Rivers, 1994 and Wydoski and Whitney, 2003). In this study, most (97 %; N=67) *C. tsiltcoosensis* had lateral line scales fewer than 80 scales (the other 3% had 80 lateral line scales). *Catostomus tsiltcoosensis* differs from *C. platyrhynchus,* which has a distinct notch at the corner between the upper and lower lips (Bond, 1994 and Wydoski and Whitney, 2003), which is absent in *C. tsiltcoosensis* (Figure 2.2).

Catostomus tsiltcoosensis tended to have fewer infraorbital pores (14-37, μ=28.9) than *C. macrocheilus* (33-50, μ= 41.4) with 97.4% (N=77) having fewer than 37 infraorbital pores while 88.1% (N=42) of *C. macrocheilus* had more than 36 infraorbital pores.

Catostomus tsiltcoosensis also tended to have relatively deeper caudal peduncle depth (CPD/DDO: 0.29-0.49, μ = 0.43) and the ratio of body width at pectoral fin bases to body depth at anterior margin of pectoral fin bases (WP1/DP1 = 0.80-1.09, μ = 0.93) than *C. macrocheilus* (CPD/DDO = 0.25-0.39, μ = 0.33; WP1/DP1= 0.74-0.92, μ = 0.82). Most (92.2%; N=64) *C. tsiltcoosensis* had the ratio of caudal peduncle depth to body depth at the origin of the dorsal fin greater than 0.379 while 95.1% (N=41) of *C. macrocheilus* were lower than 0.380. Most (93.3%; N=60) *C. tsiltcoosensis* had the ratio body width at pectoral fin bases to body depth at anterior margin of pectoral fin greater than 0.784 while 88.1 % (N=42) of *C. macrocheilus* had the ratio less than 0.875.

Catostomus tsiltcoosensis differed from *C. sp A* as described above and from *Catostomus macrocheilus* in six, third position transitions in cytochrome b: 246 (G and T), 348 (T), 501(G), 552(G), 600(A) and 705(A) (Appendix 2.2).

Description

The morphological description was based on up to 77 specimens (80-152 mm and 251-416 mm SL): elongate body, slightly laterally compressed; head moderately long 21.0-25.2% SL (N=66); snout rounded and moderately long 9.3-12.9% SL (N=65); body depth greatest at origin of dorsal fin 16.6-22.4 %SL (N=66); shallow caudal peduncle 6.6-9.3% SL (N=64); eye 2.6-6.0% SL (N=56); interorbital width 8.1-10.4 %SL (N=59); origin of dorsal fin in mid body; dorsal fin with 11-14 fin rays (N=75); pelvic fin origin

posterior to dorsal fin origin; pelvic fin with 9-12 fin rays (N=64); infraorbital pores 14-37; mouth subterminal with large fleshy lips, covered with papillae; 1-2 rows of papillae across the symphysis of the lower lip; 2-3 rows of papillae on the upper lip (N=63); 4-8 rows of papillae on the lower lip (N=63); lateral gill rakers of the first gill arch 23-31; medial gill rakers of the first gill arch 27-38; lateral line scales 65-80 (N=67); scales above lateral line 11-17 (N=64); scales below lateral line 8-14 (N=63); post-Weberian vertebrae 43-46 (μ =44.2; N=72); peritoneum dark grey to black; dark body coloration on the dorsal side; light body coloration on the ventral side; light body coloration on lower half of the lateral side (Figure 2.3 A-C).

Males tended to have fewer vertebrae anterior to origin of anal fin (VAO, $P<0.001$), fewer supraorbital pores (SUPORBPOR, $P=0.043$), shorter snout length (LAE as %SL, $P=0.009$), shorter preanal length (LOA as %SL, $P=0.010$), shorter length from the most anterior infraorbital pore to the anterior end of the eye (AIOPAE as %SL, $P=0.003$) and shallower body depth at the origin of the pectoral fin (DP1 as %SL, $P=0.017$) than females.

Based on cytochrome b sequences from 33 specimens (4 Millicoma, 6 Coos, 9 Siuslaw, 2 Woahink, and 12 Umpqua), there were three unique haplotypes (TS1, TS2 and TS3) in Siuslaw River and Woahink Lake (Appendix 2.2) and eight unique base pair positions (72(C), 246(G), 249(T), 318(T), 555(A), 876(G), 957 (A), 1041(C). All positions except position 72 were third position transitions. Position 72 was a third position transversion. The Umpqua population had three unique haplotypes (TU1, TU2 and TU3) (Appendix 2.2) and four unique third positions transitions 369(G), 609(G), 996(G) and 1050(C) (Appendix 2.2). Coos and Millicoma population had two unique

haplotypes (TMC1 and TMC2) and two unique base pair positions (480(G) and 901(C)). Position 480 was a third position transition and position 901 was first position transition. *Catostomus tsiltcoosensis* (Siuslaw, Woahink, Umpqua, Coos and Millicoma) formed a monophyletic sister group to *C. macrocheilus* (Willamette and Columbia) (Figure 2.4). *Catostomus tsiltcoosensis* in each coastal drainage was monophyletic with Umpqua sister to Coos and Millicoma, and more northerly, Siuslaw drainage sister to Umpqua, Coos and Millicoma.

Distribution

Catostomus tsiltcoosensis are found in coastal streams and lakes in Siuslaw River, Woahink Lake, Tsiltcoos Lake, Umpqua River, Coos River and Millicoma River. Their distribution is adjacent to the distribution of the *C. macrocheilus* and *C. sp A.*

Catostomus macrocheilus Girard, 1856

Partial Synonymy

Catostomus macrocheilus Girard, 1856:175 (Astoria, Oregon)

*Catostomus macrocheilus***,** Snyder 1908:165-169; Carl 1936:20; Lindsey 1956:765; McCart and Aspinwall 1970:1154; Nelson 1974:101; Reimers and Baxter 1976:3; Dauble and Buschbom 1981:802; Lee et al 1980:383; Dauble 1986:356; Nelson 1986:101; McAllister 1990:55; Bond 1994:27; La Rivers 1994:340; Gilbert 1998:168; Scott and Crossman 1998:544; Wydoski and Whitney 2003:147; Nelson 2004:78; McPhail 2007:181-186.

Material examined

Columbia River

*UW49018 (1), 326 mm, 8 November 2003, M. Paquin; ∂OS17858 (1), 400mm, 9 October 2004, P. Luke

Nehalem River

USNM58115 (7), 79.41-172.20 mm, 1879, J. Snyder.

Willamette River (Mainstem)

OS10591 (4), 130.15-182.46 mm, 24 August 1981, J. Long; OS14388 (1), 139.65 mm, 7 September 1993; OS 15718 (2), 138.65-153.47 mm, 1 July 1996, C. Hill; OS16654 (1), 183.85 mm, 29 August 1997, B. Gerth; OS16656 (2), 152.38-216mm, 25 June 1997, B. Gerth; OS 17111 (2), 144.08-152.61 mm, 25 August 1998, S. Corbett; OS17860 (1), 326 mm, 24 June 2004, J. Kettratad; OS17862 (4), 306-345 mm, 6 April 2004, J. Adams; *OS17863 (6), 275-360 mm, 2 April 2004, J. Adams; *OS17873 (4), 370-435 mm, 5 April 2004, J. Adams; ●OSUF43(1), 159 mm, 20 July 1992, P. Harris; ●OSUF44(1), 165 mm, 20 July 1992, P. Harris.

Willamette River (Mary's River)

OS16107 (1), 109.15 mm, 1 July 1998, C. Hill.

Willamette River (Pudding River)

OS14389 (1), 112.43 mm, 21 September 1993, I. Waite.

Willamette River (Long Tom River)

OS16821 (1), 158mm, 15 July 1998, M. Terwilliger; OS13135 (3), 160.14-175.29 mm, 11 July 1991, P. Petry. OS17712 (1), 125.7 mm, 22 September 1998.

Diagnosis

A northern clade species, differing from *C. columbianus*, *C. catostomus* and *C. tahoensis* in fewer lateral line scales (fewer than 80 scales). In this study, most (97.6 %; N=41) *C. macrocheilus* had lateral line with fewer than 80 scales (the other 2.4% had 80 lateral line scales). *Catostomus platyrhynchus* has a distinct lateral notch between the upper and lower lips (Bond, 1994 and Wydoski and Whitney, 2003) while *C. macrocheilus* does not (Figure 2.2). *Catostomus macrocheilus* had six fixed third positions transitions that differed from *C. tsiltcoosensis* and *C. sp. A*: 246(A), 348(C), 501(A), 552(A), 600(G) and 705(G) (Appendix 2.2)

Description

The morphological description was based on up to 42 specimens (79-435 mm SL, though only three were in the size range 184-326mm SL): elongate body, slightly laterally compressed; head moderately long 20.4-26.0% SL (N=41); snout rounded and moderately long 8.6-13.1% SL (N=41); body depth greatest at origin of dorsal fin 18.7-23.8 % SL (N=41); shallow caudal peduncle 5.1-8.7% SL (N=40); eye diameter 2.9-6.3%SL (N=39); interorbital width 8.2-10.5%SL (N=41); origin of dorsal fin in mid body; dorsal fin with 12-15 fin rays; pelvic fin origin posterior to dorsal fin origin; pelvic fin with 9-12 fin rays (N=40); infraorbital pores 33-50; mouth subterminal with large fleshy lips, covered with papillae; 1-2 rows of papillae across the symphysis of the lower lip; 2-5 rows of papillae on the upper lip (N= 40); 4-8 rows of papillae on the lower lip

(N=40); lateral gill rakers of the gill arch 25-35; medial gill raker of the first gill arch 31-43; lateral line scales 65-80 (N=41); scales above the lateral line 11-15 (N=40); scales below the lateral line 7-12 (N= 40); post-Weberian vertebrae 43-46 (μ =44.2); peritoneum dark grey to black; dark body coloration on the dorsal side; light body coloration on the ventral side; light body coloration on lower half of the lateral side (Figure 2.3).

Spawning males tended to be smaller than spawning females. Spawning males have a dark black lateral stripe from snout to the caudal region. The stripe is poorly developed or absent in females (McCart and Aspinwall, 1970). Males have a longer anal fin than females. McCart and Aspinwall (1970) reported that males from Stave Lake British Columbia, Canada had nuptial tubercles cover anal fin, pectoral fins, and pelvic fins during the spawning season. Wydoski and Whitney (2003) reported that the Columbia males have tubercles on caudal and anal fins while the males in British Columbia, Canada have tubercles on all fins.

There were 8 cytochrome b haplotypes (CMW1-CMW8) from 13 specimens, (Appendix 2.2). Haplotype CMW7 was found in both Columbia River and Willamette River. Haplotype CMW8 was found in Columbia River. Six haplotypes (CMW1, CMW2, CMW3, CMW4, CMW 5 and CMW 6) were found in the Willamette System. *Catostomus macrocheilus* formed a monophyletic sister group to *Catostomus tsiltcoosensis* (Figure 2.4).

Distribution

Catostomus macrocheilus has a wide distribution. In the Pacific Northwest, it is found in major drainages between Peace River in British Columbia in the north and the

Willamette River in Oregon in the south and from western Montana toward the Pacific Ocean. They are also found in Harney Basin of western Oregon, western Utah and northern Nevada (Reimers, 1976; McPhail and Lindsey, 1970; Lee et al, 1980; Scott and Crossman, 1998 and Wydoski & Whitney 2003). They have adjacent distribution to *C. tsiltcoosensis*.

Comparison among *Catostomus tsiltcoosensis*, *Catostomus macrocheilus* and *C. sp. A*

Due to the sampling gap between fishes that had standard length 184 mm to 251 mm, the comparisons were for specimens that had standard length from 79 mm to 184 and from 250mm to 442 mm. *Catostomus tsiltcoosensis*, *C. macrocheilus* and *C. sp. A* had very similar morphological features with similar ranges of morphometric characters. Most morphometric characters overlapped (Appendix 2.3). Morphometric PCA did not provide clean separation among *C. macrocheilus*, *C. tsiltcoosensis* and *C. sp. A* in both sexes (Figure 2.5). In male PCA, two *C. macrocheilus* embedded in the *C. tsiltcoosensis* cluster. In female PCA, one *C. macrocheilus* embedded in the *C. tsiltcoosensis* cluster. Most of *Catostomus sp. A* embedded with *C. tsiltcoosensis* in both sexes (Figure 2.5). Only few (four females and one male) embedded with *C. macrocheilus*. Characters that loaded heavily on principal component one (PC1) in both sexes were characters measuring body depth (DDO, ID_OP2 and OD_OP2) (Figure 2.5). PC1 partially separated *C. macrocheilus* from *C. tsiltcoosensis* in both sexes (Figure 2.5). In male morphometric PCA, additional morphometric characters contributing to PC1, which separated *C. macrocheilus* from *C. tsiltcoosensis* and *C. sp. A* were body depth at pectoral fin origin and length from origin of dorsal fin to the insertion of the pelvic fin (Figure 2.5 B). In female morphometric PCA, characters that loaded heavily on principal component

2 (PC2) were predorsal length, length from the tip of the snout to the dorsal fin insertion and length from the tip of the snout to the posterior end of the eye, which further helped separating *C. macrocheilus* form *C. tsiltcoosensis* (Figure 2.5 A). In male morphometric PCA, PC2 did not separated *C. macrocheilus* from *C. tsiltcoosensis* (Figure 2.5 B). Base on principal component score plot and the eigenvectors from both sexes, *C. macrocheilus* tended to have deeper body depth (DDO, ID_OP2 and OD_OP2) than *C. tsiltcoosensis* (Figure 2.5). Body depth at the dorsal fin origin (DDO) as a proportion of SL (P=0.257) and the length from the origin of the dorsal fin to the origin of pelvic fin as a proportion of SL (P=0.421) did not increase with SL. The length from the dorsal fin insertion to the origin of pelvic fin (ID_OP2) as a proportion to SL increased with SL (p= 0.003).

A plot between the ratio of body width at pectoral fin bases to body depth at anterior margin of pectoral fin bases and the ratio of caudal peduncle depth to body depth at the origin of the dorsal fin partially separated the *C. macrocheilus*, *C. tsiltcoosensis* and *C. sp. A* (Figure 2.6 A). *Catostomus macrocheilus* had a significantly lower mean ratio of caudal peduncle depth the body depth at dorsal fin origin than *C. tsiltcoosensis* and C. sp. A (P<0.001). The ratio of caudal peduncle depth to body depth at the origin of the dorsal fin increased with SL (P<0.001). The differences in the ratio of caudal peduncle depth to body depth dorsal fin origin between *C. macrocheilus* and *C. sp. A* - *C. tsiltcoosensis* were consistent in all size (Figure 2.6 B). *Catostomus tsiltcoosensis* had a significantly higher mean ratio of body width at the pectoral fin base to the body depth at the anterior margin pectoral fin base than *C. macrocheilus* and *C. sp. A* (P<0.001). The ratio of body width at pectoral fin bases to body depth at anterior margin of pectoral fin bases increased with SL (P<0.001). The differences in the ratio of body width at the

pectoral fin base to the body depth at the pectoral fin origin were consistent in all size (Figure 2.6 C).

Six sexually dimorphic morphometric characters (LOA, P=0.0053; DP1, P=0.0023; LAE, P=0.0060; IW, P<0.001 and LOP1_LOP2, P=0.0056) were detected. Body depth at the origin of pectoral fin (DP1) was the only sexually dimorphic character that exhibited differences between *C. macrocheilus* and *C. tsiltcoosensis* in both sexes. Both males and females *C. macrocheilus* had significantly higher mean body depth relative to the standard length at the origin of pectoral fin than *C. tsiltcoosensis* (both sexes P<0.001).

Meristic characters were more useful than morphometric characters. However, *C. tsiltcoosensis*, *C. macrocheilus* and *C. sp. A* also had similar range of meristic characters and most overlapped (Appendix 2.4). Meristic PCA revealed three distinct clusters in both sexes corresponding to *C. macrocheilus*, *C. tsiltcoosensis* and *C. sp. A* (Figure 2.7). Characters that loaded heavily on PC1 in both sexes included dorsal fin rays, infraorbital pores and medial gill rakers (Figure 2.7). In both sexes PC1 provided partial separation of *C. macrocheilus* from *C. tsiltcoosensis* and *C. sp. A.* Characters with heavy loading on the PC2 in both sexes included precaudal vertebrae and vertebrae anterior to the pelvic fin origin, which provided further separation between *C. tsiltcoosensis* and *C. sp. A*.

For each sex, additional meristic characters contributing PC1, which separated *C. macrocheilus* from *C. tsiltcoosensis* and *C. sp. A* were vertebrae anterior to the dorsal fin origin and lateral gill rakers in males, and supraorbital pores in females. In each sex, additional meristic characters contributing to PC2 were caudal vertebrae for males and

vertebrae anterior to the anal fin origin and vertebrae anterior to the dorsal fin origin for females.

The first two discriminant functions based on 8 meristic characters (PCV, VDO, VAO, VPO, GILRKPOST, DORSALRAYS, INFORBPOR and SUPORBPOR for female and PCV, CV, VDO, VPO, GILRKANT, GILRKPOST, DORSALRAYS and INFORBPOR for male) explained 100 % of the total variance found among the three species in both sexes ($P<0.01$ in both sexes). DFA correctly classified 90.41% of females and 96.77% of males. *Catostomus macrocheilus* had the highest correct classification rate (96.15% in female and 100% in male). Only 3.85% (N=26) of female *C. macrocheilus* was misclassified as *C. tsiltcoosensis*. *Catostomus tsiltcoosensis* had the highest misclassification rate (12.90% (N=31) of females were classified as *C. sp. A* and 7.14 % (N=14) of males were classified as *C. sp. A*). Females *Catostomus sp. A* had 12.5% (N=16) misclassification rate (misclassified as female *C. tsiltcoosensis*). Males of *C. sp. A* and *C. tsiltcoosensis* had 100 % correct classification rate. Similar to PCA, DFA from both sexes suggested that *C. macrocheilus* had more dorsal fin rays, medial gill rakers and infraorbital pores than *C. tsiltcoosensis* and *C. sp. A*. Based on DFA from both sexes, DFA also suggested that *C. tsiltcoosensis* had more vertebrae anterior to the dorsal fin origin and more medial gill rakers than *C. sp. A* (Figure 2.8).

Despite overlap, *C. macrocheilus* had a significantly higher mean number of dorsal fin rays than *C. sp. A* and *C. tsiltcoosensis* ($P<0.001$). *Catostomus tsiltcoosensis* had significantly higher mean numbers of vertebrae anterior to the dorsal fin ($P<0.001$), precaudal vertebrae ($P<0.001$) and vertebrae anterior to the pelvic fin origin ($P<0.001$) than *C. sp. A.* and C. *macrocheilus*. The number of medial gill rakers (GILRKPOST) on

the 1st arch had an ontogenetic or size signal (Figure 2.9). The relationship for each species was expressed as gill rakers = A + B/SL. The coefficients, *r2,* and sample sizes *(N)* were *C. macrocheilus* A = 39.69, B = -438.729, *r2* = 14.12% *N* = 42; *C. tsiltcoosensis* A = 36.559, B = -382.297, *r2* = 35.05% *N* = 76 and *C. sp. A* A = 34.97, B = -292.12, *r2* = 25.65% *N* =31. All species approached an asymptote at about 200 mm SL. *Catostomus macrocheilus* had a significantly higher mean number of gill rakers count than *C. tsiltcoosensis* ($P<0.001$; *C. macrocheilus*, $\mu= 29.33$; *C. tsiltcoosensis*, $\mu= 26.68$).

Three sexually dimorphic meristic characters (VAO, P=0.0294; SUPORBPOR, P=0.0209 and INFORBPOR, P=0.0059) were detected. The differences among species of the sexually dimorphic characters were mainly shown in females. Female *C. tsiltcoosensis* had a significantly higher ($P<0.001$) mean number of vertebrae anterior to the anal fin (VAO) than female of *C. sp. A* and *C. macrocheilus*. Female *C. macrocheilus* also had a significantly higher ($P<0.001$) mean number of supraorbital pores (SUPORBPOR) than female of *C. tsiltcoosensis* and *C. sp. A*. Despite being sexually dimorphic, infraorbital pore (INFORBPOR) differed greatly between *C. macrocheilus* and *C. tsiltcoosensis*-*C. sp. A. Catostomus macrocheilus* had a significantly higher ($P<0.001$ in both sexes) mean number of infraorbital pores than *C. tsiltcoosensis* and *C. sp. A*.

Phylogenetic analysis of cytochrome b revealed two distinct clades: a northern clade containing *Catostomus macrocheilus*, *C. tsiltcoosensis*, *C. sp. A*, *C. columbianus* and *C. tahoensis* and a southern clade containing *C. rimiculus*, *C. sp. B*, *C. occidentalis*, *C. snyderi*, *Deltistes luxatus* and *Chasmistes brevirostris*. In the Oregon Coastal

Subprovince, *C. sp. A* was sister to a group containing *C. macrocheilus*, *C. tsiltcoosensis*, *C. tahoensis* and *C. columbianus* (Figure 2.4).

Remarks on *C. macrocheilus* group

Three separate taxa formerly referred to *C. macrocheilus* (*sensu lato*) are recognized herein: *C. macrocheilus* (*sensu stricto*), *C. tsiltcoosensis* and *C. sp A*. *Catostomus tsiltcoosensis* was first described by Evermann and Meek (1898) who only compared it to *C. occidentalis*. Later Snyder (1908) concluded that there were no differences in morphological data between *C. tsiltcoosensis* and *C. macrocheilus*.

Even though morphological data were less useful than molecular data, morphological data strongly supported the recognition of *C. macrocheilus*, *C. tsiltcoosensis* and *C. sp. A*. *Catostomus tsiltcoosensis* and *C. sp. A* have a very similar overall morphological features. In both sexes, majority of *C. sp. A* embedded with *C. tsiltcoosensis* in the score plots of morphometric PCA and meristic PCA (Figure 2.5 and Figure 2.7). In meristic DFA, most of the misclassified fish of *C. tsiltcoosensis* were misclassified as *C. sp. A* and most of the misclassified fish of *C. sp. A* were misclassified as *C. tsiltcoosensis*. Despite the overall similarity, two morphometric ratios (ratio of caudal peduncle depth to the body depth at the dorsal fin origin and the ratio of body width at the base of the pectoral fin to body depth at the base of the pectoral fin) provided partially separation among *C. tsiltcoosensis*, *C. sp. A* and *C. macrocheilus* (Figure 2.6). Both *C. tsiltcoosensis* and *C. sp. A* have higher means ratio of caudal peduncle depth to body depth at the dorsal fin origin than *C. macrocheilus* (both with $P<0.001$). *Catostomus tsiltcoosensis* had a significantly higher mean ratio of body width at the pectoral fin base to the body depth at the anterior margin of the pectoral fin base than *C. sp. A* and *C.*

macrocheilus (P<0.001). Meristic data were more useful than morphometric data for identifying *C. tsiltcoosensis* and *C. sp. A* from *C. macrocheilus* but overlapped still evidenced (Figure 2.5 and Figure 2.7). Three meristic characters (infraorbital pores, gill rakers and dorsal fin rays) were useful in identifying *C. tsiltcoosensis* and *C. sp. A* from *C. macrocheilus*. *Catostomus tsiltcoosensis* and *C. sp. A* tended to have fewer infraorbital pores, gill rakers and dorsal fin rays than *C. macrocheilus* (all with P<0.001). Precaudal vertebrae counts contributed in separating *C. sp. A* from *C. macrocheilus* in PCA. *Catostomus sp. A* had lower means of precaudal vertebrae counts and vertebrae anterior to the pelvic fin than *C. tsiltcoosensis* (both with P<0.001). However, due to extreme overlapped, they were less useful as diagnostic characters.

The differences of morphological characters among the three species of suckers found in this study were confounded by size and sex. Both the ratio of caudal peduncle depth to the body depth at the dorsal fin origin and the ratio of body width at the pectoral fin base to the body depth at the pectoral fin base increased with SL (Figure 2.6). Infraorbital pores count was affected by sex. Females had a higher mean number of infraorbital pores than males (P=0.018). Medial gill rakers on the first gill arch were affected by size. Medial gill rakers had ontogenic signal. Medial gill rakers count increased with SL. All species approached an asymptote at about 200 mm SL (Figure 2.9).

Molecular data was more useful than morphological data in separating *C. tsiltcoosensis* and *C. sp. A* from *C. macrocheilus*. *Catostomus tsiltcoosensis* had 6 autapomorphic basepair positions in cytochrome b and formed a monophyletic sister group to *C. macrocheilus*. Sequence divergence within *C. tsiltcoosensis* ranged from

0.097 % to 1.793% (=1.09%) and within *C. macrocheilus* ranged from 0.097 % to 0.802 % (= 0.349%). McPhail and Taylor (1999), using a shorter 300-basepair cytochrome b sequence, suggested that average cytochrome b sequence divergence among British Columbia and Washington *Catostomus macrocheilus* was 6.7%. Sequence divergence from my 1042 basepair sequence ranged from 1.36 % - 2.47% between *C. macrocheilus* and *C. tsiltcoosensis* (Appendix 2.5) and is in the range expected for species pairs (1-25%, Johns and Avise, 1998). Both morphological data and molecular data suggested that *C. tsiltcoosensis* is a separate species from *C. macrocheilus*.

Catostomus sp. A had 14 autapomorphic base pair position in cytochrome b sequence. In phylogenetic analysis, *C. sp. A* was sister group containing *C. tahoensis*, *C. columbianus*, *C. tsiltcoosensis* and *C. macrocheilus*. This makes *Catostomus macrocheilus* (*sensu lato*) paraphyletic. *Catostomus sp. A* formed their own monophyletic group. The sequence divergence within *C. sp. A* ranges from 0.097%-0.704% (μ=0.377%). The sequence divergence between *C. macrocheilus* and *C. sp. A* ranged from 3.18%-3.78% (Appendix 2.5). The sequences divergence between *C. tsiltcoosensis* and *C. sp. A* ranged from 2.63%-4.08% (Appendix 2.5). These sequence divergence is greater than the sequence divergence between *C. macrocheilus* and *C. tsiltcoosensis*. Therefore, based on both morphological data and molecular data, *C. sp A* is a separate species from *C. macrocheilus and C. tsiltcoosensis*.

The Sixes River sucker is the most southerly distributed taxon of the group previously known as *C. macrocheilus* (*sensu lato*). Sixes River sucker is morphologically similar to *C. sp. A* (Figures 2.5-2.7). Therefore, it is likely that they are *C. sp A*. However,

due to the lacking of the molecular data, it is still uncertain about the species status of this taxon.

Catostomus sp. B

Material examined

Holotype

315 mm, Elk Creek, Rogue River, Oregon, 13 May 2005, B. Crowe

Paratype

Elk Creek, tributary of Rogue River

*OS17875 (3), 420-460 mm, 13 May 2005, B. Crowe; *OS17876 (1), 110 mm, May 2005, P. Samarin

Rogue River mainstem

OS8090 (6), 109.35-133.10 mm, 4 October 1979, S. Cramer; OS11013 (11), 96.80-126.80 mm, 11 September 1981, Suj; *OS 15913 (23), 315-405 mm, 23 August 1993, ODFW; OS17135 (5), 79-111.5 mm, 15 September 1998, S. Corbett; *OS17878 (1), 350 mm, 16 May 2006, J. Kettratad; *OS17883 (1), 92 mm, 20 May 2005, J. Vogue

Rough and Ready Creek, tributary of Rogue River

OS16161 (3), 72.5-130.7 mm, 14 August 1997, EPA

Bear Creek, tributary of Rogue River

OS OS17874 (1), 158 mm, 10 May 2005, P. Samarin

Diagnosis

A southern clade member which had 33 fixed differences (66(A), 69(C), 75(C), 111(A), 153(A), 198(T), 225(T), 312(T), 360(C), 361(C), 378(C), 381(C), 465(T), 591(A), 603(T), 642(T), 657(T), 666(C), 687(C), 726(C), 741(T), 756(C), 789(A), 795(C), 798(T), 801(T), 804(C), 852(T), 933(A), 997(A), 998(T), 1023(G) and 1038(A)) from the Northern clade. In this study there are 6 species that belonged to the Southern clade lineage: *Catostomus occidentalis*, *C. snyderi*, *C. sp. B*, *C. rimiculus*, *Chasmistes brevirostris* and *Deltistes luxatus*. *Catostomus sp. B* differs from *C. occidentalis* in the number of lateral line scales. In this study, most (91.1%N=45) of *Catostomus sp B*. had more than 79 lateral line scales while 97% (N=86) of *C. occidentalis* had lateral line scales fewer than 80 scales (*C. occidentalis* data from Kettratad, 2001).

Catostomus sp. B differs from *C. snyderi* in the ratio of snout length (LAE) to the head depth (HD) and the number of gill rakers. *Catostomus sp. B* tended to have higher ratio of snout length to head depth and fewer gill raker than *C. snyderi* (Markle et al, 2005). Based on data from Markle et al (2005), *C. sp. B* had the ratio of snout length to the head depth higher than 0.79 (N=30) while 99.1% (N=110) of *C. snyderi* had the ratio of snout length to the head depth lower than 0.79. In specimens larger than 200 mm SL, *C. sp B.* had gill raker fewer than 28 while *C. snyderi* had gill rakers greater than 28 (Markle et al 2005)

Catostomus sp. B differs from *Chasmistes brevirostris* and *Deltistes luxatus* in the position of the posterior margin of the lower lip relative to the ventroposterior corner of the maxilla. The posterior margin of the lower lip *Catostomus sp. B* was posteriad of the

ventroposterior corner of the maxilla while the posterior margin of the lower lips of *Chasmistes brevirostris* and *Deltistes luxatus* were even or anteriad (Markle et al 2005)

Catostomus sp. B differed from *C. rimiculus* in the ratio of predorsal length and the number of pectoral fin. *Catostomus sp. B* and *Catostomus rimiculus* were different in the ratio of predorsal length to the standard length in larger specimens. For specimens larger than 290 mm, most (75%; N=20) of *C. sp. B* had the ratio of predorsal length to the standard length greater than 0.499 while 88.2% (N=17) of *C. rimiculus* had the ratio of predorsal length to the standard length lower than 0.500. Sixty point five percent (N=43) of *C. sp. B* had more than 16 pectoral fin rays while 88.5% (N=52) of *C. rimiculus* had fewer than 17 pectoral fin rays.

Catostomus sp. B had 9 fixed cytochrome b positions (156(G), 219(G), 304(T), 501(A), 531(A), 753(A), 825(G), 840(C) and 996(A)) that differed from *C. rimiculus* (Appendix 2.2). All positions except position 304 were third codon transition. Position 304 was a first position transition.

Description

The morphological description was based on up to 56 specimens (72-134 mm SL and 314-400 mm SL): elongate body, slightly laterally compressed; head is moderately long 20.4-25.1% SL (N=45); snout rounded and moderately long 8.9-12.1% SL (N=45); body depth greatest at the origin of dorsal fin 16.2-23.2 %SL (N=45); shallow caudal peduncle 6.5-9.8%SL (N=45); eye 2.6-4.3%SL (μ =3.39%SL; N=38); interorbital width 8.2-10 %SL; origin of dorsal fin in mid body; dorsal fin with 11-14 (N= 45) fin rays; pelvic fin origin is posterior to dorsal fin origin; pelvic fin with 6-11 (N= 44) fin rays; pectoral fin rounded with 15-20 (N= 43) fin rays; infraorbital pores 11-27 (N=44); mouth

subterminal with large fleshy lip, covered with papillae; 1-2 rows of papillae across the symphysis of the lower lip; 2-5 (N=45) rows of papillae on the upper lip; lower lip extended past the ventroposterior corner of the maxilla with 4-9 (N=45) rows of papillae on the lower lip; lateral gill raker of the first gill arch for specimens less than or equal to 120mm 21-28 (μ= 23.7; N=22); lateral gill raker of the first gill arch for specimens larger than 120mm 19-27 (μ= 24.5; N=23); medial gill raker of the first gill arch 23-37 (N=44); lateral line scales 78-95 (N= 42; excluding three outliers at 70, 76 and 100); scales above the lateral line 14-21 (N=45); scales below the lateral line 10-16 (N=45); post Weberian vertebrae 42-48 (μ =43.45; N=55); peritoneum dusky olive green to dark brown; dark body coloration on the dorsal side; light body coloration on the ventral side; light body coloration on lower half of the on the lateral side (Figure 2.10).

Two sexually dimorphic characters were found in *C. sp. B*. Females tended to have more vertebrae anterior to the dorsal fin (VDO, P=0.037) and vertebrae anterior to the pelvic fin (VPO, P=0.016) than males.

Based on 16 specimens, *C. sp. B* had 7 cytochrome b haplotypes (Appendix 2.2). Two haplotypes, CRR1 and CRR2, were common haplotypes found in both Elk Creek and Rogue River. Haplotype CRR3, CRR4, CRR5, CRR6 and CRR7 were found one specimen. Haplotype CRR3 was found in Elk Creek, while CRR 4, CRR5, CRR6 and CRR7 were found in Rogue River.

Distribution

Catostomus sp. B is found in the Rogue River drainage. Samples were collected from Rough and Ready Creek, Rogue River mainstem, Bear Creek and Elk Creek.

Catostomus rimiculus Gilbert and Snyder, 1898

Synonymy

Catostomus rimiculus Gilbert & Snyder in Gilbert, 1898:3 (Trinity River, California)

Catostomus rimiculus, Gilbert 1998:198; Lee et al. 1980:388; Hohler 1981:1; Bond 1994:26; Gilbert 1998:198; Moyle 2002:197; Nelson et al. 2004:78; Markle et al. 2005:437-489

Material examined

Klamath River mainstem

OS15908 (4), 136-190 mm, 22 June 1993, M. Beuttner; OS15909 (8), 231-411 mm, 5 November 1993, U. S. Bureau of Reclamation *OS17877 (10), 333-408 mm, 22 July 2004, W. Tenniswood

Jenny Creek, tributary of Klamath River

OS12520 (2), 127.6-127.7 mm, 13 September 1990, J. Dambacher; OS12821 (3), 118.10-156 mm, 26 June 1979, D. Hohler; OS12822 (1), 120.95 mm, 1979, D. Hohler; OS12823 (1), 123.70 mm, 2 August 1979, D. Hohler; OS12824 (1), 130.50 mm, 16 August 1979, D. Hohler; OS12825 (1), 120.45 mm, 19 May 1979, D. Hohler; OS12826 (2), 79.35-102.40 mm, 25 October 1980, D. Hohler; OS12827 (5), 146-175 mm, 1979, D. Hohler; OS12828 (1), 112.10 mm, 19 August 1979, D. Hohler; OS12829 (2), 125.50-131.75 mm, 29 August 1979, D. Hohler; OS12831 (2), 125.7-131.7 mm, 28 August 1979, D. Hohler; OS12832 (2), 131.2-160 mm, 24 August 1979, D. Hohler; *OS13737 (3), 125.4-181.48 mm, 17 June 1992, P. Harris; OS17784 (3), 101.16-144.04 mm, 27 August 2001, S. Reid;

Spencer Creek, tributary of Klamath River

OS17785 (1), 184 mm, 24 April 1997, D. Wagman; *OS17879 (1), 180 mm, 1 April 2004, W. Tenniswood; *OS17880 (3), 236-338 mm, 29 April 2005, W. Tenniswood; *OS17881 (1), 150 mm, 18 May 2004, W. Tenniswood; *OS17882 (1), 177 mm, 20 May 2005, W. Tenniswood.

Smith River, California

OS5234 (5), 210-241mm, August 1975, California Department of Fish and Game.

Diagnosis

A southern clade member, *Catostomus rimiculus* differs from *C. occidentalis* in the number of lateral line scales. In this study, most (94.23 %; N=52) of *C. rimiculus* had more than 79 lateral line scales while 97% (N=86) of *C. occidentalis* had lateral lines fewer than 80 scales (*C. occidentalis* data from Kettratad (2001)).

Catostomus rimiculus is different from *C. snyderi* in the ratio of snout length (LAE) to the head depth (HD) and the number of gill rakers. *Catostomus rimiculus* tended to have higher ratio of snout length to head depth than *C. snyderi* (Markle et al, 2005). Based on the data from Markle et al (2005), most (91.66%; N=24) of *C. rimiculus* had the ratio of snout length to the head depth higher than 0.785 while 99.09% (N=110) of *C. snyderi* had the ratio of snout length to the head depth lower than 0.785. In specimens larger than 200 mm SL, *C. sp B.* had gill raker fewer than 28 while *C. snyderi* had gill rakers greater than 28 (Markle et al 2005)

Catostomus sp. B differs from *Chasmistes brevirostris* and *Deltistes luxatus* in the position of the posterior margin of the lower lip relative to the ventroposterior corner of

the maxilla. The posterior margin of the lower lip *Catostomus sp. B* was posteriad of the ventroposterior corner of the maxilla while the posterior margin of the lower lips of *Chasmistes brevirostris* and *Deltistes luxatus* were even or anteriade (Markle et al 2005)

Catostomus rimiculus is different from *C. sp. B* as described above. *Catostomus rimiculus* had 9 base pair positions (156(A), 219(A), 304(C), 501(G), 531(G), 753(G), 825(A), 840(T) and 996(G)) in cytochrome b sequence that were different from *C. sp. B* (Appendix 2.2). All positions except position 304 were transition changed at third codon position. Position 304 was a transition changed at the first position.

Description

The Smith River population was not included in the description. The description was based on up to 58 specimens (79-411mm SL, though only 4 were in the size range 184-291 mm SL): elongate body, slightly laterally compressed; head is moderately long 19.7-24.8% SL (N=54); snout rounded and moderately long 8.8-12.5% SL (N=54); body depth greatest at the origin of dorsal fin 16.2-23.1 %SL (N=54); shallow caudal peduncle 6.2-10%SL (N=54); origin of dorsal fin in mid body; dorsal fin with 11-12 fin rays (N= 53); pelvic fin origin is posterior to dorsal fin origin; pelvic fin with 8-11 fin rays (N=52); pectoral fin rounded with 13-17 fin rays (N=52); eye 3.4-5.0%SL (μ = 3.76%SL; N=33); interorbital width 7.7-10.2 %SL (N=53); infraorbital pores 10-26 (N=52); mouth subterminal with large fleshy lip, covered with papillae; 1-2 rows of papillae across the symphysis of the lower lip (N=52); 2-3 rows of papillae on the upper lip (N=52); lower lip extended past the ventroposterior corner of the maxilla with 4-9 rows of papillae on the lower lip (N= 52); lateral gill raker of the first gill arch for specimens less than 120 mm 21-25 (μ= 22.50; N=8); lateral gill raker of the first gill arch for specimens larger

than or equal to 120 mm 19-28 (μ= 23.19; N=16); medial gill raker of the first gill arch 21-34 (N=52); lateral line scales 78-98 (N=52); scales above the lateral line 13-20 (N=52); scales below the lateral line 8-17 (N=52); post-Weberian vertebrae 41-45 (μ=42.93; N=58); peritoneum dusky olive green to dark brown; dark body coloration on the dorsal side; light body coloration on the ventral side; light body coloration on lower half of the body on the lateral side (Figure 2.10).

Females had more vertebrae anterior to the pelvic fin (VPO, P=0.028), more vertebrae anterior to the anal fin (VAO, P=0.005) and longer belly (LOA, P<0.001) than males. Males had longer paired fins (LP1, P<0.001 and LP2, P<0.001) as a proportion to the SL than females.

Based on 16 cytochrome b sequences, *C. rimiculus* had 9 haplotypes (Appendix 2.2). Haplotype CRK1 was found in Jenny Creek and Klamath River. CRK2 was found in Klamath River and Spencer Creek. Haplotype CRK3, CRK4 and CRK5 were unique to Klamath River. Haplotype CRK6, CRK 7, CRK8 and CRK9 were unique to Spencer Creek. *Catostomus rimiculus* was more closely related to other species of suckers in the Klamath Lake than to *C. sp. B* (Figure 2.4). *Catostomus rimiculus* (*sensu lato*) formed a paraphyletic group (Figure 2.4).

Distribution

Catostomus rimiculus is found in the Klamath drainage. They are mainly found in the Lower Klamath River as well as the Smith River, CA. Only one specimen has been captured in the Upper Klamath Lake (Markle et al 2005). Currently, *C. rimiculus* are also found in the Smith River, CA system.

Comparison between *C. rimiculus* and *Catostomus sp. B*

Morphological data were not as useful as molecular data in distinguishing the two taxa because most of the morphological data overlapped. *Catostomus sp. B* and *C. rimiculus* had similar ranges of morphometric characters (Appendix 2.6). In both sexes, morphometric PCA did not provide separation between *C. sp. B* and *C. rimiculus*. *Catostomus sp. B* was embedded with *C. rimiculus* (Figure 2.11).

Catostomus sp. B and *Catostomus rimiculus* were different in the ratio of predorsal length to the standard length in larger specimens. In specimens larger than 290 mm, *Catostomus sp. B* had a significantly higher ($P<0.001$) mean ratio of predorsal fin length to standard length than *C. rimiculus*. In specimens larger than 290 mm, most (75%; N=20) of *C. sp. B* had the ratio of predorsal length to the standard length greater than 0.499, while 88.2% (N=17) of *C. rimiculus* had the ratio of predorsal length to the standard length lower than 0.500 (Figure 2.12).

Three sexually dimorphic characters (LP1, LP2 and LOA) were detected. Of these sexually dimorphic characters, male *C. rimiculus* had a significantly higher ($P=0.045$) mean of pelvic fin length (LP2) as a proportion the standard length than male *C. sp. B*.

Similar to morphometric data, meristic data overlapped. *Catostomus sp. B* had similar meristic characters to *C. rimiculus* (Appendix 2.7). Meristic PCA revealed 2 clusters corresponding to *C. sp. B* and *C. rimiculus* in both sexes (Figure 2.13). There were overlapped between the two clusters. In female meristic PCA, two females of *C. rimiculus* were embedded in *C. sp. B* cluster and two female of *C. sp. B* was embedded in *C. rimiculus* cluster. In male meristic PCA, two males of *C. rimiculus* were embedded in *C. sp. B* cluster and two males of *C. sp. B* was embedded in *C. rimiculus* cluster. PC1

provided partial separation between *C. rimiculus* and *C. sp. B*. Characters that loaded heavily on PC1 in both sexes were precaudal vertebrae, vertebrae anterior to the dorsal fin, vertebrae anterior to the anal fin and vertebrae anterior to the pelvic fin (Figure 2.13). For female, an additional character that loaded heavily on principal component one was lateral gill rakers. PC2 did not provide separation between *C. sp. B* and *C. rimiculus* in both sexes (Figure 2.13). PCA suggested that *C. sp. B* tended to have more precaudal vertebrae, vertebrae anterior to the dorsal fin, vertebrae anterior to the anal fin and vertebrae anterior to the pelvic fin than *C. rimiculus*.

The first two discriminant functions explained 100 % of the total variance found between the two species in both sexes ($P<0.01$ in both sexes). DFA correct classified 90.24% (N=41) in male DFA and 95.35% (N=42) in female DFA. *Catostomus rimiculus* had higher correct classification rate than *C. sp. B*. *Catostomus rimiculus* had 96.15% (N=26) correct classification rate for females and 94.74% (N=19) for males, while C. sp. *B* had 94.12% (N=17) correct classification rate in female and 86.36% (N=22) in males. Characters that contributed to discriminant function in both sexes are vertebrae anterior to the dorsal fin and medial gill rakers (Figure 2.14). Vertebrae anterior to the pelvic fin and pectoral fin rays were additional characters that contribute to the discriminant function were in female DFA (Figure 2.14). Similar to PCA, DFA suggested that *C. sp. B* tended to have more vertebrae anterior to the dorsal fin and medial gill rakers than *C. rimiculus*.

The number of medial gill rakers (GILRKPOST) on the 1st arch had an ontogenetic or size signal (Figure 2.15). The relationship for each species was expressed as gill rakers = A + B/SL. The coefficients, *r2,* and sample sizes *(N)* were *C. rimiculus* A = 33.15, B = -707.19, *r2* = 42.35% *N* = 52 and *C. sp.* A = 35.37, B = -534.06, *r2* =

37.77% *N* = 44. All species approach an asymptote at about 200 mm SL (Figure 2.15). *Catostomus sp. B* had higher means of medial gill rakers on the first gill arch and pectoral fin rays than *C. rimiculus* (both with P<0.001).

Three sexually dimorphic meristic characters (VDO, VPO and VAO) were detected. Based on these three characters, Male *C. sp. B* had significantly higher means of all three characters than male *C. rimiculus*. Female *C. sp. B* had significantly higher (P<0.001) means numbers of vertebrae anterior to the dorsal fin (VDO) and vertebrae anterior to the pelvic fin (VPO) than female *C. rimiculus*. Vertebrae anterior to the dorsal fin (VDO) and vertebrae anterior to the pelvic fin were the only two sexually dimorphic characters that showed differences between *C. sp. B* and *C. rimiculus* in both sexes. Most (75%; N=24) of female *C. sp. B* had vertebrae anterior to the dorsal fin more than 14, while 93.3% (N=30) of female *C. rimiculus* had vertebrae anterior to the dorsal fin fewer than 15. Most (93.1%; N=24) of male *C. sp. B* had vertebrae anterior to the dorsal fin more than 13, while 35% of male *C. rimiculus* had vertebrae anterior to the dorsal fin fewer than 14. Most (79.1%; N=24) of female *C. sp. B* had vertebrae anterior to the pelvic fin more than 19 while 73.33% (N=30) of female *C. rimiculus* had vertebrae anterior to the pelvic fin fewer than 20. Most (93.1%; N=29) of male *C. sp. B* had vertebrae anterior to the pelvic fin more than18 while 55% (N=20) of male *C. rimiculus* had vertebrae anterior to the pelvic fin fewer than 19.

Jenny Creek *C. rimiculus* had significantly lower mean numbers of caudal vertebrae and medial gill rakers on the first gill arch than either *C. sp. B* and other Klamath *C. rimiculus* (both with P<0.001). Jenny Creek *C. rimiculus* had a significantly lower mean number of lateral line scales than other Klamath *C. rimiculus* (P<0.001).

Jenny Creek *C. rimiculus* also had significantly lower means of pectoral fin rays (P<0.001), vertebrae anterior to anal fin (P<0.001), vertebrae anterior to the pelvic fin (P<0.001), and scales above the lateral line (P=0.010) than *C. sp. B*.

Remarks on *Catostomus rimiculus* group

Two taxa (*C. sp B* and *C. rimiculus*) were recognized in *Catostomus rimiculus* (*sensu lato*). Past studies suggested morphological differences (Snyder, 1908, Hohler, 1981 and Markle et al, 2005) as well as genetic differences (Tranah, 2001) between the *C. sp B* and *C. rimiculus*. Snyder (1908) found that the dorsal fin insertion and ventral fin insertion of *C. sp. B* tended to be more posterior than the one found in *C. rimiculus*. Markle et al (2005) found that *C. sp. B* had fewer caudal vertebrae, more vertebrae anterior to the dorsal fin and longer length of the snout to the origin of the dorsal fin than *C. rimiculus*. Hohler (1981) reported that Rogue *C. rimiculus* tended to have more pectoral fin rays than Klamath *C. rimiculus*. My results were similar to the past studies but there was great overlap in morphological data. I found that *C. sp B* tended to have higher counts of medial gill rakers, pectoral fin rays, vertebrae anterior to the origin of the dorsal fin and vertebrae anterior to the origin of the pelvic fin than *C. rimiculus*. I found stringer differences in cytochrome b.

Medial gill rakers on the first gill arch were affected by size (Figure 2.15). Despite the ontogenic signal, *C. sp. B* tended to have more medial gill rakers on the first gill arch than *C. rimiculus*.

Pectoral fin ray counts were one of the useful characters in distinguishing *C. sp. B* from *C. rimiculus*. *Catostomus sp. B* had a significantly higher mean number of pectoral fin rays than *C. rimiculus* (P<0.001). Sixty point five percent (N=43) of *C. sp. B* had

more than 16 pectoral fin rays while 88.5% (N=52) of *C. rimiculus* had fewer than 17 fin rays. Smaller individuals (less than 230 mm) tended to have higher counts of pectoral fin ray than larger individual (Figure 2.16). This was likely caused by sampling artifact.

Vertebrae count was suggested by PCA and DFA as an important character for identifying *C. sp. B* from *C. rimiculus*. The range of vertebrae count of *C. sp. B* and *C. rimiculus* overlapped. Vertebrae anterior to the origin of the dorsal fin and vertebrae anterior to the origin of the pelvic fin are sexually dimorphic characters. *Catostomus sp. B* had higher counts of both characters than *C. rimiculus* in both sexes ($P<0.001$). Seventy-five percent (N=24) of female *C. sp. B* had more than 14 predorsal vertebrae while 93.3% (N=30) of female *C. rimiculus* had fewer than 15 predorsal vertebrae. Ninety-three point one (N=29) of male *C. sp. B* had more than 13 predorsal vertebrae while 35% (N=20) of male *C. rimiculus* had fewer than14 predorsal vertebrae. Seventy-nine point one percent (N=24) of female *C. sp. B* had more than 19 prepelvic fin vertebrae while 73.33% (N=30) of female *C. rimiculus* had fewer than 20 prepelvic fin vertebrae. Ninety-three point one percent (N=29) of male *C. rimiculus* had more than18 prepelvic fin vertebrae while 55% (N=20) of male *C. rimiculus* had fewer than 19 prepelvic fin vertebrae.

The differences in vertebrae counts did not reflect strong differences in the length from the tip of the snout to the origin of the dorsal fin as in previous works by Snyder (1908) (*C. rimiculus*; 46.5%-50% and *C. sp. B*; 50-52.5%) and Markle et al (2005) (*C. rimiculus*; =47.8 and *C. sp. B*; = 49.20). The differences of length from the tip of the snout to the origin of the dorsal fin between *C. rimiculus* and *C. sp. B* in this study (*C. rimiculus*; 45.69-53.42%; =49.84% and *C. sp. B*; 48.24-54.12%; =50.37%) was very

subtle and only shown in specimens larger than 290 mm.

Tranah (2001) used PCA of 204 AFLP bands and PCA of Nei's genetic distance of 15 microsatellite loci to study genetic variability of four species of suckers in the Klamath and Rogue drainages. He found that the Klamath *C. rimiculus* was clearly separated from Rogue *C. rimiculus* (*C. sp. B*) in the principal component score plots. Furthermore, his data suggested hybridization among four species of suckers in the upper Klamath Basin. My phylogenetic analysis of cytochrome b showed that *C sp. B* formed a monophyletic sister group to the Klamath suckers. *Catostomus rimiculus* was more closely related to *Ch. brevirostris*, *C. snyderi* and *D. luxatus* than to *C. sp. B*. Not only does this suggested that *C. sp. B* was a different taxon from *C. rimiculus* but also supported the suggestion from previous studies about the hybridization of the four species of suckers in the Klamath system (Tranah, 2003 and Wagman, 2005). Sequence divergence between sister species in fishes ranges from 1%- 25% (Johns and Avise, 1998). Based on the study from Johns and Avise (1998), sequence divergence of sixty three percent of sister taxon pairs ranges from 1% -5%. Percent sequence divergence within *C. rimiculus* (=0.407%, 0.097 - 0.803%) and *C. sp. B* (= 0.195%, 0.097- 0.297%) was low, while divergence between them was 1.25% to 1.74%. Both morphological and molecular data strongly suggested that *C. sp B* is a separate taxon from *C. rimiculus*.

There were two taxa (Jenny Creek suckers and Smith River suckers) that had uncertain placement in this study. Jenny Creek sucker is a dwarfed population of *C. rimiculus* (Hohler, 1981). According to Hohler (1981), Jenny Creek *C. rimiculus* differedfrom Klamath River *C. rimiculus* in number of caudal vertebrae and from Rogue *C. rimiculus* in number of scales above the lateral line and the number of vertebrae. I

found Jenny Creek *C. rimiculus* tended to have fewer caudal vertebrae, medial gill rakers on the first gill arch, and lateral line scales than other Klamath *C. rimiculus*. Jenny Creek *C. rimiculus* also tended to have fewer caudal vertebrae, medial gill rakers on the first gill arch, vertebrae anterior to anal fin, vertebrae anterior to the pelvic fin, scales above the lateral line, and pectoral fin rays than *C. sp. B*. The number of medial gill rakers does not reach an asymptote until about 200 mm SL (Figure 2.17), which is around the maximum size of Jenny Creek suckers. Thus, the lower count of medial gill rakers in Jenny Creek is a function of their size and the dwarfism of this population. The cytochrome b haplotype CRK1 found in Jenny Creek was also found in the Klamath River. Jenny Creek *C. rimiculus* grouped with other Klamath *C. rimiculus* in the relationship of suckers based on the cytochrome b sequence.

The unique morphological features of Jenny Creek *C. rimiculus* probably resulted from isolation of Jenny Creek from the mainstem Klamath River. Jenny Creek is isolated from the mainstem Klamath River by a 10 meter waterfall which was probably caused by lava flow (Hohler 1981). This results in one-way gene transfer from Jenny Creek to the mainstem Klamath River. If the hybridization among four species of suckers in the Klamath basin occurred after the establishment of the water fall, the morphological features of the Jenny Creek *C. rimiculus* could be the putative morphological features of the *C. rimiculus* before hybridization with other species of catostomids occurred in the Klamath River system.

Smith River *C. rimiculus* is particularly interesting due to the fact that Smith River is located between the Klamath River and Rogue River. The Middle Fork of the Smith River is located next to the Illinois River of the Rogue drainage and the South Fork

of the Smith River is located next to the Klamath River. Morphologically, Smith River *C. rimiculus* resembles the both *C. sp*. B and *C. rimiculus*. Smith River specimens grouped with *C. rimiculus* in morphometric PCA. The Smith River *C. rimiculus* had a significantly higher count of caudal vertebrae (P=0.004) than both *C. sp B* and Klamath *C. rimiculus*. More genetic data is needed to establish the status of this taxon. There is no sucker present in rivers located between Smith River and Rogue River.

Ptychocheilus umpquae Snyder, 1908

Partial Synonymy

Ptychocheilus umpquae Snyder, 1908:170-173, Fig. 2c. (Callapooia Creek, Oakland, Oregon)

Ptychocheilus umpquae, Lee et al, 1980:350; Bond, 1994:17; Gilbert, 1998:160; Carney and Page, 1990:178-181; Mayden et al, 1991:819-834; Gold and Li, 1994:60-65.

Material examined

Siuslaw River

OS15463 (6), 119.67-181.22 mm, 7 June 1995, D. Markle; OS16450 (1), 164.14 mm, 3 September 1997, EPA; OS16802 (2), 104.27-115.19 mm, 3 June 1998, M. Terwilliger; OS16804 (1), 170.88 mm, 3 June 1998, M. Terwilliger; OS16805 (1), 183.20 mm, 3 June 1998, M. Terwilliger; OS16814 (2), 178.55-183.02 mm, 29 July 1998, M. Terwilliger; *OS17886 (11), 103-199 mm, 29 April 2004, J. Kettratad; *OS17888 (6), 178.63-213 mm, 29 April 2004, J. Kettratad; *OS17889 (23), 130-210mm, 13 July 2004, J. Kettratad

Woahink Lake

*OS17903 (1), 220 mm, 28 Feb 2004, M. Cunningham; OS 15483 (3), 175-220 mm, 31 March 1995, ODFW.

Umpqua River

OS2472 (1), 147.70 mm, 15 July 1946, R. Morgan, E. Hughes and D. Twohy; OS2473 (3), 133.32-160.68 mm, 15 July 1946, R. Morgan and R. Miller; OS9225 (3), 95.77-147.37 mm, 30 July 1970, C. Bond; OS9118 (1), 187 mm, 17 October 1979, S. Duke and P. Reimers; OS 9977 (2), 113.09-119.76 mm, 30 July 1970, C. Bond; OS10036 (3), 124.14-135.82 mm, 30 July 1970, C. Bond; OS13131 (1), 99.4 mm, 27 April 1991, D. Markle; OS13187 (2), 152.72-172.83 mm, 30 July 1970, C. Bond; OS12469 (1), 188 mm, 24 August 1989, D. Markle and J. Tomellieri; OS16335(2), 113.78-138.96 mm, 21 August 1997, EPA; OS17887 (1), 86.05 mm, 15 June 2004, S. Anderson; OS 17896 (1), 154.09 mm, 25 July 2004, S. Anderson; *OS17897 (1), 134.28 mm, 28 July 2004, S. Anderson; *OS17898 (2), 130.80-132.29 mm, 29 July 2004, S. Anderson; *OS17899 (11), 235-270 mm, 3 April 2004, J. Kettratad; *OS17900 (3), 113-138 mm, 6 May 2004, J. Kettratad.

Diagnosis

Ptychocheilus umpquae differs from *P. lucius* in number of anal fin rays (Carney and Page, 1990 and Mayden et al, 1991). In the study by Mayden et al (1991), *P. umpquae* had 8 anal fin rays while *P. lucius* had 9 anal fin rays. I found similar results to those of Mayden et al (1991) and Carney and Page (1990). In this study, most (95 %; N=80) *Ptychocheilus umpquae* had 8 anal fin rays while 2.5 % of *P. umpquae* had 7 anal fin rays and the other 2.5 % of *P. umpquae* had 9 anal fin rays.

Ptychocheilus umpquae differs from *P. grandis* in the number of dorsal fin rays. *Ptychocheilus umpquae* tended to have more dorsal fin rays than *P. grandis* (*P. umpquae* had 9 dorsal fin rays and *P. grandis* had 8 dorsal fin rays) (Mayden et al, 1991 and Carney and Page, 1990). In this study, 97.5 % (N=80) of *P. umpquae* had 9 dorsal fin rays while 2.5 % had 10 dorsal fin rays. This is consistent with the study by Mayden et al (1991) and Carney and Page (1990).

Regardless of the overlap, *Ptychocheilus umpquae* tended to have more transverse scales (*P. umpquae*, 26-37; *P. oregonensis*, 22-29), scales above the lateral line (*P. umpquae*, 16-22; *P. oregonensis*, 14-18) and scales around the caudal peduncle (*P. umpquae*, 31-44; *P. oregonensis*, 27-34) than *P. umpquae*. Most (86.3%; N=73) of *P. umpquae* had transverse scales more than 28 scales, while 96.8 % (N=31) of *P. oregonensis* had transverse scales fewer than 29 scales. Most (93.1%; N=73) of *P. umpquae* had scales above the lateral line more than 17 scales, while 96.8 % (N=31) of *P. oregonensis* had scales above the lateral line fewer than 18 scales. Most (97.2%; N=73) of *P. umpquae* had scales around caudal peduncle more than 33 scales while 96.8 % (N=31) of *P. oregonensis* had scales around caudal peduncle fewer than 34 scales. Mayden et al (1990) reported a narrower range of scales around caudal peduncle (32-33 scales) in *P. oregonensis*. This is probably due to a larger sample size in this study (this study N=73 and Mayden et al (1990) N=30).

In specimens larger than 160 mm, *P. umpquae* tended to have higher ratio of caudal peduncle depth to body depth at the origin of dorsal fin than *P. oregonensis*. In specimens larger than 160 mm, most (87.8%; N=41) of *P. umpquae* the ratio higher than

0.429 while *P. oregonensis* (N=21) had the ratio of caudal peduncle depth to body depth at the origin of dorsal fin lower than 0.430.

Ptychocheilus umpquae had 15 fixed cytochrome b positions (108(C), 198 (G), 204 (T), 300 (A), 315(G), 324 (C), 364 (C), 477 (T), 699 (G), 774 (A), 837 (G), 954 (C), 990 (A), 1026 (T), and 1047 (T)) that differed from *P. oregonensis* (Appendix 2.8). All positions except positions 300, 364, 774 and 837 were third position transitions. Positions 300, 774 and 837 were third position transversions. Position 364 was a first position transition.

Description

The morphological description was based on up to 95 specimens. *Ptychocheilus umpquae* is morphologically similar to *P. oregonensis*. Body elongate, slightly laterally compressed; head large 24.8-31.0 % SL (N=73); snout 7.6-10.3 %SL (N= 72); body depth 14.6-22.6 % SL (N=80); eyes 4.1-71 %SL (μ=5.30 %SL; N=72); snout 7.6-10.3%SL; mouth terminal large extending back to below the anterior edge of the pupil; dorsal fin with 9-10 rays located mid body and slightly posterior to origin of pelvic fin (N=80); pelvic fin longer in male ($P<0.05$) with 8-10 rays (N=73); forked caudal fin; pectoral larger and longer in male ($P<0.05$) with 11-18 rays (N=73); post Weberian vertebrae 41-43; lateral line scales 69-85 (N=73); scales above the lateral line 16-22 (N=73); transverse scales 26-37 (N=73); scales around caudal peduncle 31-49 (N=73); peritoneum speckled; olive green brown color on dorsum; light yellow on the lateral side; white on the ventral; dark stripe along the mid lateral of the body with orange pectoral and pelvic fin during the spawning season. Nuptial tubercles found on the head, dorsum, caudal region, pectoral fin, pelvic fin and caudal fin of fish in spawning season.

Females had longer snout length (LAE, P<0.001), wider interorbital (IW, P<0.001 and longer head (HL, P= 0.014) as proportion to the standard length than males. They also had more vertebrae anterior to the pelvic fin (VPO, P=0.048). Males had longer caudal region (LDOC, P=0.011 and LDIC, P=0.002) and longer paired fins (LP1, P<0.001 and LP2, P<0.001) as proportion of SL than females.

There were 10 cytochrome b haplotypes (S1-S5 and U1-U5) from 37 specimens. Three haplotypes (S1-S3) were found in Siuslaw River. Five haplotypes (U1-U5) and 2 common haplotypes (S4 and S5) were found in Umpqua River (Appendix 2.8). Haplotype S4 was found in all system (Siuslaw River, Umpqua River and Woahink Lake. Haplotype S5 was found in Siuslaw River and Umpqua River. *P umpquae* formed monophyletic sister group to *P. oregonensis* (Figure 2.18).

Distribution

Ptychocheilus umpquae are found in Siuslaw River, Umpqua River, Woahink Lake, Tahkenitch Lake and Tsiltcoos Lake (Lee et al, 1980 and Bond, 1993). They were introduced into Rogue River (Bond, 1993).

Ptychocheilus oregonensis Richardson, 1836

Partial Synonymy

Cyprinus (Leuciscus) oregonensis Richardson, 1836:305-306 (Columbia River)

Cyprinus (Leuciscus) oregonensis, Gilbert 1998:126

Ptychocheilus gracilis Agassiz and Pickering in Agassiz 1855:229 (Willamette Falls, Oregon)

Ptychocheilus gracilis, Gilbert 1998:87.

Ptychocheilus rapax Girard 1856:209 (Lower Columbia River)

Ptychocheilus rapax, Gilbert 1998:139.

Ptychocheilus oregonensis, Snyder 1908:170; Lindsey 1956:768; Thompson 1958:42-58; Hill 1962:27-44; Ueyno and Miller 1965:34; Patten and Rodman 1969:108-111; Reid 1971:1-61; Olney 1975:1-73; Smith 1975:35; Brown and Moyle 1981:104-111; Buchanan et al. 1981:360-364; Faler et al 1988:30-35; McAllister 1990:64; Carney and Page, 1990:178-181; Mayden et al. 1991: 819-834; Bond 1994:17; La Rivers 1994:376; Gold and Li 1994:60-65; Beauchamp et al. 1995:193-207; Ward et al. 1995:321-334; Gilbert 1998:126; Scott & Crossman 1998:487; Barfoot et al. 1999:107; Ward and Zimmerman 1999:1020-1035; Fuller et al. 1999:141; Gadomski et al. 2001:250-260; Naughton and Bennett 2003:19-24; Wydoski & Whitney 2003:132-135; Nelson et al. 2004:77; McPhail 2007:120-125.

Material examined

Willamette River

OS9873 (1), 143.02mm, 21 September 1982, Hughes and Giattina; OS15738 (1), 161.03 mm, 22 July 1996, C. Hill; OS10598 (1), 136.46 mm, 24 August 1981, J. Long; OS13266 (1), 196.28 mm, 27 April 1991, R. Spangler; OS15446 (3), 166.98-196.65 mm, 8 May 1973, B. Halstead; OS16593 (1), 159.15 mm, 27 August 1997, B. Gerth; OS 16605 (1), 145.44 mm, 6 August 1997, B. Gerth; OS16658 (1), 130.50 mm, 25 June 1997, B. Gerth; OS17037 (1), 131.42mm, 13 August 1998, S. Corbett; *OS17884 (10), 184-325 mm, 11-12 April 2004, J. Kettratad; *OS17785 (3), 267-305mm, 2 April 2004, J. Adams; *OS17902(2), 224-310mm, 15 April 2004, J. Kettratad.

Mohawk River

OS17061 (1), 130.17 mm, 28 July 1998, S. Corbett.

Santiam River

OS16334 (1), 107.22 mm, 4 September 1997, EPA.

Long Tom River

OS13139 (1), 237 mm, 30 April 1991, R. Sprangler; OS13134 (2), 96.36-149.68 mm, 11 July 1991, P. Petry.

Diagnosis

Ptychocheilus oregonensis differs from *P. lucius* in the number of anal fin rays and the number of lateral line scales (LaRiver, 1994; Carney and Page, 1990 and Mayden et al, 1991). Previous studies reported *P. oregonensis* had 8 anal fin rays and fewer than 80 lateral line scales while *P. lucius* had 9 anal fin rays and more than 70 lateral line scales (LaRiver, 1994; Carney and Page, 1990 and Mayden et al, 1991), which was confirmed (7-8 anal fin rays) and (62-79 lateral line scales).

Ptychocheilus oregonensis differs from *P. grandis* in having more dorsal fin rays (*P. oregonensis* had 9 dorsal fin rays and *P. grandis* had 8 dorsal fin rays) (Mayden et al, 1991 and Carney and Page, 1990). In this study, *P. oregonensis* had 9 dorsal fin rays (N=31), which is consistent with the study by Mayden et al (1991) and Carney and Page (1990).

Ptychocheilus oregonensis differs from *P. umpquae* as described above. *Ptychocheilus oregonensis* had 15 fix cytochrome b positions (108(T), 198(A), 204(C), 300(T), 315(A), 324(T), 364(T), 477(C), 699(A), 774(C), 837(C), 954(T), 990(G),

1026(C), and 1047(C)) that differed from *P. umpquae* (Appendix 2.8). All positions except positions 300, 364 and 774 were transition changed at third codon position. Positions 300 and 774 were transversion changed at the third position. Position 364 was a transition changed at first position.

Description

The morphological description was based on up to 31 specimens. Body elongate, slightly laterally compressed; head large 25.9-29.4 % SL; body depth 15.2-19.3 % SL; eyes 4.0-6.1%SL (μ= 4.87%SL, N=30); snout 7.9-9.8%SL; mouth terminal large extending back to below the anterior edge of the pupil; dorsal fin with 9 rays located mid body and slightly posterior to origin of pelvic fin; pelvic fin longer in males with 8-9 rays; forked caudal fin; pectoral fin longer in males with 12-17 rays; post Weberian vertebrae 41-43; lateral line scales 62-79; scales above the lateral line 14-18; transverse scales 22-29; scales around caudal peduncle 27-34; peritoneum speckle; olive green brown color on dorsal; light yellow on the lateral side; white on the ventral; dark stripe along the mid literal of the body with orange pectoral and pelvic fin during the spawning season (Patten and Rodman, 1969). Smaller preserved individual tended to have a black spot on base of caudal fin.

Females had more count of vertebrae anterior to the anal fin (VAO, P=0.015) than males. Males tended to have longer pelvic fin (LP2, P=0.013) as a proportion to the standard length than females.

There were 8 unique cytochrome b haplotypes (W1-W8) from 14 specimens (Appendix 2.8). *Ptychocheilus oregonensis* formed monophyletic sister group to *P. umpquae* (Figure 2.18).

Distribution

Ptychocheilus oregonensis ranges as far north as Nass River in British Columbia and as far south as Snake River drainage, Idaho. They are found as far east as the Peace River system in British Columbia. The Columbia system is the largest drainage that they occupy in the Pacific Northwest (Lee et al, 1980; Scott and Crossman, 1998 and Wydoski & Whitney 2003).

Comparison between *Ptychocheilus oregonensis* and *Ptychocheilus umpquae*

Morphological data were not as useful as molecular data in distinguishing *P. umpquae* from *P. oregonensis*. Most morphological data overlap. Morphometric characters of *Ptychocheilus umpquae* were very similar to *P. oregonensis* (Appendix 2.9). Morphometric PCA did not separate *P. oregonensis* from *P. umpquae* in both sexes (Figure 2.19).

Regardless of the overlap, body depth (both DP1 and DDO) and caudal peduncle depth (CPD) were useful in separating the two species apart. *Ptychocheilus umpquae* tended had a significantly higher mean of caudal peduncle depth as a proportion to standard length than *P. oregonensis* ($P<0.001$). *Ptychocheilus oregonensis* had significantly higher means ratio of body depth at the origin of the dorsal fin as a proportion to standard length ($P<0.001$) and body depth at the origin of pectoral fin as a proportion to standard length ($P<0.001$) than *P. umpquae*. The plot between the ratio of caudal peduncle depth to the body depth at the origin of the dorsal fin and the standard length partially separated *P. umpquae* from *P. oregonensis* in the specimens that were larger than 160 mm (Figure 2.20). *Ptychocheilus umpquae* tended to have higher ratio of caudal peduncle depth to the body depth at the origin of the dorsal fin than *P.*

oregonensis. The ratio of caudal peduncle depth to the body depth at the origin of the dorsal fin did not increase as the standard length increased (P=0.493) (Figure 2.20).

One character (interorbital width, IW) showed some difference between Siuslaw *P. umpquae* and Umpqua *P. umpquae*. The plot between the interorbital width and head length partially separated Umpqua *P. umpquae* from Siuslaw *P. umpquae* (Figure 2.21). Umpqua *P. umpquae* tended to have wider interorbital width as proportion to head length than Siuslaw *P. umpquae*. The ratio of interorbital width to head length tended to increase as standard length increased (Figure 2.22).

Seven sexually dimorphic morphometric characters (LDOC, LDIC, LP1, LP2, LAE, IW and HL) were detected. Male *P. umpquae* tended to have longer pectoral fin length (LP1, P<0.001) as a proportion to standard length than male *P. oregonensis*.

Ptychocheilus umpquae had similar meristic range as *P. oregonensis* (Appendix 2.10). Regardless of some overlap, meristic characters were more useful than morphometric characters in distinguishing *P. umpquae* from *P. oregonensis*. In both sexes, meristic PCA revealed two distinct clusters corresponding to *P. umpquae* and *P. oregonensis*. Siuslaw *P. umpquae* embedded with Umpqua *P. umpquae*. Principal component 1 provided separation between *P. umpquae* and *P. oregonensis*. Characters that loaded heavily on PC1 in both sexes were preoperculomandibular pores, transverse scales, scales above the lateral line, scales below the lateral line and scales around caudal peduncle (Figure 2.23). In males, infraorbital pores was an additional character that contributed to PC1. Based on principal component score plot and the loading in both sexes, *P. umpquae* tended to have lower counts of preoperculomandibular pore and higher counts of transverse scales, scales above the lateral line, scales below the lateral

line and scales around caudal peduncle than *P. oregonensis*. Discriminant function explained 100% of the total variance (P<0.001 in both sexes). DFA correctly classified all specimens in female DFA and correctly classified 97.67% (N=43) in male DFA. In male DFA, 3.23% (N=31) of *P. umpquae* was misclassified as *P. oregonensis*. Characters that had high coefficient in both sexes were scales above the lateral line and scales around caudal peduncle. Similar to PCA, DFA suggested that *P. umpquae* tended to higher counts of scales above the lateral line and scales around caudal peduncle than *P. oregonensis* in both sexes (Figure 2.24).

Two sexually dimorphic meristic characters (VAO and VPO) were detected. Out of these two characters, only one character (VAO) showed differences between species. Female *P. oregonensis* had a significantly higher mean number of vertebrae anterior to the anal fin than female *P. umpquae* (P=0.007).

Ptychocheilus umpquae formed monophyletic sister group to *P. oregonensis* in cytochrome b phylogenetic analysis. Umpqua *P. umpquae* embedded with Siuslaw *P. umpquae* in the cytochrome b phylogenetic analysis (Figure 2.18).

Remarks on *Ptychocheilus*

Ptychocheilus umpquae was first described by Snyder in 1908. Carl Bond questioned whether these differences were greater than differences between two populations of *P. oregonensis* but Carney and Page (1990), Mayden et al. (1991) and this study confirm that *P. umpquae* is a separate species from *P. oregonensis*. In the phylogenetic analysis, *Ptychocheilus umpquae* formed its own monophyletic group sister to *P. oregonensis*. Morphological data distinguishing *P. umpquae* from *P. oregonensis* were body depth (DP1 and DDO), caudal peduncle depth (CPD), preoperculomandibular

pores, scales above the lateral line, transverse scales and scales around caudal peduncle.

Five diagnostic characters (predorsal scales, lateral line scales, scales above the lateral line, scales around caudal peduncle, scales from origin of pelvic fin to the lateral line and cephalic pores) for *P. oregonensis* and *P. umpquae* have been suggested in past studies (Snyder, 1908; Carney and Page (1990), Mayden et al (1991) and Bond (1994)). Predorsal scales was suggested by Snyder (1908) and Carney and Page (1990) as a useful diagnostic character. Mayden et al (1991) reported that consistent and accurate predorsal scales count was difficult to obtain. Like Mayden et al (1991), I found predorsal scales to be extremely difficult to apply and difficult to get a consistent count.

The number of lateral line scales was suggested by Snyder (1908) as useful for distinguishing *P. oregonensis* (67-75) from *P. umpquae* (73-85). Despite overlap (*P. umpquae* 69-85; μ=74.19 and *P. oregonensis* 62-79; μ=71.67), I found similar result where *P. umpquae* tended to have higher counts of lateral line scales than *P. oregonensis* ($P<0.001$). However, the difference in lateral line scales between *P. umpquae* and *P. oregonensis* was not as strong as the difference in scales above the lateral line because there was more overlap in Lateral line scales count (Appendix 2.10).

The number of scales above the lateral line was a diagnostic character reported by Snyder (1908) and Carney and Page (1990) with 12 to 20 for *P. oregonensis* and 16 to 24 for *P. umpquae*. In this study, the range of the scales above the lateral line was similar to the past studies, 14 to 18 for *P. oregonensis* and 16 to 22 for *P. umpquae*. Most (93.1%; N=73) *P. umpquae* had scales above the lateral line more than 17 scales, while 96.8 % (N=31) *P. oregonensis* had scales above the lateral line fewer than 18 scales.

Scales around caudal peduncle was used by Mayden et al (1991) as an important

distinguishing character between *P. umpquae* and *P. oregonensis*. Mayden et al (1991) reported that *P. oregonensis* had 27-30 scales around caudal peduncle and *P. umpquae* had 32-36 scales around caudal peduncle. The result of this study was similar to that of Mayden et al (1991), with *P. umpquae* having 31-44 scales around caudal peduncle and *P. oregonensis* having 27-34 scales around caudal peduncle. Most (97.2%; N=73) of *P. umpquae* had scales around caudal peduncle more than 33 scales, while 96.8 % (N=31) of *P. oregonensis* had scales around caudal peduncle fewer than 34 scales. The range of scales around caudal peduncle in this study was based on 73 specimens from 16 sites while the study by Mayden (1991) was based on 30 specimens from 4 sites.

The number of scales from origin of pelvic fin to the lateral line was proposed by Bond (1994) as a diagnostic character between *P. umpquae* and *P. oregonensis*. Bond (1994) suggested that *P. oregonensis* had fewer than 21 scales from origin of pelvic fin to the lateral line and *P. umpquae* had more than 18 scales from origin of pelvic fin to the lateral line. In this study, transverse scales were the number of scales above the lateral line plus the number of scales from the origin of the pelvic fin to the lateral line. The difference in the number of scales from origin of pelvic fin to the lateral line suggested by Bond (1994) was shown in the number of transverse scales count which strongly suggested the differences between *P. umpquae* and *P. oregonensis*. In this study *P. umpquae* had 26-37 (μ=30.58) transverse scales and *P. oregonensis* had 22-29 (μ=25.93) transverse scales. Most (86.3%; N=73) of *P. umpquae* had transverse scales more than 28 scales, while 96.8 % (N=31) of *P. oregonensis* had transverse scales fewer than 29 scales.

Carney and Page (1991) reported that cephalic pore count was a useful diagnostic character for separating *P. umpquae* from *P. oregonensis*. Their data suggested that *P.*

umpquae tended to have fewer infraorbital, supraorbital and preoperculomandibular pores than *P. oregonensis*. According to their data, the range of cephalic pore counts broadly overlapped: for infraorbital pores (*P. umpquae* had 29-38 (μ=33.0) and *P. oregonensis* had 31-44 (μ=38.3); for supraorbital pores *P. umpquae* had 14-21 (μ=17.9) and *P. oregonensis* had 17-24 (μ=19.4); and for preoperculomandibular pores *P. umpquae* had 20-27 (μ=24.3) and *P. oregonensis* had 21-34 (μ=29.3). Although overlapping, *P. umpquae* had lower mean numbers of infraorbital ($P<0.001$), supraorbital ($P=0.008$) and preoperculomandibular ($P<0.001$) pores than *P. oregonensis*. Despite having significantly different means between the two species, cephalic pores canal is less useful as a diagnostic characters when compared to scales counts. The difference in the range of *P. umpquae* between this study and the study by Carney and Page (1990) was probably due to a larger sample size used in this study (this study N=73 and Carney and Page (1990) N=30). In addition to characters proposed in past studies, I found body depth, caudal peduncle depth and cytochrome b sequence to be very useful diagnostic characters for separating *P. umpquae* from *P. oregonensis*. *Ptychocheilus umpquae* tended to have shallower body depth at dorsal fin origin and deeper caudal peduncle depth than *P. oregonensis*. The differences between *P. umpquae* and *P. oregonensis* in caudal peduncle depths became obvious in specimens larger than 160mm. In specimens larger than 160 mm, *P. umpquae* tended to have higher ratio of caudal peduncle depth to body depth at the origin of dorsal fin than *P. oregonensis*.

Cytochrome b sequence was more useful than morphological data in separating *P. umpquae* from *P. oregonensis*. In cytochrome b phylogenetic analysis, *Ptychocheilus umpquae* formed monophyletic group sister *P. oregonensis*.

Previous studies suggested that *P. umpquae* was an allopatric sister taxon of *P. oregonensis* (Carney and Page, 1990 and Mayden et al., 1991). The results from this study also suggest that *P. umpquae* is a sister taxon of *P. oregonensis*. Two previous studies also indicated that Siuslaw *P. umpquae* was different from Umpqua *P. umpquae* both morphologically (Mayden et al, 1991) and genetically (Gold and Li, 1994). Mayden et al (1991) suggested that Umpqua *P. umpquae* tended to have wider interorbital width than Siuslaw *P. umpquae*. In this study, Umpqua *P. umpquae* had a significantly higher mean ratio of interorbital width to head length than Siuslaw *P. umpquae* (P=0.025). The difference in interorbital width as proportion to head length was more pronounce in specimens larger than 160 mm. The ratio of interorbital width to head length was affected by size. It increased as standard length increased. Therefore, the different observed in interorbital width as proportion to head length was probably caused by sampling artifact because there were more Umpqua *P. umpquae* specimens larger than 180 mm than Siuslaw *P. umpquae* (Figure 2.22). The differences observed in Mayden et al (1991) study could have resulted from smaller sample size (N=30) in Mayden et al. study.

Gold and Li (1994) reported that the genome size of *P. umpquae* (Siuslaw) and *P. umpquae* (Umpqua) was more different from each other than the differences between two other randomly drawn cyprinid genomes. My results suggested that Siuslaw *P. umpquae* was somewhat different from Siuslaw *P. umpquae* based on the cytochrome b haplotypes. Despite the occurrence of common haplotypes (S4 and S5), *Ptychocheilus umpquae* had five haplotypes (U1-U5) that only occurred in the Umpqua River and three haplotypes that only occurred in the Siuslaw River. However, in my phylogenetic analysis based on cytochrome b sequence, the two populations (Umpqua and Siuslaw) were embedded

within *P. umpquae* clade. There was no separation between the two populations. It is possible that the differences between the two populations is more obvious in the nuclear genome but not in the mitochondrial genome.

Discussion

A biological species is a group of interbreeding natural populations that are reproductively isolated from other such groups (Mayr, 2000). Allopatric sister taxa pose a challenge in classification for the biological species concept because of the lack of sympatric reproductive isolation (Wiley, 1981). Two alternative species concepts can aid this problem: the phylogenetic species concept and the evolutionary species concept. The phylogenetic species concept has many versions (Coyne and Orr, 2004; Wheeler and Platnick, 2000; Mishler and Theriot, 2000; Meier and Willmann, 2000) and most use the idea of the monophyletic group as part of the definition. The phylogenetic species concept may be more practical to use than the biological species concept because it focuses on practical evidence. Unlike the biological species concept, reproductive isolation is considered to be a synapomorphic character which will not affect establishment of a monophyletic group in the phylogenetic species concept (Coyne and Orr, 2004). The evolutionary species concept defines species as "a lineage of ancestral descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate" (Wiley, 1987; Wiley, 1981). Historical fate incorporates origin and extinction of taxa (Wiley, 1981; Wiley and Mayden, 2000).

The evolutionary species concept was the species concepts I applied. My criteria

for establishing taxon status involved morphological discriminant and constitution of monophyly of the taxon from molecular data. I believe that the case for recognizing *Catostomus tsiltcoosensis*, *C. sp. A* and *C. sp. B* is justified under both the evolutionary species concept and the phylogenetic species concept. The evidence is morphological discrimination of individuals and the presence of autapomorphic molecular data in each taxon. *Catostomus tsiltcoosensis* is greatly different from *C. macrocheilus* in body depth, the number of infraorbital pores and the cytochrome b sequence. *Catostomus sp. A* is greatly different from *C. macrocheilus* in the number of infraorbital pores and is greatly different from *C. tsiltcoosensis* in the ratio of body width at the base of pectoral fin to the body depth at the pectoral fin origin. *Catostomus sp.* A has 14 positions that were autapomorphic characters in cytochrome b sequence. *Catostomus sp. B* is different from *C. rimiculus* in number of vertebrae anterior to dorsal fin and cytochrome b sequence. The hybridization opportunity with other species of suckers in the Klamath River for *C. rimiculus* constitutes its evolutionary tendency and historical fate. The lack of opportunity to hybridize with other species of suckers in the Klamath River of *C. sp. B* suggests that it has a different evolutionary tendency and historical fate from *C. rimiculus*.

When *C. tsiltcoosensis* and *C sp. A* are taken into account, the Oregon Coastal Subprovince has at least 5 endemic species of primary freshwater fishes (*Catostomus tsiltcoosensis*, *C. sp. A*, *Oregonichthys kalawatseti, Ptychocheilus umpquae* and *Rhinichthys evermanni*). This brings the level of endemism up to 62.5% from 37.5%. This level of endemism is higher than the level of endemism in the Klamath (57%). This

strongly suggests that Oregon coastal province is one of the important areas of endemism in the Pacific Northwest.

Cryptic Species

Founder effect, population size, intensity of the local selection, isolation time, and the amount of gene exchange determine the level of divergence among different allopatric taxa (McPhail, 2007). The scenario in which the allopatric population will quickly diverge involves: 1) small initial population which carried a fraction of genetic diversity from the origin population got isolated, 2) long isolation time (in the geological sense), 3) genetic drift occurs, and 4) different selecting pressure from what occurred in the origin population (Frankham et al, 2002).

Within the Oregon Coastal Subprovince, Coquille River *C. sp. A* had the greatest divergence (distance 2.63-4.08%). Other suckers in the Oregon Coastal Subprovince had about half the divergence (1.36-2.47%) of the *C. sp. A*. Greater divergence of the Coquille *C. sp. A* did not result in very distinct overall morphological features from *C. tsiltcoosensis* and *C. machrocheilus*. Instead, *C. sp. A*. has very similar morphological features to *C. tsiltcoosensis*. This suggests that very similar selecting pressure has been acting on the catostomids in the Oregon Coastal Subprovince. *Catostomus tsiltcoosensis* from different coastal drainages (Siuslaw, Umpqua and Coos-Millicoma) form their own monophyletic group based on cytochrome b sequence. However, they are morphologically indistinguishable. Like the differences between *C. sp. A* and *C. tsiltcoosensis*, the lack of morphological differences also suggested that there are similar selection pressures on the catostomids in different river systems in the Oregon Coastal Subprovince. A similar scenario also occurred in the Klamath Subprovince. *Catostomus*

rimiculus and *C. sp. B* were strongly diverged molecularly but were morphologically very similar.

In the presence of similar selecting pressures in different systems, genetic drift could be the cause of the differences found in molecular data (Frankham et al., 2002). The differences in cytochrome b sequences among *Catostomus tsiltcoosensis* from the Siuslaw River, the Umpqua River and the Coos-Millicoma River could have resulted from genetic drift. Oregon Coastal Subprovince river systems are considerably smaller systems than the Columbia-Willamette river systems. It is possible that the initial population that got isolated in the Oregon Coastal Subprovince were substantially smaller than the population in the Willamette-Columbia system. This helped genetic drift to occur faster in the Oregon Coastal Subprovince than in the Willamette-Columbia system. This potentially resulted in fixed differences in cytochrome b sequences. A cryptic species complex is defined as a group of species that are reproductively isolated from each other but are morphologically indistinguishable (Paris et al., 1989). *Catostomus tsiltcoosensis* could consist of at least three cryptic species. They deserved to be recognized as evolutionary significant units. More studies are needed in order to establish the status of these taxa.

Phenotypic plasticity

Primary freshwater fishes in the Oregon Coastal Subprovince tend to share one common trait. They tend to have shallower body depth than their sister taxa in the Columbia system. *Catostomus tsiltcoosensis* and *Ptychocheilus umpquae* both have shallower body depth than their allopatric sister taxa in the Willamette River. *Rhinichthys osculus* in the coastal streams also tend to have narrower body depth than *R. osculus* in

the Willamette system (Zirges, 1973). Morphological characters within a fish species are highly correlated to environmental factors such as temperatures and stream flow (Lindsey, 1952; Taylor and McPhail 1985, Thompson et al. 1997, Taylor, 1999 and Hendry et al. 2006). A narrow body depth and a more streamlined body tend to be a convergent trait responding to swifter current (Taylor and McPhail 1985 and Hendry et al. 2006).

Phenotypic plasticity is a condition in which a given genotype can express different phenotypes in different environmental conditions. Phenotypic plasticity has been viewed as both deterring and promoting diversification. It is viewed as deterring diversification because it damped natural selection (Wright, 1931 and Schlichting, 2004). Phenotypic plasticity can promote diversification in the process called genetic assimilation (Waddington, 1942). Waddington (1942) suggested that if the new environment condition persists, some of the characters that were initially plastic could be later fixed for three different reasons: 1) there is a cost in maintaining plasticity in the new environment, 2) random mutation could disrupt the hidden plastic response, and 3) there may be selection for canalization in such a way that the original environmental conditions would no longer elicit that phenotype. Schlichting (2004) listed three conditions which genetic assimilation could promote diversification: 1) the low probability of having appropriate mutations available to deal with any particular environmental change, 2) the overwhelming likelihood of some form of environmental change, and (3) the pervasiveness of plastic responses (high likelihood of plasticity with low to moderate likelihood that plasticity is in the appropriate direction). Phenotypic plasticity could be the initial cause of the morphological differences prior to the act of natural selection in

the new environment and random mutation that later fixed the plastic character in the irreversible way. However, phenotypic plasticity cannot be ruled out as the cause of the morphological differences found in this study. Future ecological study, common garden experiments and rearing experiment of these fish would be appropriate ways to determine whether phenotypic plasticity of this trait is present in sister taxa of the Columbia Subprovince and the Oregon Coastal Subprovince.

DNA barcoding for Catostomus in Oregon Coastal Subprovince

DNA barcoding is the use of mitochondrial DNA as a species-specific identifier. Distance methods are the methods of choice. There are two main distant methods used, Blast and tree estimation. Blast used a raw similarity score to find nearest neighbor to the query sequence (Hajibabaei et al 2005). The second method involved using distances to create a tree (Hebert et al, 2004 and Ward et al. 2005). The major shortcoming of DNA barcoding is the lack of diagnostic characters, which is found in the character-based method. Another problem with DNA barcoding is that it often does not give the nearest similarity score as the closest relative (DeSalle et al (2005). In fish, Cytochrome c oxidase I is used as the gene of choice. Currently, cytochrome oxidase 1 sequences have not been sampled from fishes in the Oregon coastal province. Cytochrome b sequence was also suggested in a past study as a possible species identifier (Lemer et al., 2007). Cytochrome b sequences have also been used in past studies to detect cryptic species (Colborn et al., 2001, Kreiser, 2001; Lima et al., 2005 and Hyde et al., 2008). In this study, cytochrome b sequences are useful for identifying suckers from different systems. Cytochrome c oxidase I gene and cytochrome b gene are part of the mitochondrial genome. The evolutionary rate of cytochrome c oxidase I within the catostomid was

roughly the same as the evolutionary rate of cytochrome b in the catostomid group. The distances between *C. macrocheilus* and *C. catostomus* in CO1 gene is 0.07 while the distance between *C. macrocheilus* and *C. catostomus* is 0.09. Based on the fact that the evolutionary rate of cytochrome c oxidase I and cytochrome b are roughly the same and the fact that both of them belong to relatively small mitochondrial genome, it is likely that cytochrome c oxidase 1 sequences would be able to identify catostomids in these systems as well as the cytochrome b sequences.

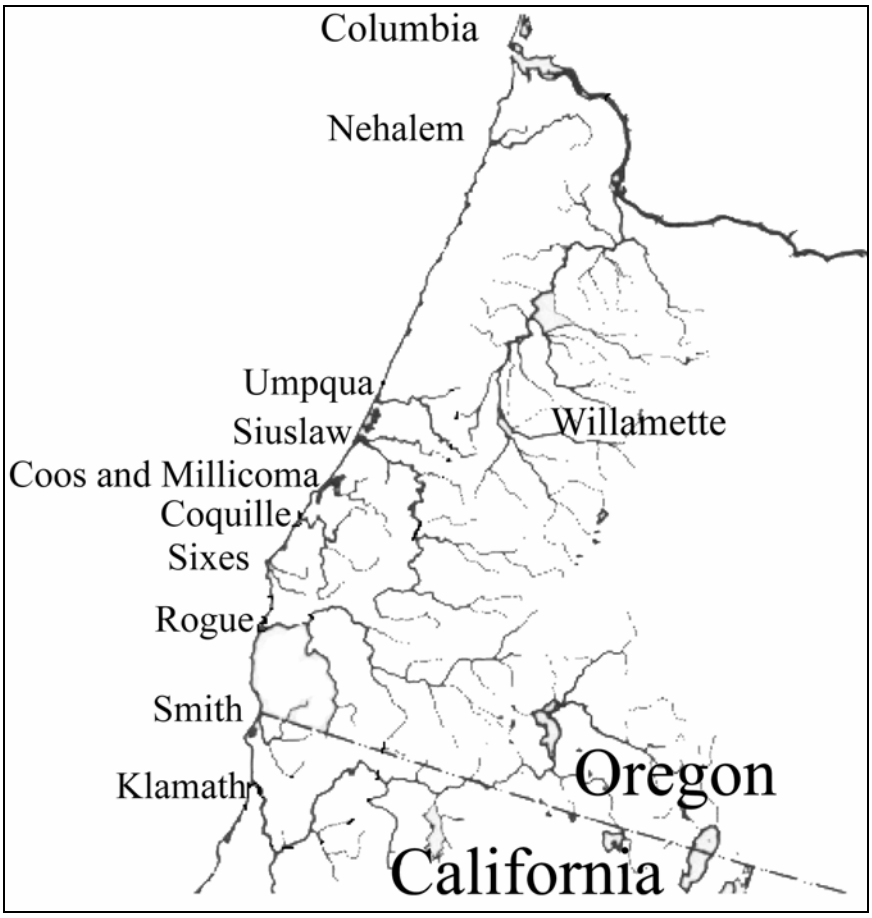

Figure 2.1 Map of the Oregon and Northern California coastal river systems used in this study.

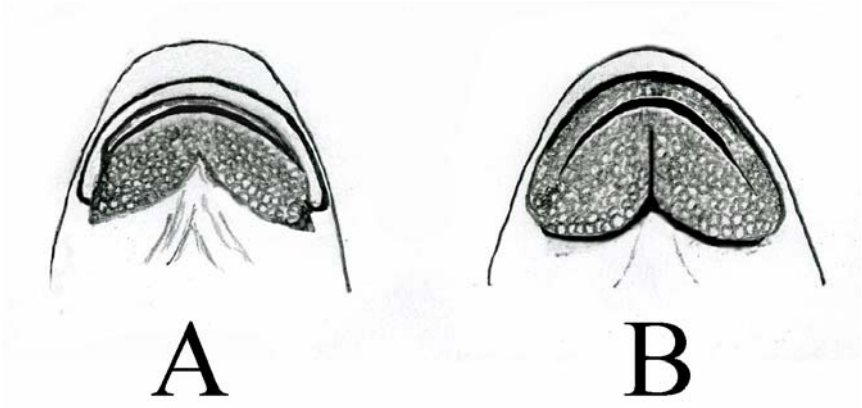

Figure 2.2. Typical ventral view of A) *Catostomus platyrhynchus*'s lip morphology and B) lip morphology found in *C. macrocheilus*, *C. tsiltcoosensis* and *C. sp. A*. Illustration is after Wydoski and Whitney (2003).

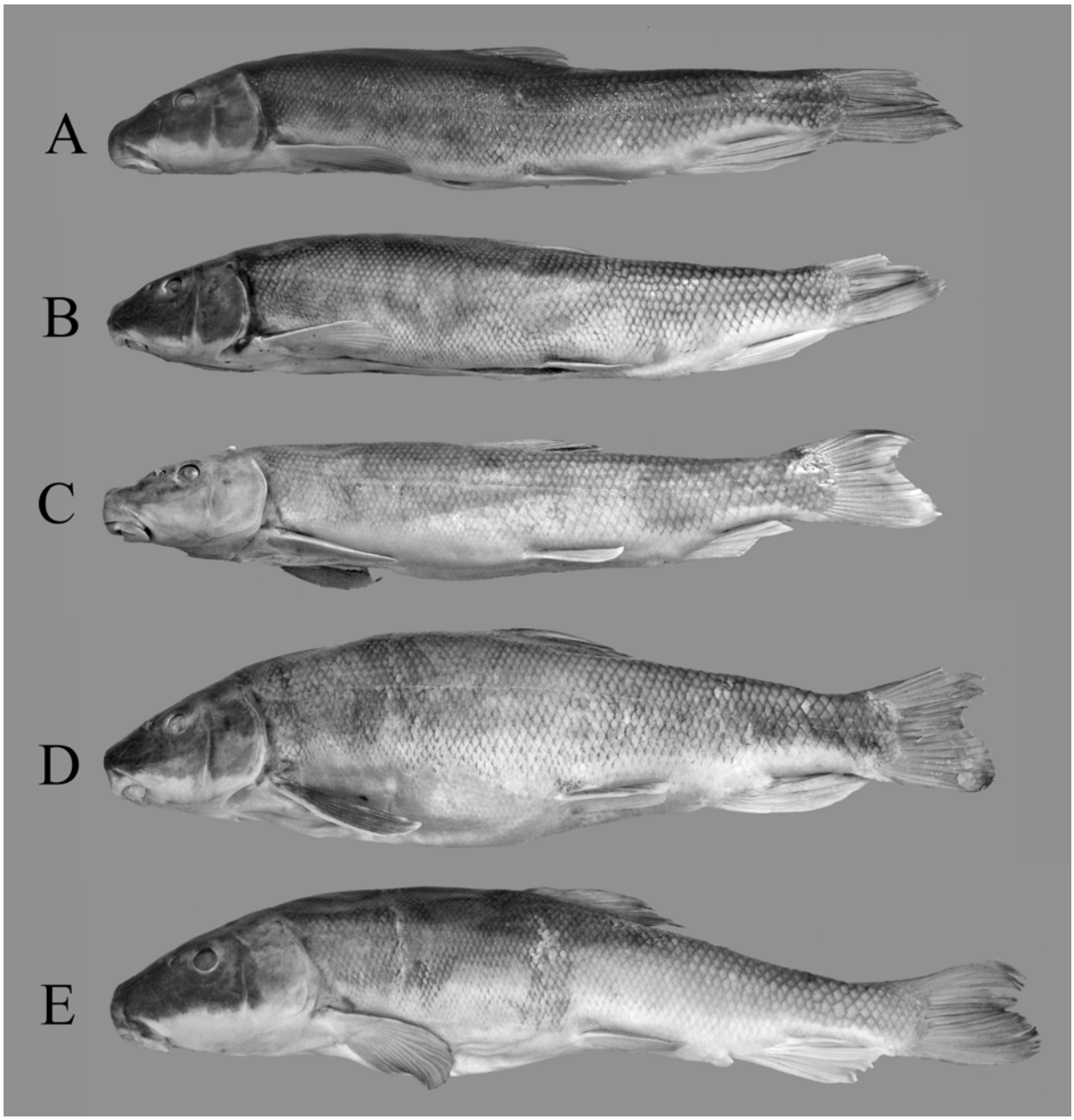

Figure 2.3 Left lateral view of A) *Catostomus tsiltcoosensis* Umpqua River (OS17872 (J), male, 303 mm in SL), B) *C. tsiltcoosensis* Coos River (OS17861 (B), female, 382 mm in SL), C) *C. tsiltcoosensis* Siuslaw River (OS15461 (6), female, 340 mm in SL), D) *C. sp. A* Coquille River (male, OS17866, 352mm in SL) and E) *C. macrocheilus* Columbia-Willamette (female, OS17873 (C), 416 mm in SL).

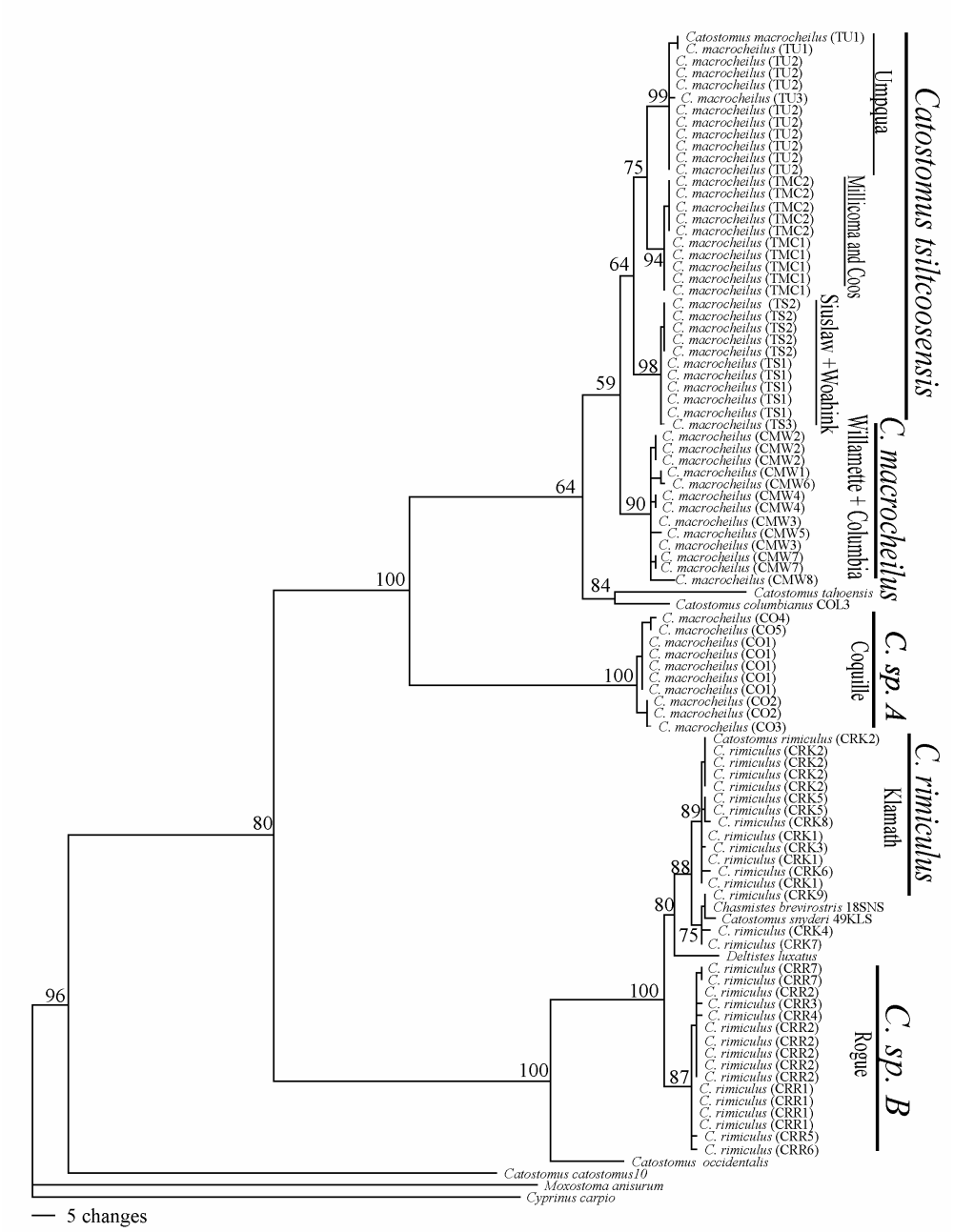

Figure 2.4 Strict consensus tree of suckers in Oregon coastal river system from 18 trees (602 steps long with consistency index (CI) = 0.7027, retention index (RI) = 0.9523 and rescaled consistency index (RC) = 0.6692) base on cytochrome b sequence (1042 base pair with 200 parsimony informative characters) from parsimony algorithm. *Moxostoma anisurum* and *Cyprinus carpio* are outgroups. The tree is 605 steps long with CI =0.6992, RI= 0.9515 and RC= 0.6653. Branch length represented changes occur on each branch. Haplotype of each sample for *C. macrocheilus, C. tsiltcoosensis, C. sp. A, C. sp. B and C. rimiculus* is given in the parenthesis. The number represents the bootstrap value at each node. Node without numbers had bootstrap values less than 50%. Scale indicates 5 bp changed.

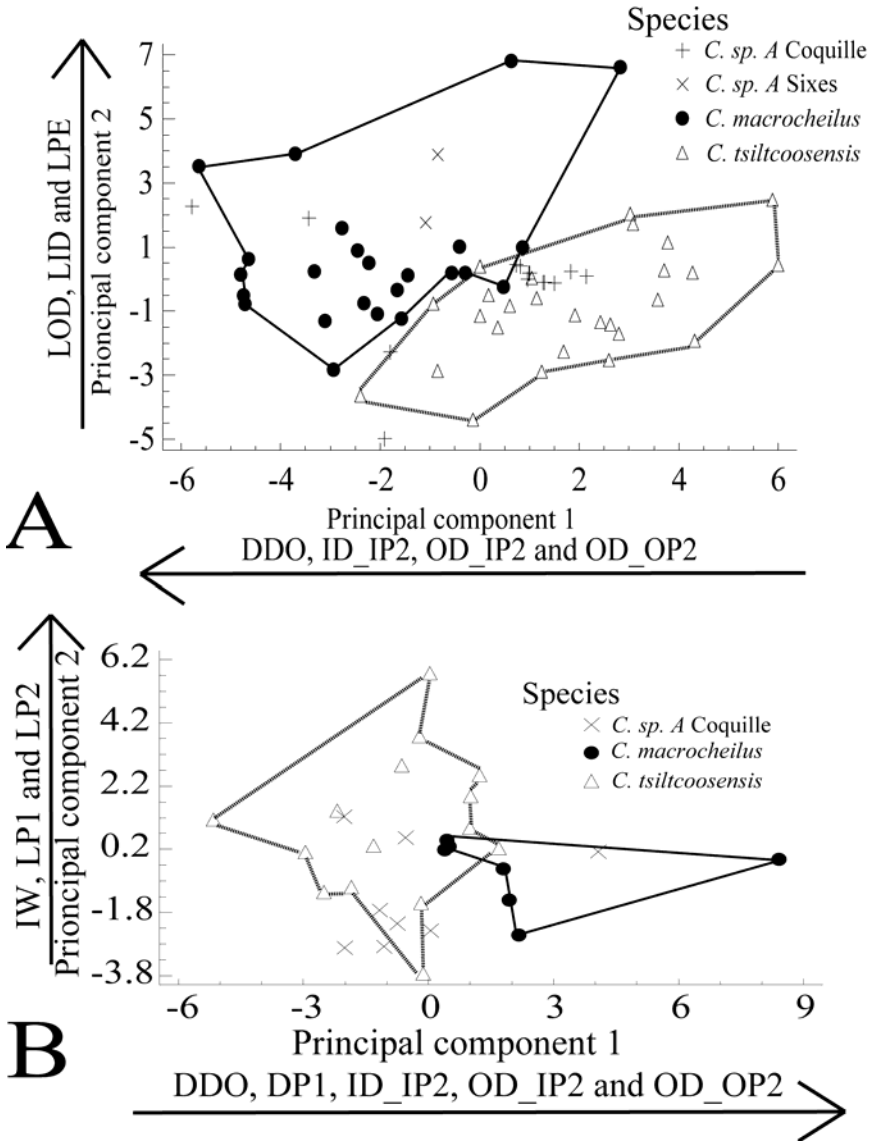

Figure 2.5 Principal component score plot of 28 morphometric characters (LAE, LPE, IW, WP1, DP1, DDO, LOP2, LOP1_LOP2, LOD, LID, LOA, LIA, LDA, LDOC, LDIC, CPD, LP1, LP2, LPEOD, SPMLL, LDMM, AIOPAE, AIOPAE, SAIOP, ED, CRD, IDOP2, ODIP2 and ODO2) for A) female suckers from *C. macrocheilus* (*sensu lato*) complex (*C. macrocheilus* (24), *C. tsiltcoosensis* (27) and Coquille River (15) and Sixes River Suckers (2)) and B) male suckers from *C. macrocheilus* (*sensu lato*) complex (*C. macrocheilus* (7), *C. tsiltcoosensis* (15) and Coquille River (8)). Axis notations identify characters with high absolute loading higher than 0.3. Arrow points in the direction of increase value of each character. Principal component 1 explains 26.35% of the total variance for female and 15.42% for male. Principal component 2 explains 15.44% of the total variance both for female and male.

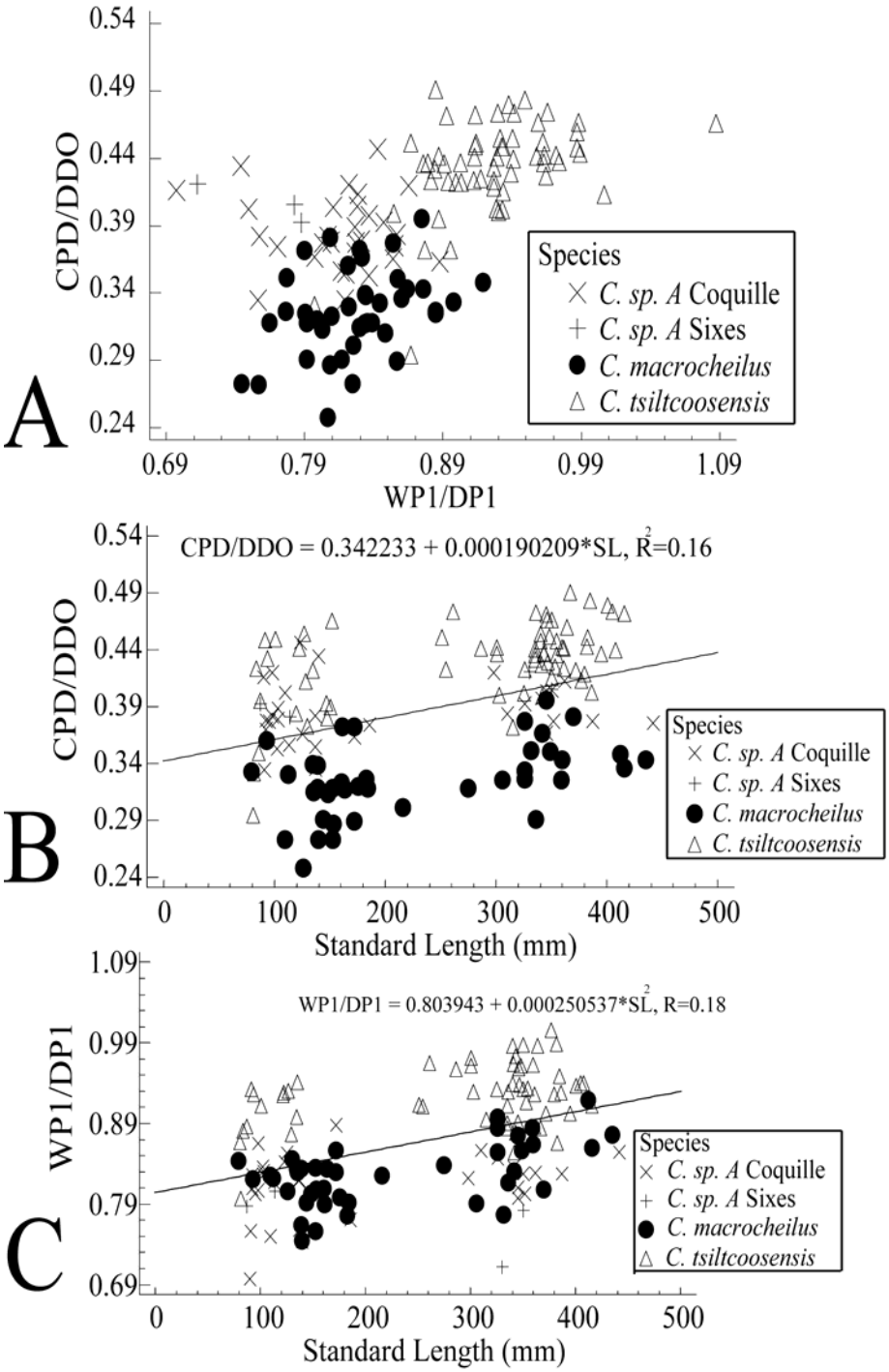

Figure 2.6 A) The ratio of caudal peduncle depth to body depth at the origin of dorsal fin (CPD/DDO) at different ratio of body width at the base of pectoral fin to body depth at the base of pectoral fin (WP1/DP1) in *Catostomus macrocheilus* (*sensu lato*) complex B) Relationship of CPD/DDO at different SL for *C. macrocheilus* (*sensu lato*) complex and C) Relationship of WP1/DP1 at different SL for *C. macrocheilus* (*sensu lato*) complex

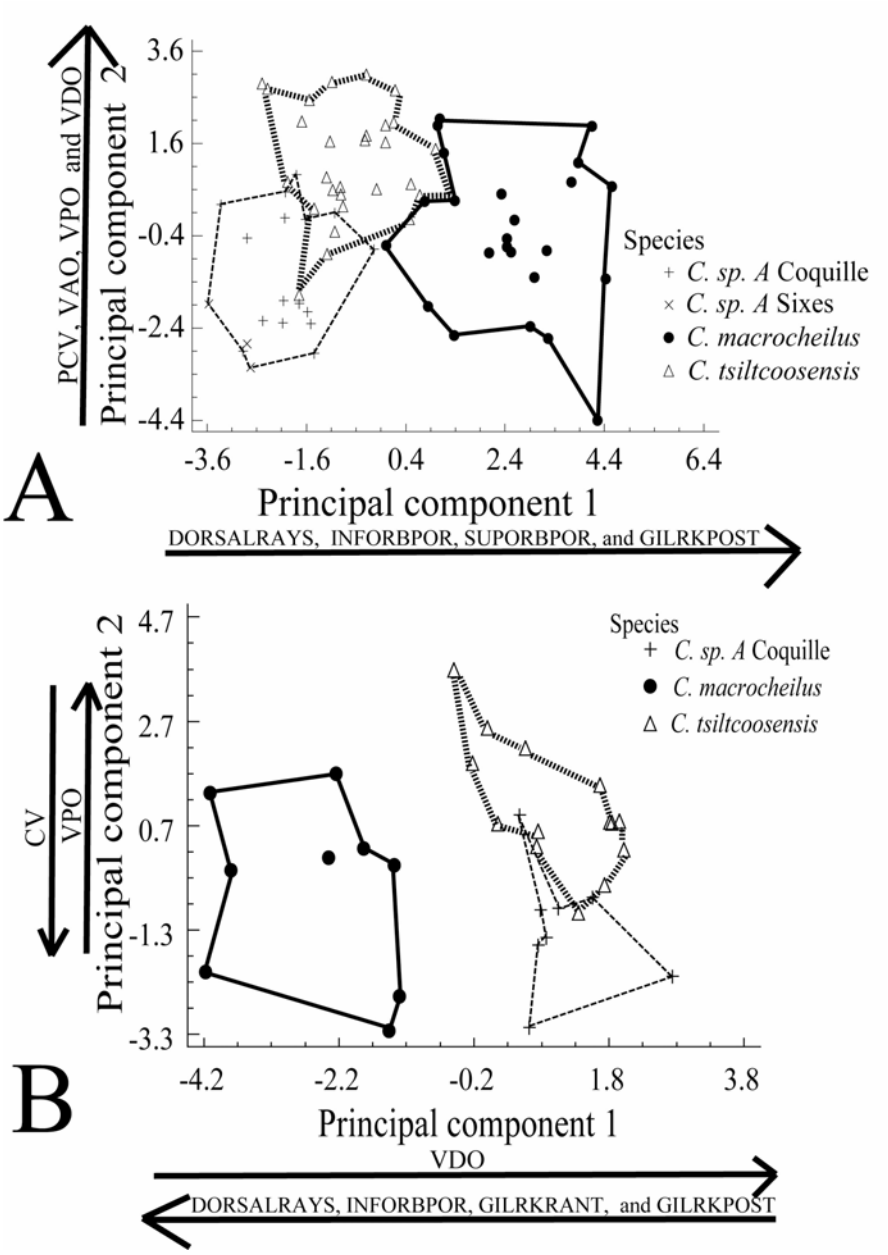

Figure 2.7 Principal component score plot of 18 meristic characters (PCV, CV, INTER1DEP, VDO, VAO, VPO, GILRKANT, GILRKPOST, PGRVAGR, INFORBPOR, IOPAE, SUPORBPOR, LLPECTPOR, SCALBELOLL, SCACADPED, DORSALRAYS, PECTRAYS and PELVRAYS) for A) female *C. macrocheilus* (*sensu lato*) complex (*C. macrocheilus* (24) and *C. tsiltcoosensis* (29) and Coquille River suckers (16)) and B) male *C. macrocheilus* (*sensu lato*) complex (*C. macrocheilus* (9) and *C. tsiltcoosensis* (14) and Coquille River suckers (8). Axis notations identify characters with high absolute loading (>0.3). Arrow points in the direction of increase value of each character. Principal component 1 explains 25.19% of the total variance for female and 19.62% for male. Principal component 2 explains 16.84% of the total variance both female and 15.27% male.

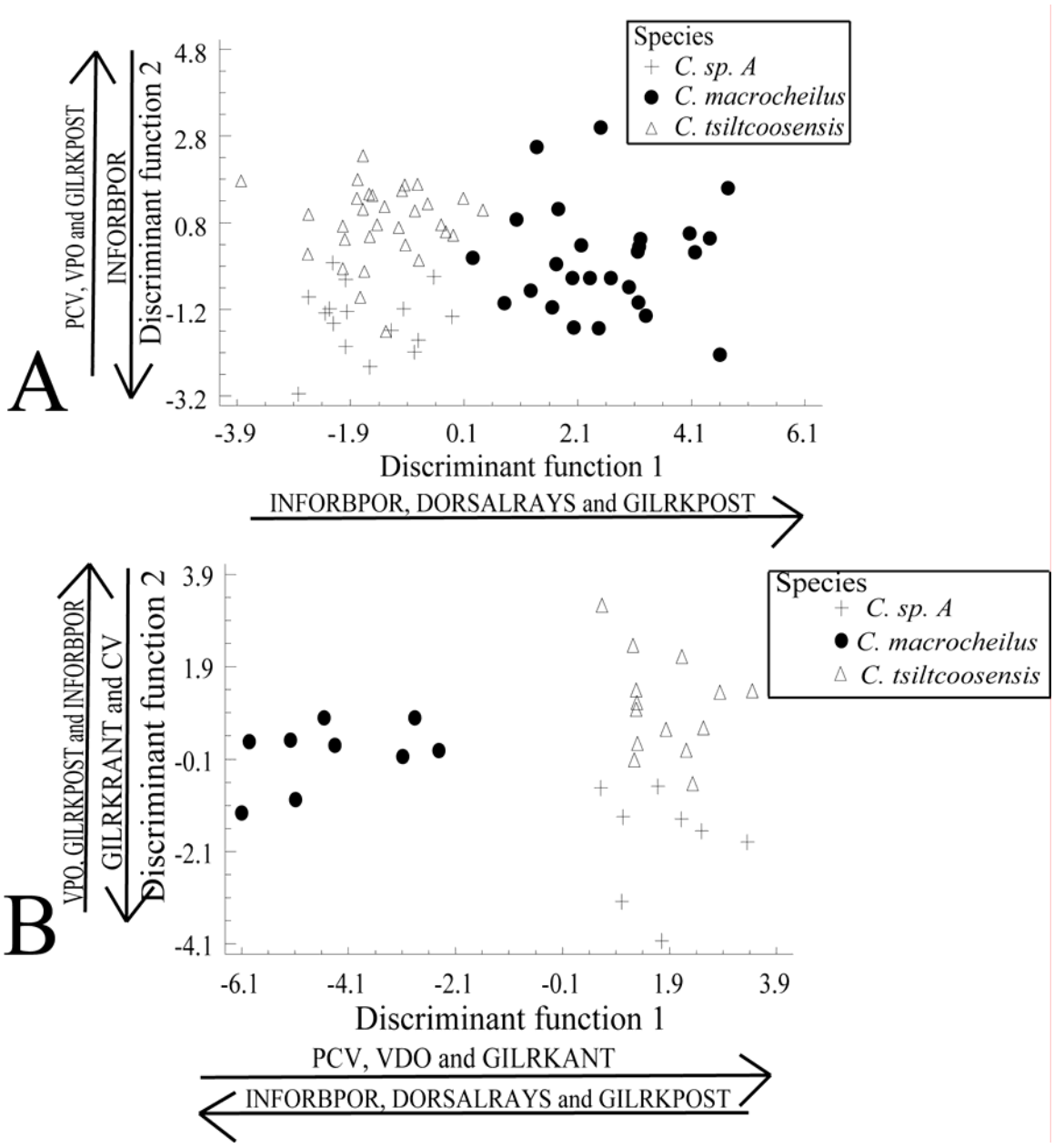

Figure 2.8 Discriminant function score plot based on 8 meristic characters (PCV, VDO, VAO, VPO, GILRKPOST, DORSALRAYS, INFORBPOR and SUPORBPOR for female and PCV, CV, VDO, VPO, GILRKANT, GILRKPOST, DORSALRAYS and INFORBPOR for male) for A) female *Catostomus macrocheilus* (*sensu lato*) complex (*C. macrocheilus* (26), *C. tsiltcoosensis* (31) and *C. sp. A* (16)) and B) for male *Catostomus macrocheilus* (*sensu lato*) complex (*C. macrocheilus* (9), *C. tsiltcoosensis* (14) and *C. sp. A* (8)). Axis notations identify characters with coefficient greater than 0.3. Arrow points in the direction of increase value of each character.

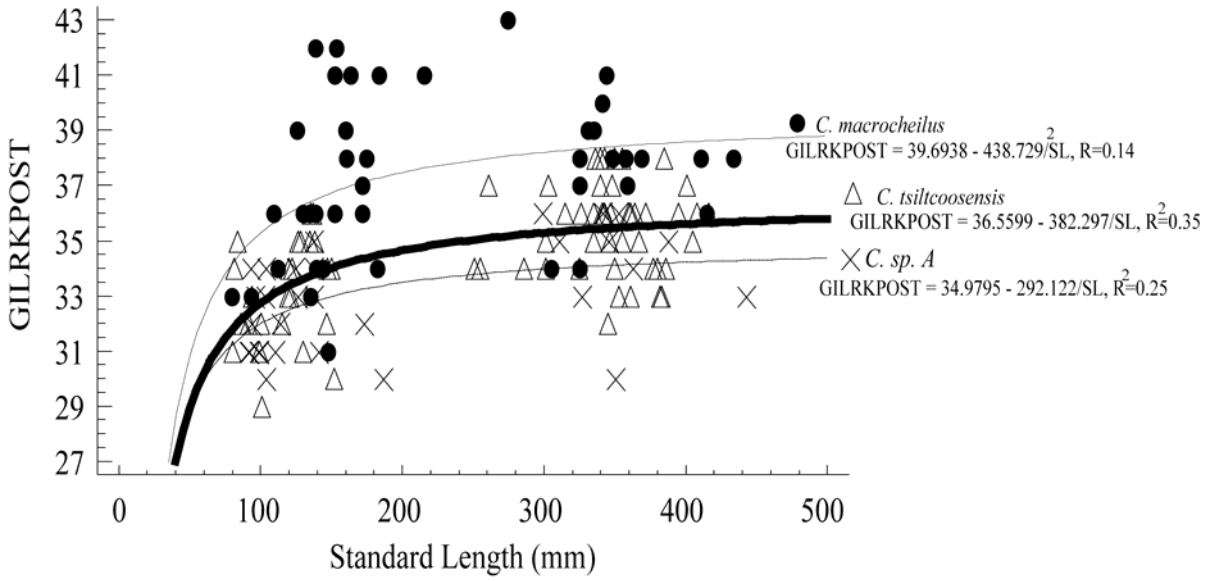

Figure 2.9 Relationship between medial gill rakers and size in *Catostomus macrocheilus, C. tsiltcoosensis* and *C. sp. A* with ontogenetic regression model.

Figure 2.10 Left lateral view of A) Male *Catostomus sp. B* (315mm in SL) and B) Male *C. rimiculus* (OS17787 (G); 295 mm in SL

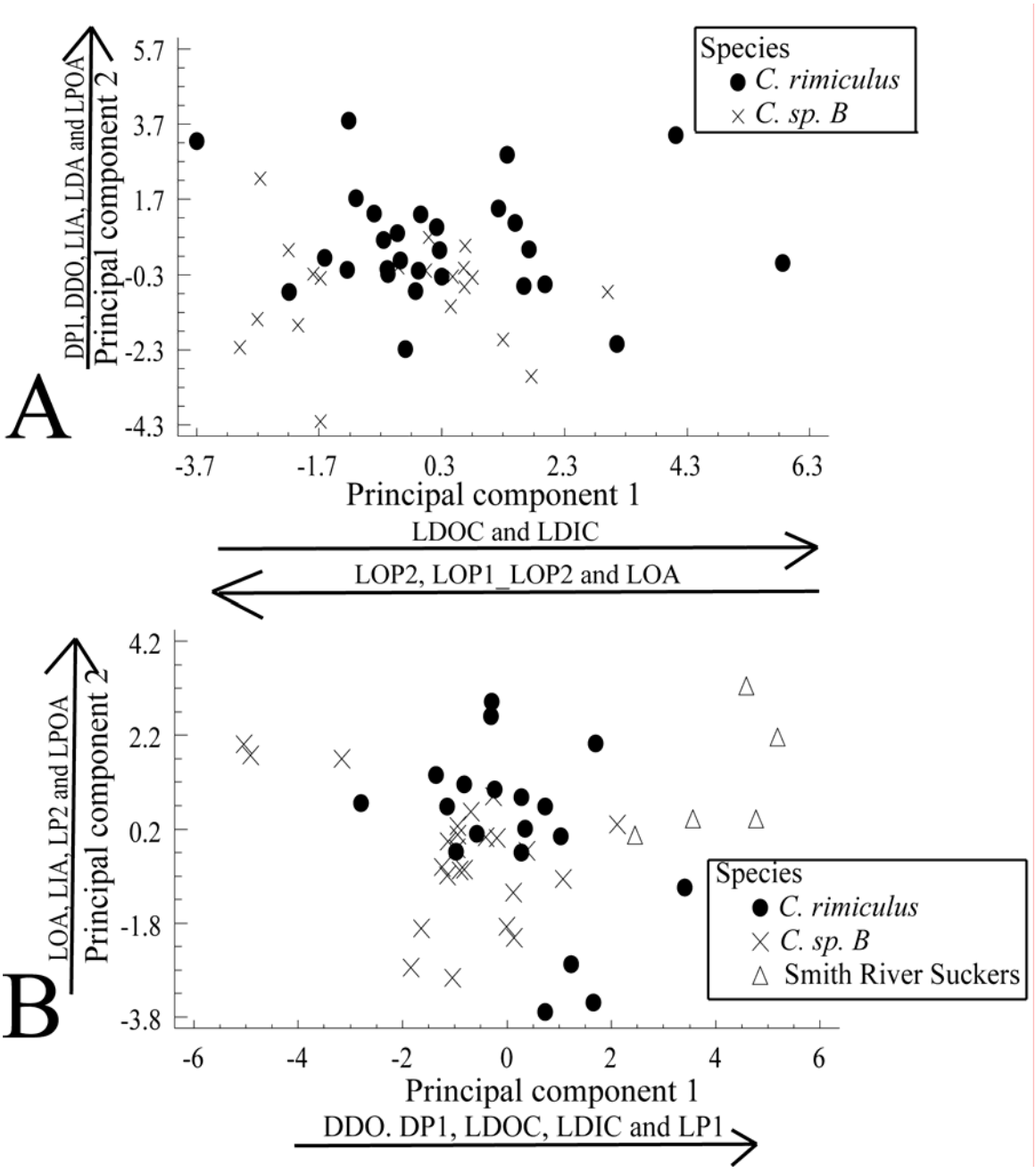

Figure 2.11 Morphometric principal component score plot of 12 morphometric characters (DP1, DDO, LOP2, LOP1_LOP2, LOA, LIA, LDA, LDOC, LDIC, LP1, LP2 and LPOA) for A) female *C. rimiculus* (*sensu lato*) (*C. sp. B* (20) and Klamath *C. rimiculus* (*sensu stricto*) (28)) and B) male *C. rimiculus* (*sensu lato*) (*C. sp. B* (24), Klamath *C. rimiculus* (*sensu stricto*) (19) and Smith River suckers (5)). Axis notations identify characters with high absolute loading (>0.3). Arrow points in the direction of increase value of each character. Principal component 1 explains 28.65% of the total variance for female and 36.35% for male. Principal component 2 explains 21.05% of the total variance for female and 21.19% for male.

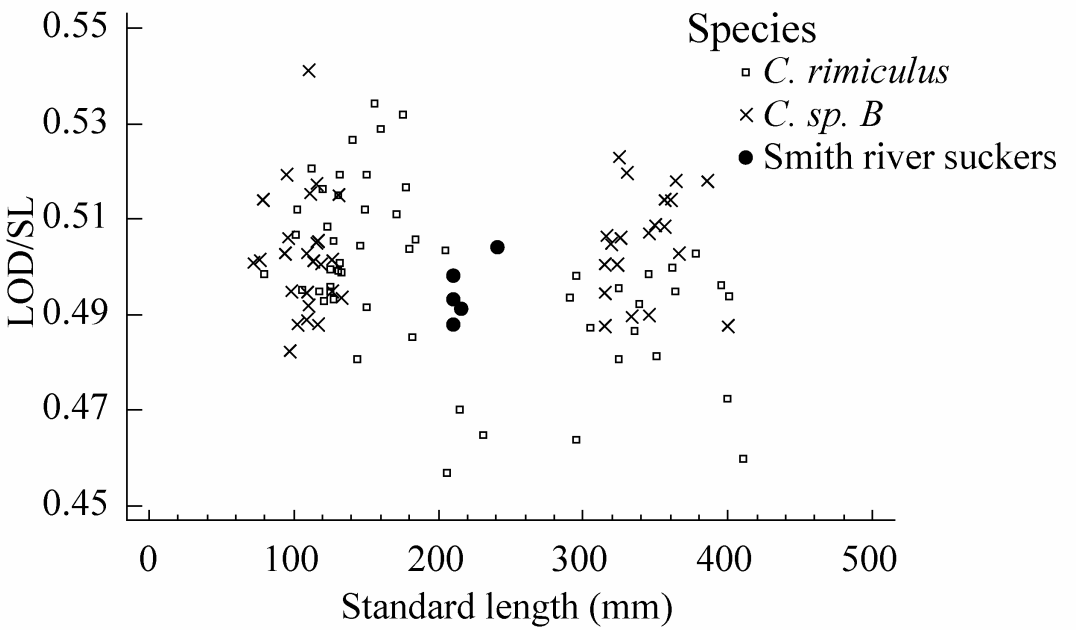

Figure 2.12 The ratio of predorsal fin length to the standard length for *C. rimiculus* and *C. sp. B* at different standard length (SL).

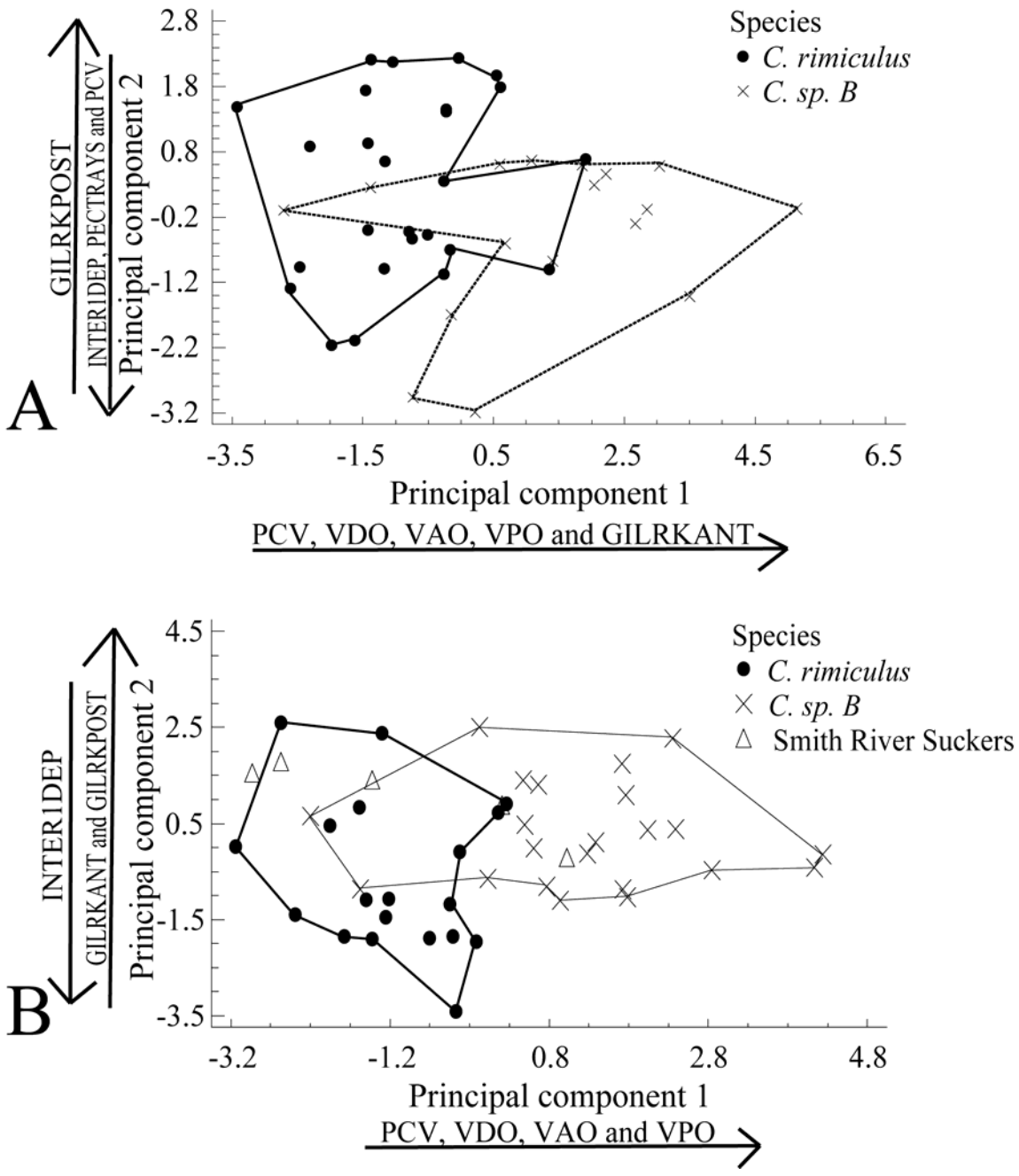

Figure 2.13 Meristic principal component score plot of 11 meristic characters (PCV, INTER1DEP, VDO, VAO, VPO, GILRKANT, GILRKPOST, LLSCALES, PAPUPRLIP, DORSALRAYS and PECTRAYS) for A) female *C. rimiculus* (*sensu lato*) (*C. sp. B* (17) and Klamath and *C. rimiculus* (*sensu stricto*) (26)) and B) male *C. rimiculus* (*sensu lato*) (*C. sp. B* (22), Klamath *C. rimiculus* (*sensu stricto*) (19) and Smith River suckers (5)). Axis notations identify characters with high absolute loading (>0.3). Arrow points in the direction of increase value of each character. Principal component 1 explains 31.49% of the total variance for female and 29.15% for male. Principal component 2 explains 16.64% of the total variance for female and 17.41% for male.

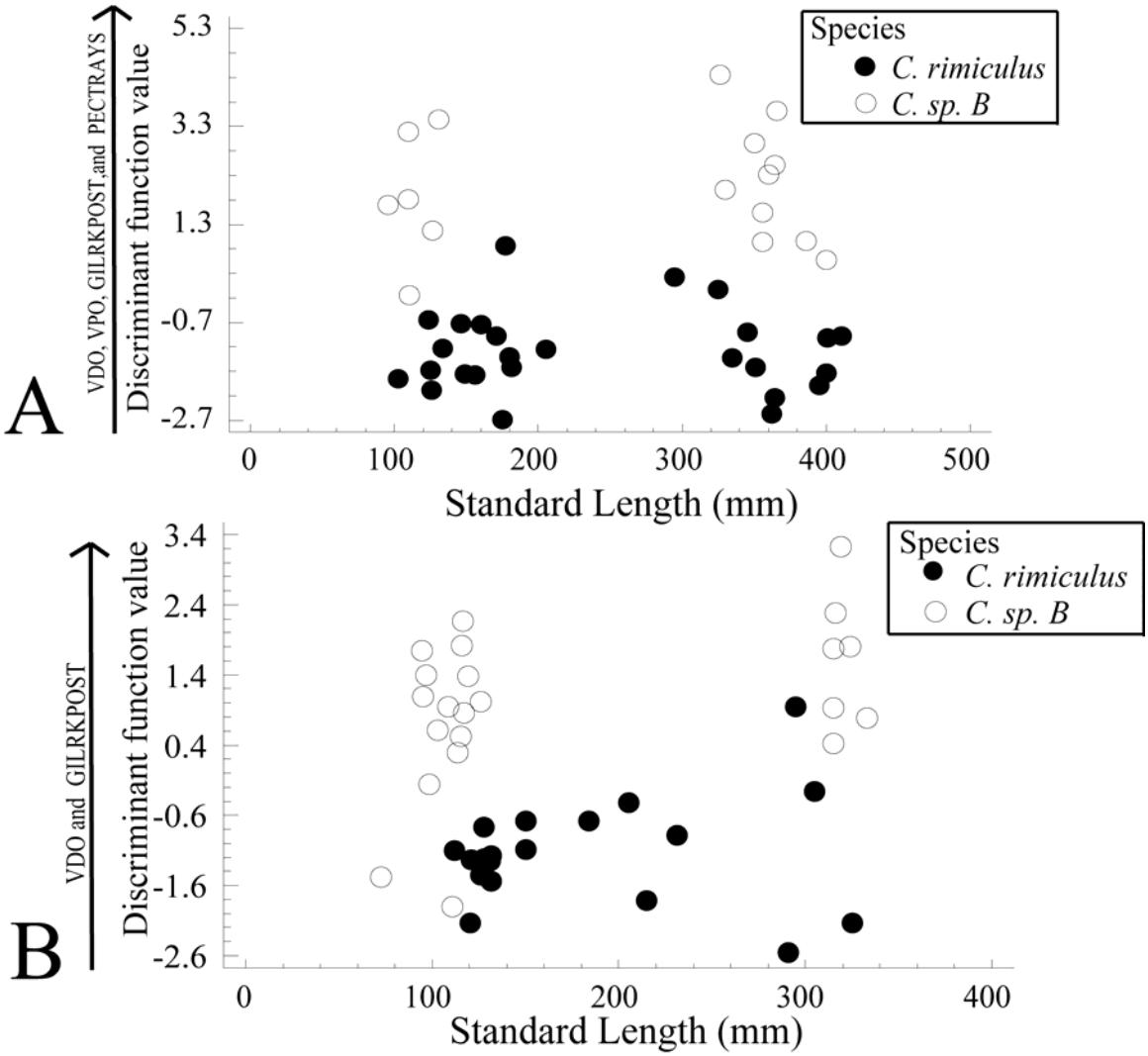

Figure 2.14 Discriminant function score from DFA of 7 meristic characters (PCV, INTER1DEP, VDO, VPO, GILRKANT, GILRKPOST and PECTRAYS for female and PCV, INTER1DEP, VDO, VAO, VPO, GILRKANT and GILRKPOST for male) for A) female *Catostomus rimiculus* (*sensu lato*) complex (Klamath *C. rimiculus* (*sensu stricto*) (26) and *C. sp. B* (17)) and B) for male *Catostomus rimiculus* (*sensu lato*) complex (Klamath *C. rimiculus* (*sensu stricto*) (19) and *C. sp. B* (22)) at different standard length. Axis notations identify characters with coefficient greater than 0.3. Arrow points in the direction of increase value of each character.

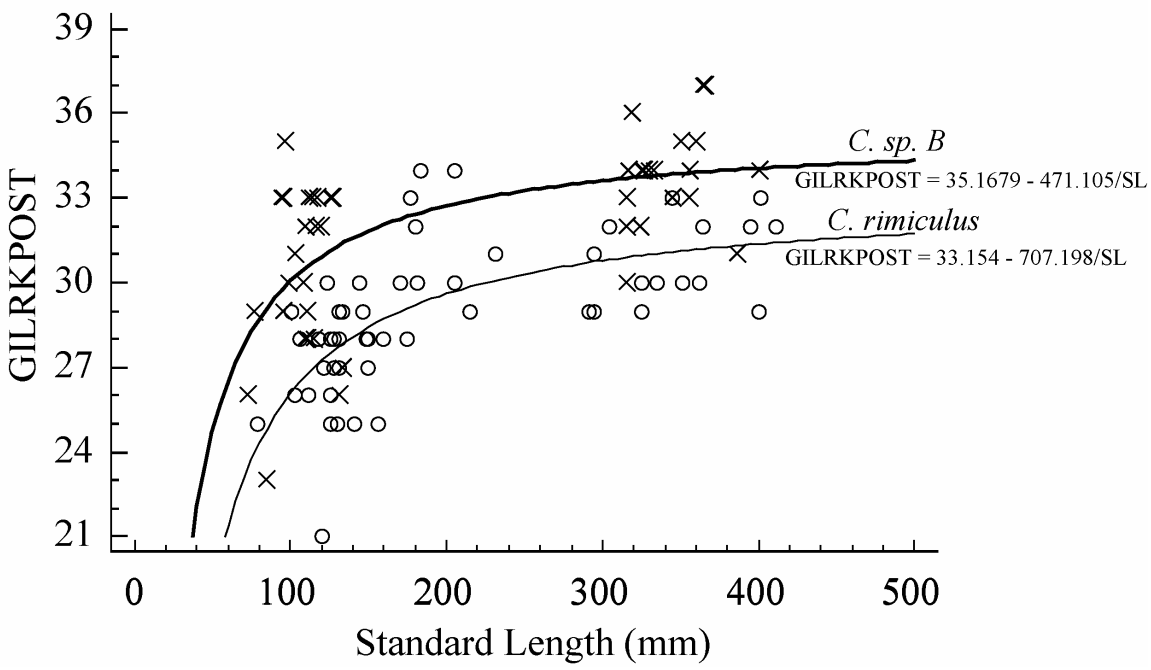

Figure 2.15 Relationship between medial gill rakers and size in *Catostomus rimiculus* (N=52) and *C. sp. B* (N=43) with ontogenetic regression model.

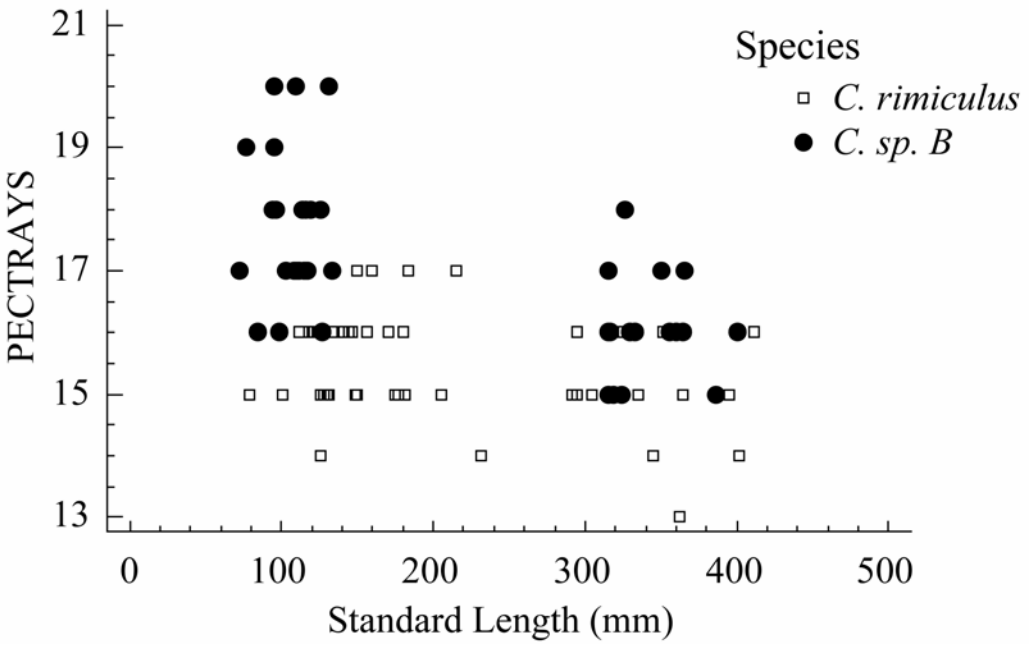

Figure 2.16 Pectoral fin rays counts of *C. rimiculus* and *C. sp. B* at different standard length (SL).

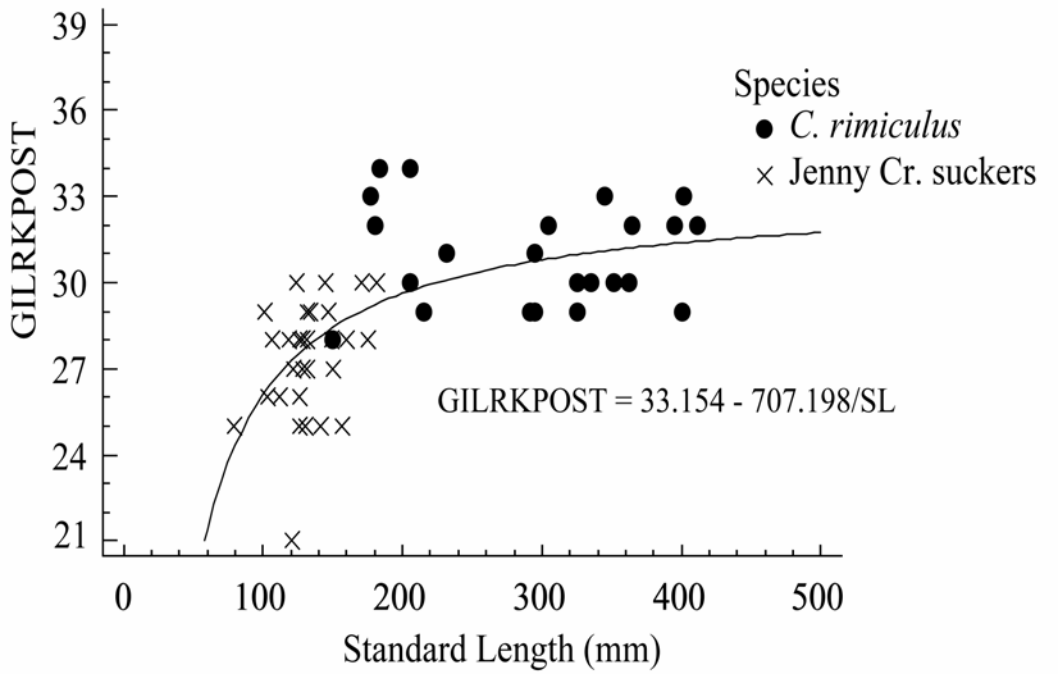

Figure 2.17 Relationship between gill rakers and standard length (SL) in Jenny creek *C. rimiculus* and *Catostomus rimiculus* from Klamath mainstem with ontogenetic regression model.

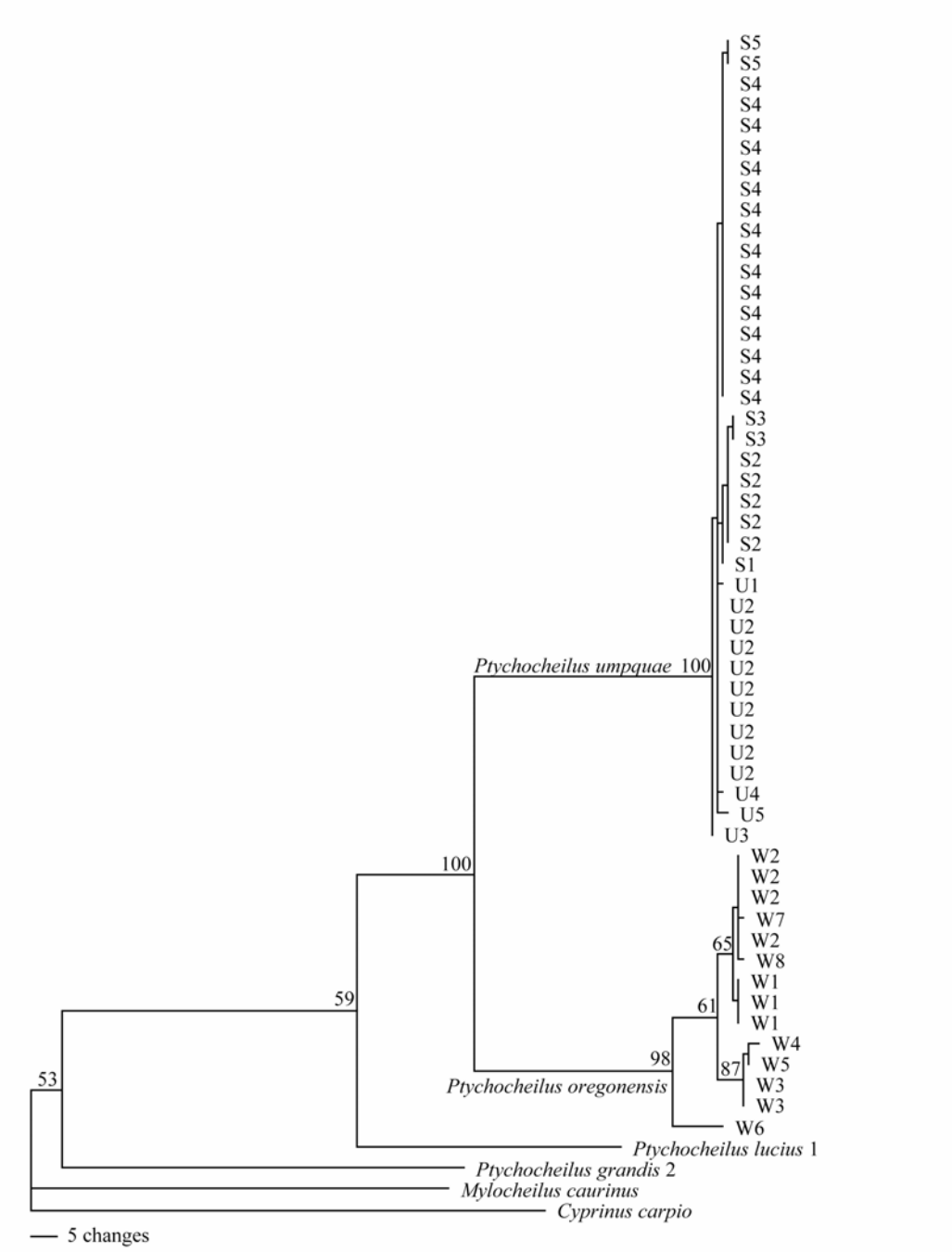

Figure 2.18 Relationship among 4 species of *Ptychocheilus* base on cytochrome b sequence (1041 base pair with 134 parsimony informative characters) from parsimony algorithm. *Mylocheilus caurinus* is the outgroup. Haplotype for each sample is labled at the terminal branch for *P. oregonensis* and *P. umpquae*. The tree is 394 steps long with CI = 0.8503, RI=0.8778, and RC=0.7464. Branch length represents changes occurred on each branch. The number represents the bootstrap value at each node. Node without number had bootstrap value less than 50

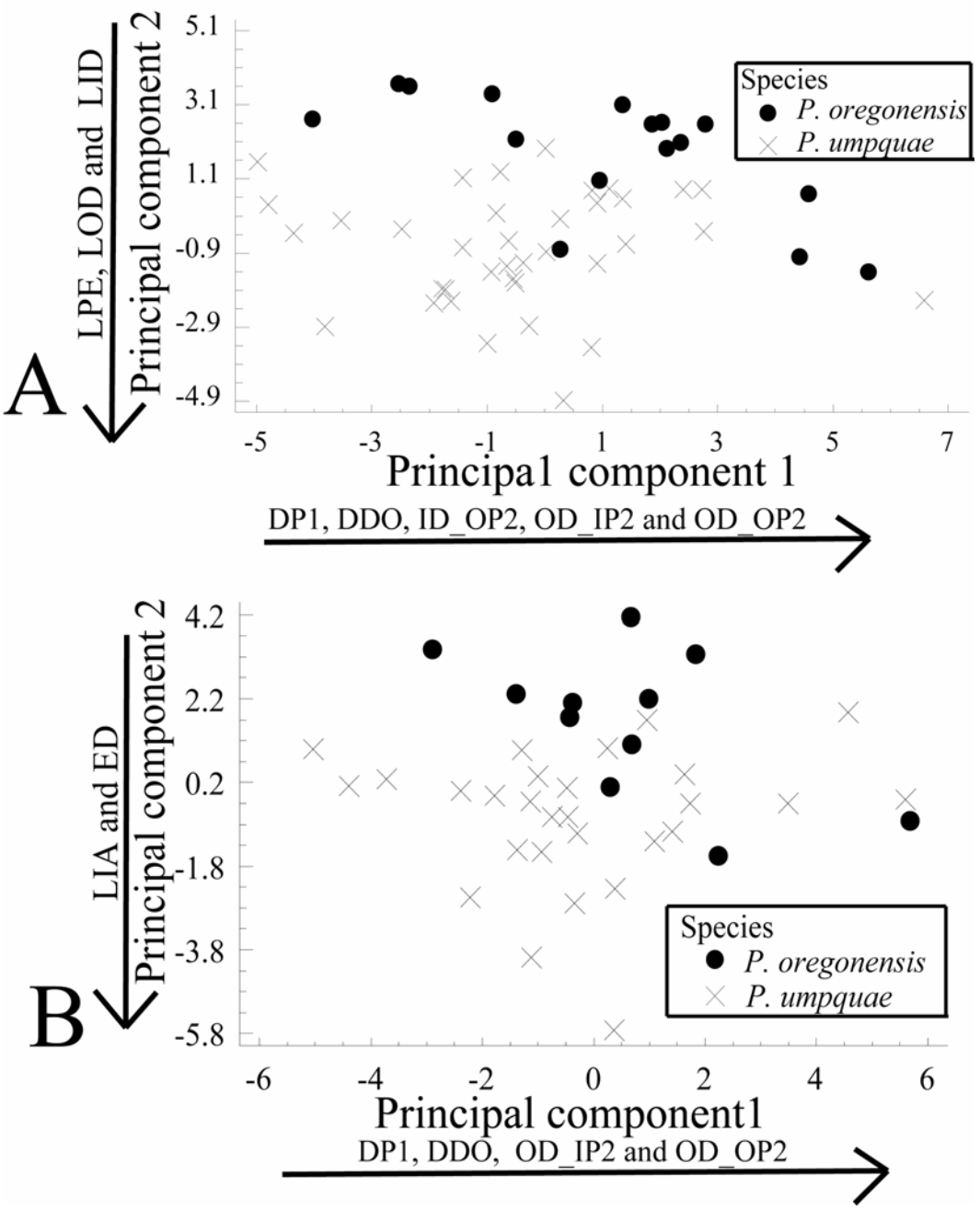

Figure 2.19 Principal component score plot of 23 morphometric characters (LAE, LPE, IW, WP1, DP1, DDO, LOD, LID, LOA, LIA, LDA, LDOC, LDIC, CPD, LP1, LP2, LPEOD, ED, CRD, ID_OP2, OD_IP2, OD_OP2 and ID_IP2) for A) female *Ptychocheilus umpquae* (37) and *P. oregonensis* (16) and B) male *Ptychocheilus umpquae* (28) and *P. oregonensis* (11). Axis notations identify characters with high absolute loading (>0.3). Arrow points in the direction of increase value of each character. Principal component 1 explains 26.99% of the total variance for female and 23.98% for male and principal component 2 explains 17.15% of the total variance for female and 16.86% for male.

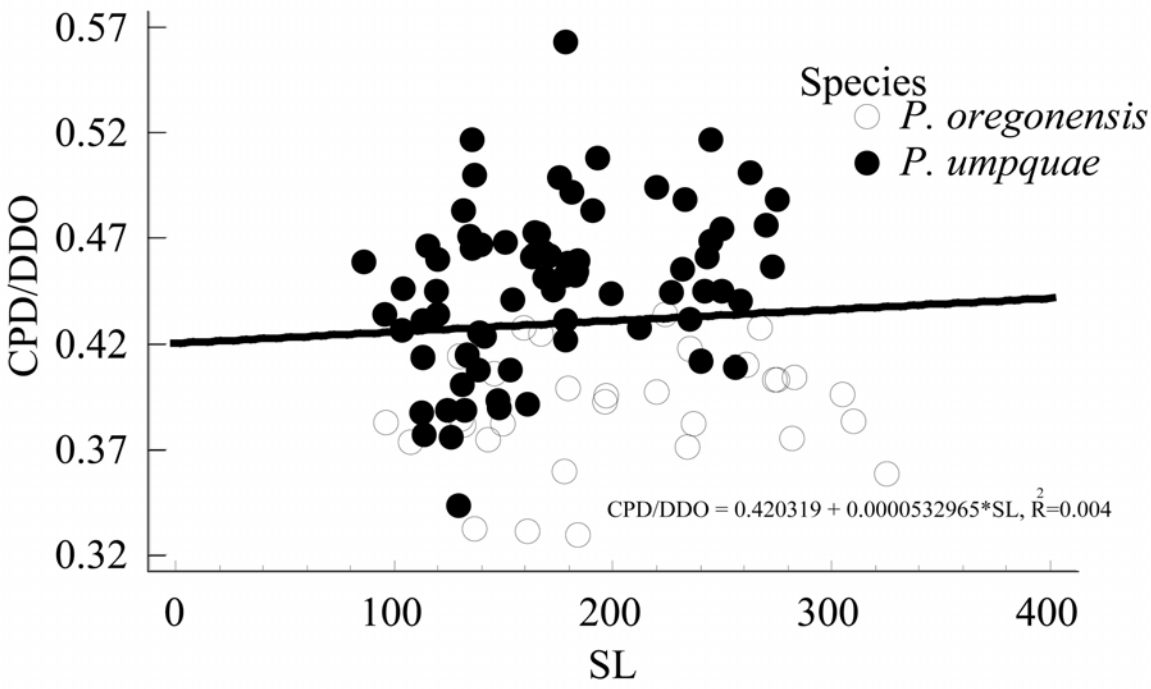

Figure 2.20 Linear relationship of the ratio of caudal peduncle depth to the body depth at the origin of the dorsal fin and the standard length of *Ptychocheilus oregonensis* and *P. umpquae*.

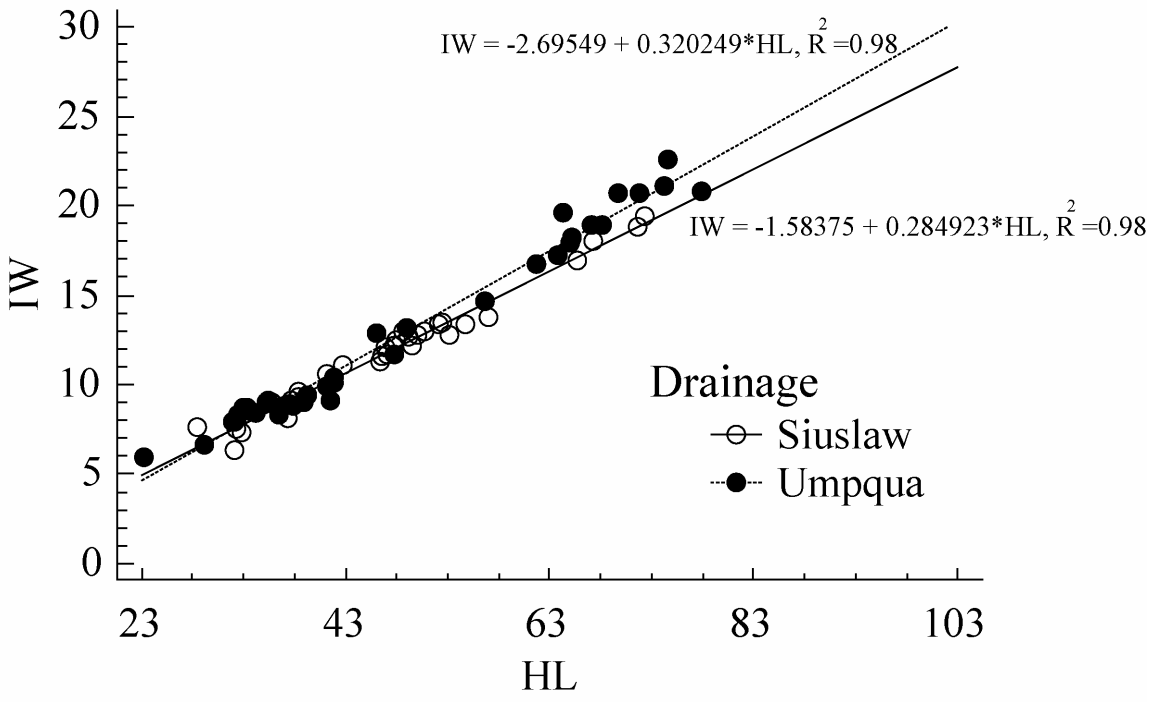

Figure 2.21 Linear regression relationship of interorbital width (IW) and head length (HL) of *Ptychocheilus umpquae* (34 Siuslaw specimens and 35 Umpqua specimens).

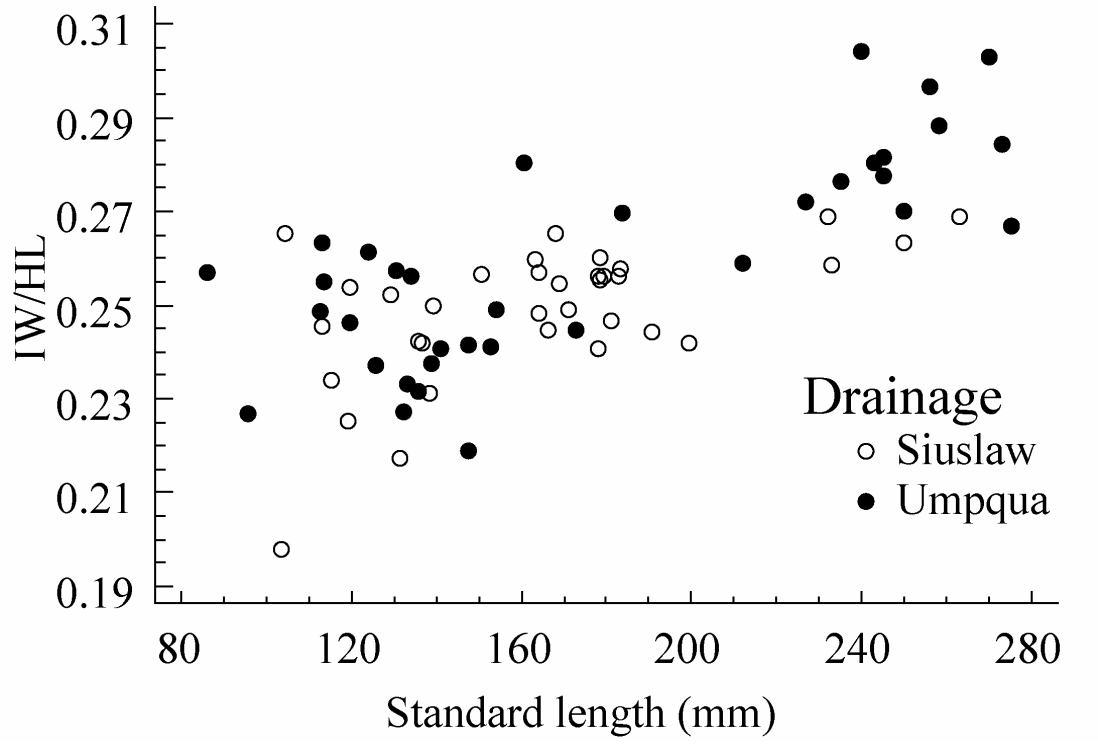

Figure 2.22 The ratio of interorbital width to head length (IW/HL) at different standard length of *Ptychocheilus umpquae* (34 Siuslaw specimens and 35 Umpqua specimens).

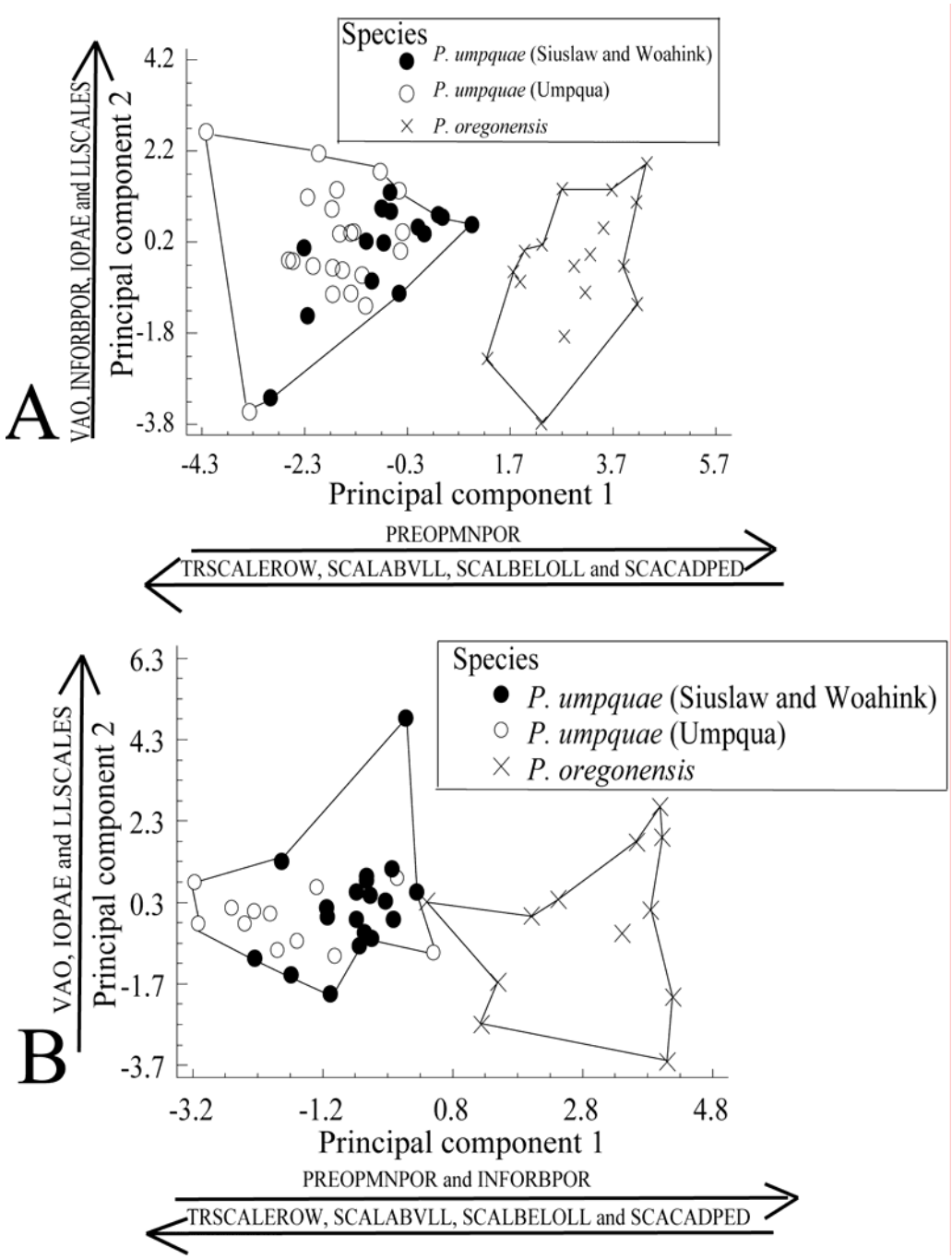

Figure 2.23 Principal component score plot of 12 meristic characters (VAO, GILRKPOST, PREOPMNPOR, INFORBPOR, IOPAE, SUPORBPOR, LLSCALES, LLPECTOR, TRSCALEROW, SCALABVLL, SCALBELOLL and SCASCADPED) for A) female *Ptychocheilus umpquae* (38) and *P. oregonensis* (17) and B) male *Ptychocheilus umpquae* (31) and *P. oregonensis* (12). Axis notations identify characters with absolute loading greater than 0.3. Arrow points in the direction of increase value of each character. Principal component 1 explains 41.62% of the total variance for female and 37.33% for male. Principal component 2 explains 14.29% of the total variance for female and 16.61% for male.

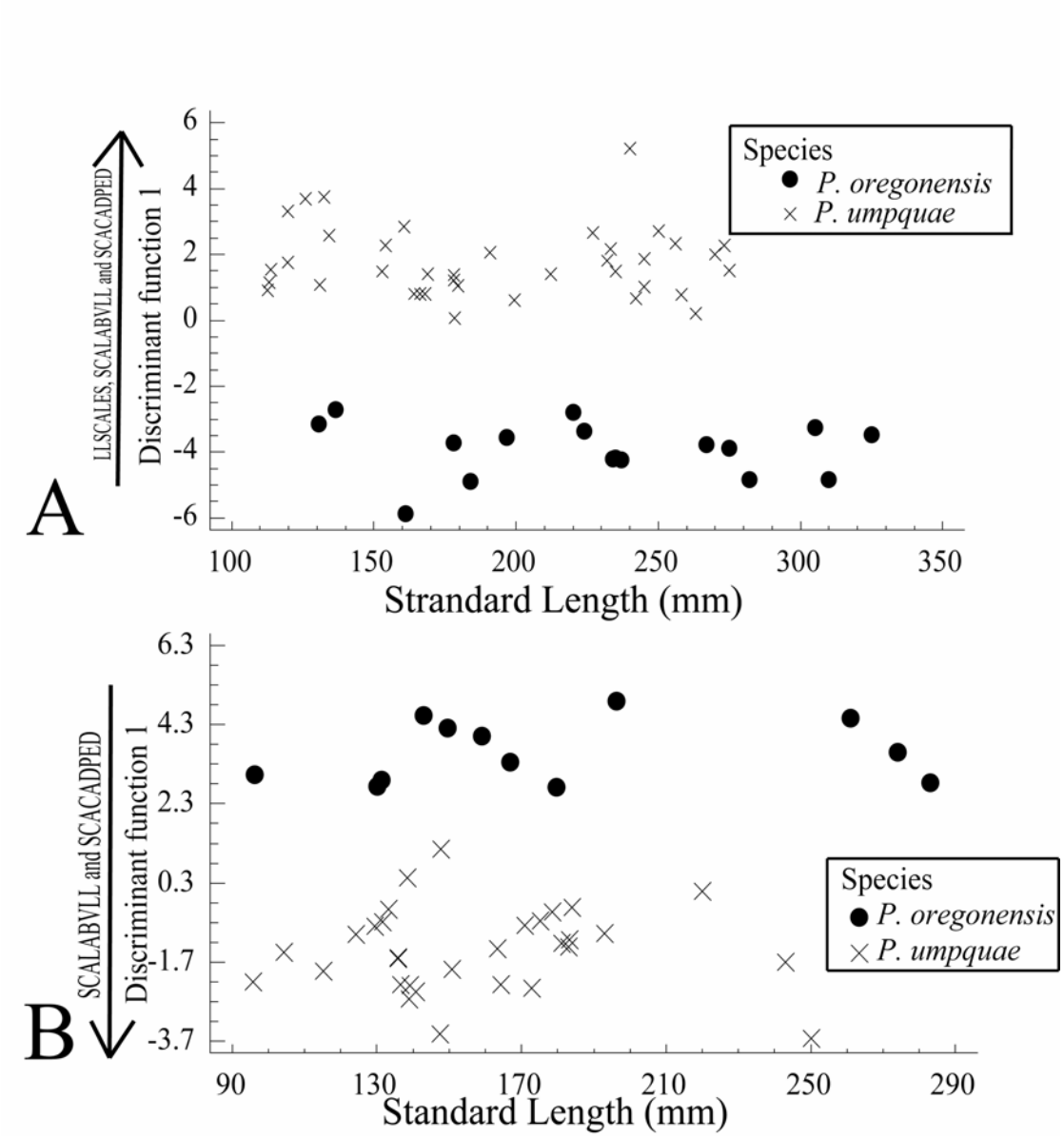

Figure 2.24 Discriminant function score from DFA of 9 meristic characters (VAO, PREOPMNPOR, INFORBPOR, IOPAE, LLSCALES, TRSCALEROW, SCALABVLL, SCALBELOLL and SCACADPED) for A) female *Ptychocheilus oregonensis* (17) and *P. umpquae* (38) and B) male *Ptychocheilus oregonensis* (12) and *P. umpquae* (31) at different SL. Axis notations identify characters with absolute coefficient greater than 0.3. Arrow points in the direction of increase value of each character.

Appendix 2.1 Acronyms of all the counts and measurements used in this study. All characters except PAPUPRLIP and PAPLORLIP were used in the *Ptychocheilus* analysis. All characters except MAXL, LPTC and IDIP2 were used in *C. tsiltcoosensis*, *C. sp. A* and *C. macrocheilus* analysis. All characters except SCACADPED, LPTC, IDIP2 and MAXL were used in *C. rimiculus* and *C. sp. B* analysis.

Meristic characters	Description
PCV	Post-Weberian precaudal vertebrae without a definite haemal spine even if a haemal arch was present.
CV	Post-Weberian caudal vertebrae with a definite haemal spine including urostyle.
INTER1DEP	Number of vertebrae anterior to first dorsal fin pterygiophore including vertebrae immediately posterior to point of interdigitation with neural spines
VDO	Number of vertebrae anterior to a vertical from base of first dorsal fin ray including vertebra intersected by the vertical.
VAO	Number of vertebrae anterior to a vertical from base of first anal fin ray including vertebra intersected by the vertical.
VPO	Number of vertebrae anterior to a vertical from base of first pelvic fin ray including vertebra intersected by the vertical.
GILRKRANT	Number of gill rakers on lateral surface of first gill arch.
GILRKPOST	Number of gill rakers on the medial surface of the first gill arch
PGRVAGR	Number of gill rakers on medial surface of first arch anteriad of all gill rakers on the lateral surface of the arch.
PREOPMNPOR	Preoperculomandibular pores.
INFORBPOR	Infraorbital pores.
IOPAE	Number of infraorbital pores anterior to the anterior edge of the eye
SUPORBPOR	Supraorbital pores.
LLSCALES	Lateral line scales.
LLPECTPOR	Number of lateralis pores on cleithrum from supratemporal canal to first lateral line scales.
TRSCALES	Number of scales above the lateral line plus the number of scales from the origin of the pelvic fin to the lateral line.
SCALABVLL	Scales above lateral line.
SCALBELOLL	Scales below lateral line.
PAPUPRLIP	Rows of papillae at symphysis of upper lip.
PAPLORLIP	Rows of papillae at symphysis of lower lip.
SCACADPED	Scales around caudal peduncle
DORSALRAYS	Dorsal fin rays
ANF	Anal fin rays
PECTRAYS	Pectoral fin rays
PELVRAYS	Pelvic fin rays

Appendix 2.1 Acronyms of all the counts and measurements used in this study (Continued)

Morphometric characters	Description
SL	Standard length (measurements made on preserved specimens)
LAE	Snout length.
LPE	Distance from tip of snout to posterior margin of eye.
HL	Head length.
IW	Interorbital width at the least bony measurement at the narrowest point.
WP1	Distant from from the origin (the base of the anterior most rays) of the pectoral fin of the left side to the right side of the fish
DP1	Body depth att eh orgin of the pectoral fin (measure at the vertical line that cross the base of the most anterior most rays).
DDO	Depth of body at dorsal-fin origin.
LOP2	Distance from tip of snout to pelvic fin origin.
LOP1_LOP2	Distance from pectoral fin origin to pelvic fin origin.
LOD	Pre-dorsal length.
LID	Distance from tip of snout to insertion of dorsal fin.
LOA	Pre-anal length.
LIA	Distance from tip of snout to insertion of anal fin.
LDA	Distance from dorsal fin origin to anal fin origin.
LPTC	Length from tip of snout to median point of postemporal sensory canal
LDOC	Distance from dorsal fin origin to middle of caudal fin base.
LDIC	Distance from dorsal fin insertion to middle of caudal fin base.
DCAUDPED	Caudal peduncle depth.
LP1	Distance from the tip of the posterior margin of the longest pectoral fin ray to the origin (the base of the anterior most rays) of the pectoral fin
LP2	Distance from the tip of the posterior margin of the longest pelvic fin ray to the origin (the base of the anterior most rays) of the pelvic fin.
LPEOD	Distance from posterior margin of eye to origin of dorsal fin.
LPOA	Distance from pelvic fin origin to anal fin origin.
SPMLL	Projected distance from tip of snout to posterior margin of lower lip with mouth closed.
LDMM	Distance from symphysis of lower jaw to lateroposterior margin of lower lip lobe.
GAPLMM	Distance from symphysis of lower jaw to point where lower lip lobes separate.
AIOPAE	Distance from anteriormost infraorbital pore to anterior margin of eye.
MAXL	Distance from the tip of snout to posterior edge of Maxilla
SAIOP	Distance from tip of snout to anteriormost infraorbital pore.

Appendix 2.1 Acronyms of all the counts and measurements used in this study (Continued)

Morphometric characters	Description
IDOP2	Distance from the insertion of the dorsal fin to the origin of the pelvic fin
CR-D	Distance from the base of the first scales on the dorsal surface of the cranial to the origin of the dorsal fin
ODIP2	Distance from the origin of the dorsal fin to the insertion of the pelvic fin
ODOP2	Distance from the origin of the dorsal fin to the origin of the pelvic fin
IDIP2	Distance from the insertion of the dorsal fin to the insertion of the pelvic fin
ED	Eye diameter

Appendix 2.2 Haplotypes found in the *Catostomus tsiltcoosensis* (TS, TU and TMC), *C. macrocheilus* (CMW), *C. sp. A* (CO), *C. sp. B* (CRR) and *C. rimiculus* (CRK). Haplotypes TS1-TS3 were found in Siuslaw River and Woahink Lake. Haplotypes TU1-TU3 were found in Umpqua River. Haplotypes TMC1 and TMC2 were found in Coos River and Millicoma River. Haplotypes CO1-CO5 were found in Coquille River. Haplotypes CMW1-CMW10 were found in Willamette River and Columbia River. Haplotypes CRR1-CRR7 were from Rogue River. Haplotypes CRK1-CRK9 were from Klamath River

Haplotype		Positions												
	N	46	72	84	92	102	132	145	156	219	225	228	240	244
TS1	5	G	G	A	G	A	A	C	A	A	T	G	T	G
TS2	5	-	-	-	-	-	-	-	-	-	-	-		-
TS3	1	-	-	-	-	-	-	-	-	-	-	-	-	-
TU1	2	-	-	-	-	-	-	-	-	-	-	-	-	-
TU2	9	-	-	-	-	-	-	-	-	-	-	-	-	-
TU3	1	-	-	-	-	-	-	-	-	-	-	-	-	-
TMC1	5	-	-	-	-	-	-	-	-	-	-	-	-	-
TMC2	5	-	-	-	-	-	-	-	-	-	-	-	-	-
CO1	5	-	-	-	-	-	-	-	-	-	-	A	-	-
CO2	2	-	-	-	-	-	-	-	-	-	-	A	-	-
CO3	1	-	-	-	-	-	-	-	-	-	-	A	-	-
CO4	1	-	-	G	-	-	-	-	-	-	-	A	-	T
CO5	1	-	-	-	-	-	-	-	-	-	-	A	-	T
CMW1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW2	3	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW3	2	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW4	1	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW5	1	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW6	1	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW7	1	-	-	-	-	-	-	-	-	-	-	-	-	-
CMW8	1	-	-	-	-	G	-	-	-	-	-	-	-	-
CRR1	4	-	C	G	-	G	-	-	G	G	C	-	-	-
CRR2	6	-	C	G	-	G	G	-	G	G	C	-	-	-
CRR3	1	-	C	G	-	G	G	-	G	G	C	-	-	-
CRR4	1	A	C	G	-	G	G	-	G	G	C	-	-	-
CRR5	1	-	C	-	-	G	G	-	G	G	C	-	-	-
CRR6	1	-	C	G	-	G	-	-	G	G	C	-	-	-
CRR7	2	-	C	G	-	-	G	-	G	G	C	-	-	-
CRK1	3	-	C	G	-	-	-	T	-	-	C	-	-	-
CRK2	5	-	C	G	-	-	-	T	-	-	C	-	-	-
CRK3	1	-	C	G	-	-	-	T	-	-	C	-	-	-

Appendix 2.2 Haplotype found in the *Catostomus tsiltcoosensis*, *C. macrocheilus*, *C. sp. A*, *C. sp. B* and *C. rimiculus* (continued)

Haplotype	Positions													
	N	46	72	84	92	102	132	145	156	219	225	228	240	244
CRK4	1	-	C	G	A	-	-	-	-	-	C	-	-	-
CRK5	2	-	C	G	-	-	-	T	-	-	C	-	-	-
CRK6	1	-	C	G	-	-	-	T	-	-	C	-	A	-
CRK7	1	-	C	G	-	-	-	-	-	-	C	-	-	-
CRK8	1	-	C	G	-	-	-	T	-	-	C	-	-	-
CRK9	1	-	C	G	-	-	-	-	-	-	C	-	-	-

Appendix 2.2 Haplotype found in the *Catostomus tsiltcoosensis*, *C. macrocheilus*, *C. sp. A*, *C. sp. B* and *C. rimiculus* (continued)

Haplotype	Positions												
	246	249	255	303	304	318	330	348	363	369	393	396	405
TS1	G	T	T	A	C	T	A	T	A	A	T	A	A
TS2	-	-	-	-	-	-	-	-	-	-	-	-	-
TS3	-	-	-	-	-	-	-	-	-	-	-	-	-
TU1	T	C	-	-	-	A	-	-	-	G	-	-	-
TU2	T	C	-	-	-	A	-	-	-	G	-	-	-
TU3	T	C	-	-	-	A	-	-	-	G	-	-	-
TMC1	T	C	-	-	-	A	-	-	-	-	-	G	-
TMC2	T	C	-	-	-	A	-	-	-	-	-	G	-
CO1	A	-	C	-	-	A	-	-	-	-	-	G	-
CO2	A	-	C	-	-	A	-	-	G	-	-	G	-
CO3	A	-	C	-	-	A	-	-	G	-	-	G	-
CO4	A	-	C	-	-	A	-	-	-	-	-	G	-
CO5	A	-	C	-	-	A	-	-	-	-	-	G	-
CMW1	A	-	-	-	-	A	G	C	-	-	-	G	G
CMW2	A	-	-	-	-	A	G	C	-	-	-	G	-
CMW3	A	-	-	-	-	A	G	C	-	-	-	G	-
CMW4	A	-	-	-	-	A	G	C	-	-	-	G	-
CMW5	A	-	-	-	-	A	G	C	-	-	-	-	-
CMW6	A	-	-	-	-	A	G	C	-	-	-	G	G
CMW7	A	-	-	-	-	A	-	C	-	-	-	G	-
CMW8	A	-	-	-	-	A	G	C	-	-	-	-	-
CRR1	-	-	-	-	T	A	-	-	G	-	C	-	-
CRR2	-	-	-	-	T	A	-	-	G	-	C	-	-
CRR3	-	-	-	-	T	A	-	-	G	-	-	-	-
CRR4	-	-	-	G	T	A	-	-	G	-	C	-	-
CRR5	-	-	-	-	T	A	-	-	G	-	C	-	-
CRR6	-	-	-	-	T	A	-	-	G	-	C	-	-
CRR7	-	-	-	-	T	A	-	-	G	-	C	-	-
CRK1	-	-	-	-	-	A	-	-	G	-	C	-	-
CRK2	-	-	-	-	-	A	-	-	G	-	C	-	-
CRK3	-	-	-	-	-	A	-	-	G	-	C	-	-
CRK4	-	-	-	-	-	A	-	-	G	-	C	-	-
CRK5	C	-	-	-	-	A	-	-	G	-	C	-	-
CRK6	-	-	-	-	-	A	-	-	G	-	C	-	-
CRK7	-	-	-	-	-	A	-	-	G	-	C	-	-
CRK8	C	-	-	-	-	A	-	-	G	-	C	-	-
CRK9	A	-	-	-	-	A	-	-	G	-	C	-	-

Appendix 2.2 Haplotype found in the *Catostomus tsiltcoosensis*, *C. macrocheilus*, *C. sp. A*, *C. sp. B* and *C. rimiculus* (continued)

Haplotype	Positions												
	408	432	450	480	501	515	522	525	531	540	552	555	564
TS1	A	A	A	A	G	A	C	A	A	C	G	A	A
TS2	-	-	-	-	-	-	-	-	-	-	-	-	-
TS3	-	-	-	-	-	-	-	-	-	-	-	-	-
TU1	G	-	-	-	-	-	-	-	-	-	-	G	-
TU2	-	-	-	-	-	-	-	-	-	-	-	G	-
TU3	-	-	-	-	-	-	-	-	-	-	-	G	-
TMC1	-	-	-	G	-	-	-	-	-	-	-	G	-
TMC2	-	-	-	G	-	G	-	-	-	-	-	G	-
CO1	-	-	G	-	A	-	-	-	-	G	-	G	-
CO2	-	-	G	-	A	-	-	-	-	G	-	G	-
CO3	-	-	G	-	A	-	-	-	-	G	-	G	-
CO4	-	-	G	-	A	-	-	-	-	G	-	G	-
CO5	-	-	G	-	A	-	-	-	-	G	-	G	-
CMW1	-	-	-	-	A	-	-	-	-	-	A	G	-
CMW2	-	-	-	-	A	-	T	-	-	-	A	G	-
CMW3	-	-	-	-	A	-	-	-	-	-	A	G	-
CMW4	-	-	-	-	A	-	-	G	-	-	A	G	-
CMW5	-	-	-	-	A	-	-	-	-	-	A	G	G
CMW6	-	-	-	-	A	-	-	-	-	-	A	G	-
CMW7	-	-	-	-	A	-	-	-	-	-	A	G	-
CMW8	-	-	-	-	A	-	-	-	-	-	A	G	-
CRR1	-	-	-	T	A	-	-	-	-	-	A	-	-
CRR2	-	-	-	T	A	-	-	-	-	-	A	-	-
CRR3	-	-	-	T	A	-	-	-	-	-	A	-	-
CRR4	-	-	-	T	A	-	-	-	-	-	A	-	-
CRR5	-	-	-	T	A	-	-	-	-	-	A	-	-
CRR6	-	-	-	T	A	-	-	-	-	-	A	-	-
CRR7	-	-	-	T	A	-	-	-	-	-	A	-	-
CRK1	-	G	-	T	-	-	-	-	G	-	A	-	-
CRK2	-	G	-	T	-	-	-	-	G	-	A	-	-
CRK3	-	G	-	T	-	-	-	-	G	-	A	-	-
CRK4	-	-	-	T	-	-	-	-	G	-	A	-	-
CRK5	-	G	-	T	-	-	-	-	G	-	A	-	-
CRK6	-	G	-	T	-	-	-	-	G	-	A	-	-
CRK7	-	-	-	T	-	-	-	-	G	-	A	-	-
CRK8	-	G	-	T	-	-	-	-	G	-	A	-	-
CRK9	-	-	-	T	-	-	-	-	G	-	A	-	-

Appendix 2.2 Haplotype found in the *Catostomus tsiltcoosensis*, *C. macrocheilus*, *C. sp. A*, *C. sp. B* and *C. rimiculus* (continued)

Haplotype	Positions												
	573	582	600	606	609	612	615	627	645	675	700	705	750
TS1	A	T	A	A	A	A	C	A	G	A	G	A	A
TS2	-	-	-	-	-	-	-	-	-	-	-	-	-
TS3	-	-	-	-	-	-	-	-	-	-	-	-	-
TU1	-	-	-	-	G	G	-	-	-	-	-	-	-
TU2	-	-	-	-	G	G	-	-	-	-	-	-	-
TU3	-	-	-	-	G	G	-	-	-	-	-	-	-
TMC1	-	-	-	-	-	G	-	-	-	-	-	-	-
TMC2	-	-	-	-	-	G	-	-	-	-	-	-	-
CO1	G	-	-	-	-	-	A	-	-	C	-	-	G
CO2	G	-	-	-	-	-	A	-	-	C	-	-	G
CO3	G	-	-	-	-	-	A	-	-	C	-	-	G
CO4	G	C	-	-	-	-	A	-	-	C	-	-	G
CO5	G	C	-	-	-	-	A	-	-	C	-	-	G
CMW1	-	-	G	-	-	-	-	-	-	-	-	G	-
CMW2	-	-	G	-	-	-	-	-	-	-	-	G	-
CMW3	-	-	G	-	-	-	-	-	-	-	-	G	-
CMW4	-	-	G	-	-	-	-	-	-	-	-	G	-
CMW5	-	-	G	-	-	-	-	-	-	-	-	G	-
CMW6	-	-	G	-	-	-	-	-	-	-	-	G	-
CMW7	-	-	G	-	-	-	-	-	-	-	-	G	-
CMW8	-	-	G	-	-	G	-	-	A	-	-	G	-
CRR1	G	C	-	G	-	-	-	G	A	-	-	-	-
CRR2	G	C	-	G	-	-	-	G	A	-	-	-	-
CRR3	G	C	-	G	-	-	-	G	A	-	-	-	-
CRR4	G	C	-	G	-	-	-	G	A	-	-	-	-
CRR5	G	C	-	G	-	-	-	G	A	-	-	-	-
CRR6	G	C	-	G	-	-	-	G	A	-	-	-	-
CRR7	G	C	-	G	-	-	-	G	A	-	-	-	-
CRK1	G	C	-	G	-	-	-	G	A	-	-	-	-
CRK2	G	C	-	G	-	-	-	G	A	-	-	-	-
CRK3	G	C	-	-	-	-	-	G	A	-	-	-	-
CRK4	G	C	-	G	-	-	-	-	A	-	-	-	-
CRK5	G	C	-	G	-	-	-	G	A	-	-	-	-
CRK6	G	C	-	G	-	-	-	G	A	-	-	-	-
CRK7	G	C	-	G	-	-	-	-	A	-	-	-	-
CRK8	G	C	-	G	-	-	-	G	A	-	A	-	-
CRK9	G	C	-	G	-	-	-	-	A	-	-	-	-

Appendix 2.2 Haplotype found in the *Catostomus tsiltcoosensis*, *C. macrocheilus*, *C. sp. A*, *C. sp. B* and *C. rimiculus* (continued)

Haplotype	Positions												
	753	771	816	825	840	846	867	876	879	897	901	906	909
TS1	A	C	A	G	C	A	A	G	A	T	T	A	A
TS2	-	-	-	-	-	-	-	-	-	-	-	-	-
TS3	-	T	-	-	-	-	-	-	-	-	-	-	-
TU1	-	-	G	-	-	-	-	A	-	-	-	-	-
TU2	-	-	-	-	-	-	-	A	-	-	-	-	-
TU3	-	-	-	-	-	-	-	A	-	-	-	-	-
TMC1	-	-	-	-	-	-	-	A	-	-	C	-	-
TMC2	-	-	-	-	-	-	-	A	-	-	C	-	-
CO1	-	-	-	-	-	G	G	A	G	-	-	G	G
CO2	-	-	-	-	-	-	G	A	G	-	-	G	G
CO3	-	-	-	-	-	-	G	A	G	-	-	G	G
CO4	-	-	-	-	-	G	G	A	G	-	-	G	G
CO5	-	-	-	-	-	G	G	A	G	-	-	G	G
CMW1	-	-	-	-	-	-	-	A	-	C	-	-	-
CMW2	-	-	-	-	-	-	-	A	-	-	-	-	-
CMW3	-	-	-	-	-	-	-	A	-	-	-	-	-
CMW4	-	-	-	-	-	-	-	A	-	-	-	-	-
CMW5	-	-	-	-	-	-	-	A	-	-	-	-	-
CMW6	-	-	-	-	-	-	-	A	-	C	-	-	-
CMW7	-	-	-	-	-	-	-	A	-	-	-	-	-
CMW8	-	-	-	-	-	-	-	A	-	-	-	-	-
CRR1	-	-	G	-	-	-	-	A	-	-	C	-	-
CRR2	-	-	G	-	-	-	-	A	-	-	C	-	-
CRR3	-	-	G	-	-	-	-	A	-	-	C	-	-
CRR4	-	-	G	-	-	-	-	A	-	-	C	-	-
CRR5	-	-	G	-	-	-	-	A	-	-	C	-	-
CRR6	-	-	G	-	-	-	-	A	-	-	C	G	-
CRR7	-	-	G	-	-	-	-	A	-	-	C	-	-
CRK1	G	-	G	A	T	-	-	A	-	-	C	-	-
CRK2	G	-	G	A	T	-	-	A	-	-	C	-	-
CRK3	G	-	G	A	T	-	-	A	-	-	C	-	-
CRK4	G	-	G	A	T	-	-	A	-	-	C	-	-
CRK5	G	-	G	A	T	-	-	A	-	-	C	-	-
CRK6	G	-	G	A	T	-	-	A	-	-	C	-	-
CRK7	G	-	G	A	T	-	-	A	-	-	C	-	-
CRK8	G	-	G	A	T	-	-	A	-	-	C	-	-
CRK9	G	-	G	A	T	-	-	A	-	-	C	-	-

Appendix 2.2 Haplotype found in the *Catostomus tsiltcoosensis*, *C. macrocheilus*, *C. sp. A*, *C. sp. B* and *C. rimiculus* (continued)

Haplotype	Positions										
	957	960	990	996	1017	1041	1045	1050	1053	1077	1101
TS1	A	C	T	A	G	C	G	T	A	C	A
TS2	-	-	-	-	-	-	-	-	-	-	G
TS3	-	-	-	-	-	-	-	-	-	-	-
TU1	G	-	-	G	-	T	-	C	-	-	G
TU2	G	-	-	G	-	T	-	C	-	-	G
TU3	G	-	-	G	-	T	-	C	G	-	G
TMC1	G	-	-	-	-	T	-	-	-	-	-
TMC2	G	-	-	-	-	T	-	-	-	-	-
CO1	G	G	-	-	-	T	A	-	-	-	-
CO2	G	G	-	-	-	T	A	-	G	-	-
CO3	G	A	-	-	-	T	A	-	G	-	-
CO4	G	G	-	-	-	T	A	-	-	-	-
CO5	G	G	-	-	-	T	A	-	-	-	-
CMW1	G	-	-	-	-	T	-	-	-	-	-
CMW2	G	-	-	-	-	T	-	-	-	-	-
CMW3	G	-	-	-	-	T	-	-	-	-	-
CMW4	G	-	-	-	-	T	-	-	-	-	-
CMW5	G	-	-	-	-	T	-	-	-	-	-
CMW6	G	-	C	-	-	T	-	-	-	-	-
CMW7	G	-	-	-	-	T	-	-	-	-	-
CMW8	G	-	-	-	-	T	-	-	-	-	-
CRR1	-	T	-	-	-	T	A	C	-	T	-
CRR2	-	T	-	-	-	T	A	C	-	T	-
CRR3	-	T	-	-	-	T	A	C	-	T	-
CRR4	-	T	-	-	-	T	A	C	-	T	-
CRR5	-	T	-	-	-	T	A	C	-	T	-
CRR6	-	T	-	-	-	T	A	C	-	T	-
CRR7	-	T	-	-	-	T	A	C	-	T	-
CRK1	-	T	-	G	-	T	A	C	-	T	-
CRK2	-	T	-	G	A	T	A	C	-	T	-
CRK3	-	T	-	G	-	T	A	C	-	T	-
CRK4	-	T	-	G	-	T	G	C	-	C	-
CRK5	-	T	-	G	-	T	A	C	-	T	-
CRK6	-	T	-	G	-	T	A	-	-	T	-
CRK7	-	T	-	G	-	T	G	C	-	T	-
CRK8	-	T	-	G	-	T	A	C	-	T	-
CRK9	-	T	-	G	-	T	G	C	-	T	-

Appendix 2.2.Haplotype found in the *Catostomus tsiltcoosensis*, *C. macrocheilus*, *C. sp. A*, *C. sp. B* and *C. rimiculus* (continued)

Haplotype	Positions	
	1110	1113
TS1	G	A
TS2	-	-
TS3	-	-
TU1	-	-
TU2	-	-
TU3	-	-
TMC1	-	-
TMC2	-	-
CO1	-	-
CO2	-	-
CO3	-	-
CO4	-	-
CO5	-	-
CMW1	-	-
CMW2	-	-
CMW3	-	G
CMW4	-	-
CMW5	-	-
CMW6	-	-
CMW7	-	-
CMW8	A	-
CRR1	A	-
CRR2	A	-
CRR3	A	-
CRR4	A	-
CRR5	A	-
CRR6	A	-
CRR7	A	-
CRK1	A	-
CRK2	A	-
CRK3	A	-
CRK4	A	-
CRK5	A	-
CRK6	A	-
CRK7	A	-
CRK8	A	-
CRK9	A	-

Appendix 2.3 Summary of morphometric measurements as ratios to SL for *Catostomus macrocheilus*, *C. tsiltcoosensis* and *C. sp.* A.

Characters	Species	N	Mean	SD	Minimum	Maximum
LAE	*macrocheilus*	41	0.0991	0.0096	0.086	0.131
	tsiltcoosensis	65	0.1101	0.0077	0.093	0.129
	Species A	31	0.1013	0.0064	0.090	0.116
LPE	*macrocheilus*	41	0.1424	0.0077	0.130	0.168
	tsiltcoosensis	65	0.1479	0.0078	0.125	0.162
	Species A	31	0.1453	0.0068	0.130	0.157
HL	*macrocheilus*	41	0.2290	0.0112	0.204	0.260
	tsiltcoosensis	66	0.2309	0.0101	0.210	0.252
	Species A	31	0.2342	0.0107	0.213	0.253
IW	*macrocheilus*	40	0.0922	0.0049	0.082	0.105
	tsiltcoosensis	59	0.0921	0.0054	0.081	0.104
	Species A	31	0.0886	0.0041	0.082	0.097
WP1	*macrocheilus*	41	0.1468	0.0092	0.124	0.167
	tsiltcoosensis	60	0.1478	0.0060	0.132	0.160
	Species A	31	0.1351	0.0097	0.121	0.158
DP1	*macrocheilus*	41	0.1776	0.0095	0.165	0.206
	tsiltcoosensis	66	0.1596	0.0075	0.140	0.182
	Species A	31	0.1661	0.0110	0.148	0.198
DDO	*macrocheilus*	41	0.2127	0.0127	0.187	0.238
	tsiltcoosensis	66	0.1903	0.0133	0.166	0.224
	Species A	31	0.1950	0.0175	0.158	0.236
LOP2	*macrocheilus*	40	0.5664	0.0135	0.543	0.596
	tsiltcoosensis	65	0.5841	0.0126	0.563	0.615
	Species A	31	0.5718	0.0152	0.521	0.601
LOP1_LOP2	*macrocheilus*	40	0.3400	0.0134	0.314	0.372
	tsiltcoosensis	59	0.3584	0.0151	0.327	0.414
	Species A	31	0.3438	0.0160	0.309	0.373

Appendix 2.3 Summary of morphometric measurements as ratios to SL for *Catostomus macrocheilus*, *C. tsiltcoosensis* and *C. sp.* A. (Continued)

Characters	Species	N	Mean	SD	Minimum	Maximum
LOD	*macrocheilus*	40	0.4991	0.0124	0.477	0.529
	tsiltcoosensis	65	0.5094	0.0111	0.489	0.534
	Species A	31	0.5105	0.0160	0.486	0.547
LID	*macrocheilus*	40	0.6682	0.0131	0.642	0.702
	tsiltcoosensis	58	0.6597	0.0147	0.626	0.709
	Species A	31	0.6501	0.0145	0.622	0.680
LOA	*macrocheilus*	40	0.7758	0.0172	0.739	0.814
	tsiltcoosensis	65	0.7895	0.0165	0.749	0.831
	Species A	31	0.7807	0.0160	0.746	0.809
LIA	*macrocheilus*	39	0.8527	0.0102	0.828	0.877
	tsiltcoosensis	58	0.8663	0.0133	0.831	0.890
	Species A	31	0.8535	0.0185	0.820	0.908
LDA	*macrocheilus*	39	0.3530	0.0143	0.317	0.386
	tsiltcoosensis	65	0.3423	0.0137	0.315	0.376
	Species A	31	0.3413	0.0186	0.299	0.375
LDOC	*macrocheilus*	39	0.5703	0.0165	0.542	0.615
	tsiltcoosensis	58	0.5567	0.0157	0.513	0.600
	Species A	31	0.5531	0.0168	0.516	0.596
LDIC	*macrocheilus*	39	0.3866	0.0140	0.352	0.420
	tsiltcoosensis	58	0.4007	0.0119	0.373	0.424
	Species A	31	0.4092	0.0202	0.356	0.455
CPD	*macrocheilus*	40	0.0694	0.0071	0.051	0.087
	tsiltcoosensis	64	0.0817	0.0059	0.066	0.093
	Species A	31	0.0748	0.0065	0.064	0.088
LP1	*macrocheilus*	39	0.1766	0.0151	0.142	0.204
	tsiltcoosensis	58	0.1660	0.0123	0.131	0.191
	Species A	30	0.1832	0.0183	0.145	0.213

Appendix 2.3 Summary of morphometric measurements as ratios to SL for *Catostomus macrocheilus*, *C. tsiltcoosensis* and *C. sp.* A. (Continued)

Characters	Species	N	Mean	SD	Minimum	Maximum
LP2	*macrocheilus*	39	0.1370	0.0103	0.115	0.160
	tsiltcoosensis	58	0.1302	0.0082	0.112	0.153
	Species A	31	0.1446	0.0165	0.117	0.182
LPEOD	*macrocheilus*	40	0.3770	0.0097	0.347	0.394
	tsiltcoosensis	58	0.3803	0.0107	0.359	0.409
	Species A	31	0.3870	0.0142	0.359	0.419
LPOA	*macrocheilus*	40	0.2169	0.0121	0.184	0.238
	tsiltcoosensis	58	0.2153	0.0109	0.189	0.244
	Species A	31	0.2218	0.0138	0.199	0.254
SPMLL	*macrocheilus*	39	0.0546	0.0075	0.040	0.075
	tsiltcoosensis	46	0.0601	0.0070	0.046	0.076
	Species A	31	0.0540	0.0061	0.039	0.068
LDMM	*macrocheilus*	39	0.0470	0.0060	0.034	0.061
	tsiltcoosensis	58	0.0506	0.0046	0.040	0.061
	Species A	31	0.0509	0.0050	0.043	0.061
GAPLMM	*macrocheilus*	39	0.0290	0.0061	0.012	0.042
	tsiltcoosensis	58	0.0311	0.0043	0.021	0.041
	Species A	31	0.0301	0.0039	0.022	0.038
AIOPAE	*macrocheilus*	39	0.0678	0.0061	0.055	0.078
	tsiltcoosensis	56	0.0754	0.0040	0.067	0.084
	Species A	30	0.0702	0.0052	0.061	0.079
SAIOP	*macrocheilus*	39	0.0384	0.0056	0.028	0.055
	tsiltcoosensis	57	0.0405	0.0052	0.031	0.053
	Species A	30	0.0350	0.0051	0.026	0.047
ED	*macrocheilus*	39	0.0392	0.0078	0.029	0.063
	tsiltcoosensis	56	0.0352	0.0083	0.027	0.060
	Species A	30	0.0436	0.0104	0.027	0.057

Appendix 2.3 Summary of morphometric measurements as ratios to SL for *Catostomus macrocheilus*, *C. tsiltcoosensis* and *C. sp.* A. (Continued)

Characters	Species	N	Mean	SD	Minimum	Maximum
CR-D	*macrocheilus*	40	0.3229	0.0122	0.292	0.348
	tsiltcoosensis	65	0.3213	0.0103	0.299	0.342
	Species A	31	0.3327	0.0142	0.301	0.366
ID-OP2	*macrocheilus*	41	0.2006	0.0145	0.170	0.234
	tsiltcoosensis	65	0.1813	0.0122	0.156	0.214
	Species A	31	0.1749	0.0188	0.141	0.215
OD-IP2	*macrocheilus*	41	0.2356	0.0120	0.214	0.263
	tsiltcoosensis	65	0.2209	0.0121	0.191	0.258
	Species A	31	0.2184	0.0132	0.183	0.244
OD-OP2	*macrocheilus*	41	0.2273	0.0110	0.206	0.247
	tsiltcoosensis	65	0.2096	0.0112	0.183	0.230
	Species A	31	0.2069	0.0159	0.171	0.245

Appendix 2.4 Meristic variation of *Catostomus tsiltcoosensis*, *C. macrocheilus* and *C. sp*. A

Characters	Species	N	Mean	SD	Minimum	Maximum
PCV	*macrocheilus*	42	24.6	0.80	22	26
	tsiltcoosensis	72	25.0	0.66	24	26
	Species A	31	24.3	0.60	23	25
CV	*macrocheilus*	42	19.7	0.84	18	22
	tsiltcoosensis	73	19.2	0.71	18	21
	Species A	31	19.3	0.60	18	20
INTER1DEP	*macrocheilus*	42	10.5	0.97	8	12
	tsiltcoosensis	72	10.2	0.80	9	12
	Species A	31	9.7	0.73	9	11
VDO	*macrocheilus*	42	13.6	0.73	12	15
	tsiltcoosensis	73	14.2	0.69	12	16
	Species A	31	13.8	0.72	12	15
VAO	*macrocheilus*	42	31.4	0.74	30	33
	tsiltcoosensis	72	32.0	0.63	30	33
	Species A	31	31.5	0.81	30	33
VPO	*macrocheilus*	42	19.6	1.06	18	21
	tsiltcoosensis	72	20.0	0.77	18	21
	Species A	31	19.2	0.65	18	20
PREOPMNPOR	*macrocheilus*	42	20.1	3.33	14	29
	tsiltcoosensis	77	18.6	5.32	3	34
	Species A	31	18.0	4.59	12	33
INFORBPOR	*macrocheilus*	42	41.4	4.32	33	50
	tsiltcoosensis	77	29.0	4.90	14	37
	Species A	31	30.7	4.81	19	38
IOPAE	*macrocheilus*	42	13.2	2.74	9	21
	tsiltcoosensis	77	11.3	2.86	4	18
	Species A	31	10.7	2.32	4	17

Appendix 2.4 Meristic variation of *Catostomus tsiltcoosensis*, *C. macrocheilus* and *C. sp*. A (continued).

Characters	Species	N	Mean	SD	Minimum	Maximum
SUPORBPOR	*macrocheilus*	42	20.3	4.08	9	31
	tsiltcoosensis	76	17.2	3.04	12	27
	Species A	31	17.2	2.09	13	22
GILRKRANT	*macrocheilus*	42	29.3	2.02	25	35
	tsiltcoosensis	77	26.7	1.55	23	31
	Species A	31	27.9	1.15	26	30
GILRKPOST	*macrocheilus*	42	37.3	2.89	31	43
	tsiltcoosensis	77	34.6	2.17	27	38
	Species A	31	33	1.88	30	36
PGRVAGR	*macrocheilus*	42	3.2	0.59	2	4
	tsiltcoosensis	77	2.7	0.66	1	4
	Species A	31	2.1	0.54	1	3
LLSCALES	*macrocheilus*	41	71.1	3.49	65	80
	tsiltcoosensis	67	71.7	3.32	65	80
	Species A	31	73.0	3.37	67	79
LLPECTPOR	*macrocheilus*	40	2.9	1.27	0	7
	tsiltcoosensis	66	3.0	1.53	0	8
	Species A	31	3.7	0.79	2	5
TRSCALES	*macrocheilus*	40	25.1	2.18	22	31
	tsiltcoosensis	63	25.5	2.38	21	32
	Species A	31	25.1	1.52	22	29
SCALABVLL	*macrocheilus*	40	12.9	1.02	11	15
	tsiltcoosensis	64	13.1	1.07	11	17
	Species A	31	13.2	0.90	11	15

Appendix 2.4 Meristic variation of *Catostomus tsiltcoosensis*, *C. macrocheilus* and *C. sp*. A (continued).

Characters	Species	N	Mean	SD	Minimum	Maximum
SCALBELOLL	*macrocheilus*	40	9.5	1.04	7	12
	tsiltcoosensis	63	10.3	1.25	8	14
	Species A	31	10.5	1.12	8	13
PAPUPRLIP	*macrocheilus*	40	2.2	0.56	2	5
	tsiltcoosensis	63	2.0	0.18	2	3
	Species A	31	2.1	0.43	2	4
SCACADPED	*macrocheilus*	42	21.2	1.59	19	25
	tsiltcoosensis	67	21.9	2.15	19	28
	Species A	31	22.9	1.95	19	27
PAPLORLIP	*macrocheilus*	40	6.2	1.06	4	8
	tsiltcoosensis	63	5.7	0.95	4	8
	Species A	31	5.7	1.17	2	8
DORSALRAYS	*macrocheilus*	42	13.6	0.73	12	15
	tsiltcoosensis	75	12.2	0.61	11	14
	Species A	31	11.9	0.63	10	13
ANF	*macrocheilus*	40	7.0	0.23	6	8
	tsiltcoosensis	65	7.0	0.18	6	8
	Species A	31	6.9	0.25	6	7
PECTRAYS	*macrocheilus*	40	18.4	0.93	16	20
	tsiltcoosensis	63	17.8	0.77	16	20
	Species A	31	17.8	0.79	16	20
PELVRAYS	*macrocheilus*	40	11.0	0.64	9	12
	tsiltcoosensis	64	10.7	0.68	9	12
	Species A	31	10.6	0.62	9	11

Appendix 2.5 Percent sequence divergence between catostomids that was previously recognized as same species. The percent sequence divergence was estimated from likelihood model GRT+I+G.

Comparisons	Percent sequence divergence		
	Range	Mean	SE
Catostomus sp B vs. *C. rimiculus*	1.25-1.74	1.499	0.015
C. macrocheilus vs. *C. tsiltcoosensis*	1.357-2.467	1.937	0.029
C. macrocheilus vs. *C. sp A*	2.85-3.814	3.366	0.036
C. tsiltcoosensis vs. *C. sp A*	2.63-4.083	3.410	0.058

Appendix 2.6 Summary of morphometric measurements as ratios to SL for *Catostomus rimiculus* and *C. sp. B.*

Characters	Species	N	Mean	SD	Minimum	Maximum
LAE	*rimiculus*	54	0.1067	0.0084	0.088	0.125
	sp. B	45	0.1070	0.0081	0.089	0.122
LPE	*rimiculus*	54	0.1427	0.0078	0.122	0.155
	sp. B	45	0.1433	0.0068	0.131	0.164
HL	*rimiculus*	54	0.2257	0.0109	0.197	0.248
	sp. B	45	0.2265)	0.0101	0.204	0.251
IW	*rimiculus*	53	0.0899	0.0055	0.077	0.102
	sp.B	45	0.0908	0.0042	0.083	0.100
WP1	*rimiculus*	54	0.1533	0.0110	0.127	0.178
	sp. B	45	0.1529	0.0073	0.136	0.173
DP1	*rimiculus*	54	0.1608	0.0099	0.141	0.188
	sp. B	45	0.1628	0.0095	0.143	0.187
DDO	*rimiculus*	54	0.2005	0.0168	0.162	0.231
	sp. B	45	0.2004	0.0168	0.162	0.233
LOP2	*rimiculus*	54	0.5679	0.0175	0.505	0.607
	sp. B	45	0.5743	0.0121	0.543	0.594
LOP1_LOP2	*rimiculus*	54	0.3469	0.0177	0.309	0.397
	sp. B	45	0.3548	0.0155	0.314	0.378
LOD	*rimiculus*	54	0.4984	0.0174	0.457	0.534
	sp. B	45	0.5037	0.0119	0.482	0.541
LID	*rimiculus*	54	0.6342	0.0175	0.600	0.674
	sp. B	45	0.6337	0.0217	0.561	0.694
LOA	*rimiculus*	54	0.7867	0.0169	0.742	0.818
	sp. B	45	0.7776	0.0212	0.727	0.812
LIA	*rimiculus*	54	0.8630	0.0154	0.830	0.892
	sp. B	45	0.8507	0.0185	0.806	0.886

Appendix 2.6 Summary of morphometric measurements as ratios to SL for *Catostomus rimiculus* and *C. sp. B* (continued).

Characters	Species	N	Mean	SD	Minimum	Maximum
LDA	*rimiculus*	54	0.3556	0.0138	0.334	0.389
	sp. B	45	0.3483	0.0162	0.283	0.376
LDOC	*rimiculus*	54	0.5765	0.0171	0.537	0.618
	sp. B	45	0.5725	0.0112	0.548	0.606
LDIC	*rimiculus*	54	0.4304	0.0156	0.397	0.468
	sp. B	45	0.4302	0.0200	0.375	0.469
CPD	*rimiculus*	54	0.0829	0.0079	0.063	0.100
	sp. B	45	0.0831	0.0059	0.065	0.097
LP1	*rimiculus*	54	0.1765	0.0179	0.135	0.207
	sp. B	45	0.1677	0.0152	0.121	0.210
LP2	*rimiculus*	54	0.1331	0.0117	0.109	0.165
	sp. B	45	0.1303	0.0134	0.100	0.170
LPEOD	*rimiculus*	54	0.3762	0.0157	0.337	0.411
	sp. B	45	0.3816	0.0089	0.357	0.399
LPOA	*rimiculus*	54	0.2292	0.0119	0.203	0.255
	sp. B	45	0.2180	0.0173	0.190	0.252
LDMM	*rimiculus*	54	0.0573	0.0082	0.036	0.077
	sp. B	45	0.0561	0.0066	0.046	0.071
GAPLMM	*rimiculus*	54	0.0331	0.0081	0.018	0.051
	sp. B	45	0.0316	0.0071	0.017	0.047
AIOPAE	*rimiculus*	54	0.0679	0.0054	0.057	0.078
	sp. B	45	0.0677	0.0059	0.054	0.079
SAIOP	*rimiculus*	54	0.0452	0.0065	0.031	0.057
	sp. B	45	0.0463	0.0069	0.031	0.061
ED	*rimiculus*	52	0.0341	0.0039	0.028	0.040
	sp. B	38	0.0339	0.0057	0.027	0.043

Appendix 2.6 Summary of morphometric measurements as ratios to SL for *Catostomus rimiculus* and *C. sp. B* (continued).

Characters	Species	N	Mean	SD	Minimum	Maximum
CR-D	*rimiculus*	54	0.3185	0.0169	0.286	0.355
	sp. B	45	0.3294	0.0121	0.301	0.355
ID-OP2	*rimiculus*	54	0.1853	0.0157	0.153	0.229
	sp. B	45	0.1774	0.0131	0.150	0.210
OD-IP2	*rimiculus*	54	0.2284	0.0140	0.191	0.268
	sp. B	45	0.2279	0.0178	0.197	0.258
OD-OP2	*rimiculus*	54	0.2169	0.0146	0.190	0.254
	sp. B	45	0.2164	0.0178	0.176	0.263

Appendix 2.7 Meristic Variation of *Catostomus sp. B* and *Catostomus rimiculus*.

Characters	Species	N	Mean	SD	Minimum	Maximum
PCV	*rimiculus*	58	24.5	0.80	23	26
	sp. B	55	24.8	0.73	23	26
CV	*rimiculus*	58	18.5	1.13	17	21
	sp. B	55	18.7	0.80	18	22
INTER1DEP	*rimiculus*	58	10.3	0.96	9	13
	sp. B	55	10.7	0.80	9	12
VDO	*rimiculus*	58	13.7	0.70	12	16
	sp. B	55	14.5	0.74	12	16
VAO	*rimiculus*	58	31.0	0.92	29	34
	sp. B	55	31.5	0.90	30	34
VPO	*rimiculus*	58	18.8	0.95	17	22
	sp. B	55	19.8	0.91	18	22
GILRKRANT	*rimiculus*	52	23.1	1.71	19	28
	sp. B	45	24.1	1.78	19	28
GILRKPOST	*rimiculus*	52	28.9	2.64	21	34
	sp.B	44	31.8	3.04	23	37
PGRVAGR	*rimiculus*	52	2.8	0.62	2	5
	sp. B	45	2.8	0.75	1	4
PREOPMNPOR	*rimiculus*	51	11.1	2.42	5	18
	sp. B	44	10.4	1.93	6	15
INFORBPOR	*rimiculus*	52	17.8	5.22	10	26
	sp. B	44	17.6	4.44	11	27
IOPAE	*rimiculus*	52	7.8	1.21	5	11
	sp. B	43	7.7	1.39	5	12

Appendix 2.7 Meristic Variation of *Catostomus sp. B* and *Catostomus rimiculus* (continued).

Characters	Species	N	Mean	SD	Minimum	Maximum
SUPORBPOR	*rimiculus*	52	13.2	2.21	9	18
	sp. B	41	12.8	2.01	9	18
LLSCALES	*rimiculus*	52	87.3	4.81	78	98
	sp. B	45	86.3	5.14	70	100
LLPECTPOR	*rimiculus*	53	3.0	1.36	0	6
	sp. B	45	3.5	1.47	0	6
TRSCALES	*rimiculus*	52	30.7	3.04	25	39
	sp. B	44	31.4	3.63	21	41
SCALABVLL	*rimiculus*	52	15.9	1.58	13	20
	sp. B	45	16.5	1.59	14	21
SCALBELOLL	*rimiculus*	52	12.8	1.47	8	17
	sp. B	45	13.0	1.45	10	16
PAPUPRLIP	*rimiculus*	52	2.1	0.35	2	3
	sp. B	45	2.4	0.73	2	5
SCACADPED	*rimiculus*	48	28.2	2.31	23	35
	sp. B	25	29.9	3.45	26	40
PAPLORLIP	*rimiculus*	52	5.9	1.13	4	9
	sp. B	45	6.2	1.22	4	9
DORSALRAYS	*rimiculus*	53	10.9	0.51	10	12
	sp. B	45	11.3	0.84	10	14
ANF	*rimiculus*	50	7.0	0.20	7	8
	sp. B	40	7.0	0.39	5	8
PECTRAYS	*rimiculus*	52	15.5	0.87	13	17
	sp. B	43	17.0	1.29	15	20
PELVRAYS	*rimiculus*	52	9.5	0.73	8	11
	sp. B	44	9.6	1.30	6	11

Appendix 2.8 Haplotypes of *Ptychocheilus umpquae* and *P. oregonensis*. Haplotypes S1-S3 were found in Siuslaw River and Woahink River. Haplotypes S4 and S5 were found in both Siuslaw River and Umpqua River. Haplotypes U1-U5 were only found in Umpqua River. Haplotypes W1-W8 were found in Willamette River.

Haplotype		Position																					
	N	108	141	147	159	198	204	225	300	315	324	364	444	477	580	637	652	699	774	783	798	837	867
S1	1	C	A	G	A	G	T	T	A	G	C	C	C	T	A	T	A	G	A	C	A	G	A
S2	5	C	-	-	G	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S3	2	C	-	-	G	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S4	14	C	-	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S5	2	C	-	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U1	1	C	-	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U2	9	C	-	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U3	1	C	-	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U4	1	C	-	A	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-
U5	1	C	-	A	-	G	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-
W1	2	T	-	A	-	A	C	C	T	A	T	T	-	C	-	-	-	A	C	-	-	C	-
W2	4	T	-	A	-	A	C	C	T	A	T	T	-	C	-	-	-	A	C	-	G	C	-
W3	2	T	-	A	-	A	C	C	T	A	T	T	-	C	-	-	-	A	C	-	-	C	-
W4	1	T	G	A	-	A	C	C	T	A	T	T	-	C	-	G	-	A	C	-	-	C	-
W5	1	T	-	A	-	A	C	C	T	A	T	T	-	C	-	-	-	A	C	-	-	C	-
W6	1	T	-	A	-	A	C	-	T	A	T	T	-	C	-	-	-	A	C	-	-	C	G
W7	1	T	-	A	-	A	C	C	T	A	T	T	T	C	-	-	-	A	C	-	G	C	-
W8	1	T	-	A	-	A	C	C	T	A	T	T	-	C	G	-	-	A	C	-	-	C	-

Appendix 2.8. Haplotype of *Ptychocheilus umpquae* and *P. oregonensis* (continued)

Haplotype	Position											
	951	954	984	990	1023	1026	1045	1047	1053	1065	1076	1080
S1	C	C	G	A	A	T	G	T	T	G	T	G
S2	-	-	-	-	-	-	-	-	-	-	-	-
S3	-	-	-	-	-	-	-	-	-	-	C	-
S4	-	-	A	-	-	-	-	-	-	-	-	-
S5	-	-	A	-	-	-	-	-	-	-	C	-
U1	-	-	-	-	G	-	-	-	-	-	-	-
U2	-	-	-	-	-	-	-	-	-	-	-	A
U3	-	-	-	-	-	-	-	-	-	-	-	-
U4	-	-	-	-	-	-	-	-	-	-	-	-
U5	-	-	-	-	-	-	-	-	-	-	-	-
W1	-	T	-	G	-	C	A	C	-	-	-	A
W2	-	T	-	G	-	C	A	C	-	-	-	A
W3	T	T	A	G	-	C	-	C	C	-	-	A
W4	T	T	A	G	-	C	-	C	A	-	-	A
W5	-	T	-	G	-	C	A	C	C	-	-	A
W6	-	T	-	G	-	C	-	C	-	A	-	A
W7	-	T	-	G	-	C	A	C	-	-	-	A
W8	-	T	-	G	-	C	A	C	-	-	-	A

Appendix 2.9 Summary of morphometric characters of *Ptychocheilus umpquae* and *P. oregonensis* as a ratio to SL.

Characters	Species	N	Mean	SD	Minimum	Maximum
LAE	*umpquae*	72	0.0893	0.0055	0.076	0.103
	oregonensis	31	0.0873	0.0053	0.079	0.098
LPE	*umpquae*	72	0.1435	0.0073	0.131	0.165
	oregonensis	31	0.1385	0.0055	0.131	0.150
HL	*umpquae*	73	0.2782	0.0103	0.248	0.310
	oregonensis	31	0.2771	0.0078	0.259	0.294
IW	*umpquae*	73	0.0709	0.0050	0.061	0.084
	oregonensis	31	0.0734	0.0049	0.065	0.084
WP1	*umpquae*	78	0.1278	0.0095	0.105	0.152
	oregonensis	31	0.1315	0.0101	0.116	0.156
DP1	*umpquae*	78	0.1579	0.0090	0.127	0.181
	oregonensis	31	0.1691	0.0098	0.152	0.193
DDO	*umpquae*	80	0.1856	0.0147	0.146	0.226
	oregonensis	31	0.2017	0.0142	0.182	0.250
LOP2	*umpquae*	78	0.5556	0.0193	0.513	0.600
	oregonensis	31	0.5495	0.0187	0.504	0.578
LOP1_LOP2	*umpquae*	80	0.2849	0.0146	0.246	0.314
	oregonensis	31	0.2836	0.0130	0.265	0.317
LOD	*umpquae*	73	0.5769	0.0140	0.548	0.607
	oregonensis	31	0.5708	0.0111	0.551	0.599
LID	*umpquae*	73	0.6835	0.0113	0.658	0.704
	oregonensis	31	0.6772	0.0134	0.653	0.705
LOA	*umpquae*	72	0.7252	0.0178	0.690	0.766
	oregonensis	31	0.7192	0.0140	0.691	0.745

Appendix 2.9 Summary of morphometric characters of *Ptychocheilus umpquae* and *P. oregonensis* as a ratio to SL (continued).

Characters	Species	N	Mean	SD	Minimum	Maximum
LIA	*umpquae*	73	0.8138	0.0139	0.784	0.846
	oregonensis	31	0.8089	0.0109	0.785	0.828
LDA	*umpquae*	73	0.2277	0.0110	0.197	0.262
	oregonensis	31	0.2400	0.0081	0.223	0.252
LDOC	*umpquae*	73	0.4763	0.0138	0.448	0.519
	oregonensis	31	0.4869	0.0114	0.468	0.515
LDIC	*umpquae*	73	0.3676	0.0140	0.334	0.403
	oregonensis	31	0.3763	0.0073	0.365	0.393
CPD	*umpquae*	73	0.0831	0.0064	0.067	0.098
	oregonensis	31	0.0782	0.0041	0.070	0.085
LP1	*umpquae*	80	0.1757	0.0176	0.147	0.214
	oregonensis	31	0.1653	0.0118	0.140	0.184
LP2	*umpquae*	80	0.1338	0.0108	0.112	0.155
	oregonensis	31	0.1331	0.0077	0.117	0.148
LPEOD	*umpquae*	73	0.4484	0.0118	0.421	0.478
	oregonensis	31	0.4438	0.0099	0.426	0.464
LPOA	*umpquae*	80	0.1781	0.0113	0.146	0.199
	oregonensis	31	0.1812	0.0093	0.165	0.201
ED	*umpquae*	72	0.0530	0.0067	0.041	0.072
	oregonensis	30	0.0487	0.0063	0.040	0.061
CR-D	*umpquae*	73	0.3727	0.0144	0.341	0.415
	oregonensis	31	0.3661	0.0104	0.350	0.393
ID-OP2	*umpquae*	77	0.2145	0.0125	0.181	0.252
	oregonensis	31	0.2226	0.0114	0.202	0.256

Appendix 2.9 Summary of morphometric characters of *Ptychocheilus umpquae* and *P. oregonensis* as a ratio to SL (continued).

Characters	Species	N	Mean	SD	Minimum	Maximum
OD-IP2	*umpquae*	78	0.1738	0.0155	0.132	0.219
	oregonensis	31	0.1884	0.0122	0.168	0.218
OD-OP2	*umpquae*	78	0.1897	0.0155	0.146	0.248
	oregonensis	30	0.2019	0.0109	0.188	0.227

Appendix 2.10 Summary of meristic characters of *Ptychocheilus umpquae* and *P. oregonensis.*

Characters	Species	N	Mean	SD	Minimum	Maximum
PCV	*umpquae*	95	22.17	0.429	21	23
	oregonensis	31	22.26	0.514	21	23
CV	*umpquae*	95	19.56	0.54	19	21
	oregonensis	31	19.65	0.608	18	21
INTER1DEF	*umpquae*	95	13.57	0.613	12	15
	oregonensis	31	13.55	0.568	12	14
VDO	*umpquae*	95	17.32	0.775	14	19
	oregonensis	31	17.13	0.562	16	18
VAO	*umpquae*	95	26.14	0.709	24	28
	oregonensis	31	26.45	0.85	25	28
VPO	*umpquae*	95	16.04	0.771	14	18
	oregonensis	31	15.9	1.012	14	18
GILRKRANT	*umpquae*	73	9.85	1.163	7	13
	oregonensis	31	9.97	0.912	8	12
GILRKPOST	*umpquae*	73	14.99	1.007	11	17
	oregonensis	31	14.16	0.688	13	16
PGRVAGR	*umpquae*	73	1.33	0.554	0	2
	oregonensis	31	1.26	0.575	0	2
PREOPMNPOR	*umpquae*	73	23.14	2.874	13	35
	oregonensis	31	27.94	4.487	15	37
INFORBPOR	*umpquae*	73	30.53	3.72	17	37
	oregonensis	31	34.52	4.289	25	43
IOPAE	*umpquae*	73	10.21	1.675	4	14
	oregonensis	31	11.23	1.146	9	13

Appendix 2.10 Summary of meristic characters of *Ptychocheilus umpquae* and *P. oregonensis* (continued).

Characters	Species	N	Mean	SD	Minimum	Maximum
SUPORBPOR	*umpquae*	73	16.88	2.489	10	22
	oregonensis	31	18.39	2.883	12	25
LLSCALES	*umpquae*	73	74.19	2.675	69	85
	oregonensis	31	71.68	3.525	62	79
LLPECTPOR	*umpquae*	73	3.77	1.1	2	7
	oregonensis	31	4.9	1.106	3	7
TRSCALES	*umpquae*	73	30.59	2.229	26	37
	oregonensis	31	25.94	1.482	22	29
SCALABVLL	*umpquae*	73	19.05	1.201	16	22
	oregonensis	31	16.03	0.948	14	18
SCALBELOLL	*umpquae*	73	13.44	1.236	11	18
	oregonensis	31	11.03	0.912	9	12
SCACADPED	*umpquae*	73	38.15	2.498	31	44
	oregonensis	31	30	1.461	27	34
DORSALRAYS	*umpquae*	80	9.03	0.157	9	10
	oregonensis	31	9	0	9	9
ANF	*umpquae*	80	8	0.225	7	9
	oregonensis	31	7.97	0.18	7	8
PECTRAYS	*umpquae*	73	16.1	1.095	11	18
	oregonensis	31	16.06	0.998	12	17
PELVRAYS	*umpquae*	73	9.03	0.372	8	10
	oregonensis	31	8.97	0.18	8	9

Chapter 3

Biogeography of primary freshwater fishes in the Oregon Coastal Subprovince based on the phylogenetic relationships of *Catostomus* and *Ptychocheilus*

Abstract

There are three main areas of fish diversity in Oregon coastal rivers: the Columbia Subprovince, the Oregon Coastal Subprovince (Siuslaw-Umpqua-Coos) and the Klamath Subprovince. The direction of dispersal of primary freshwater fishes in the Oregon Coastal Subprovince has previously been explained as either origination in or dispersal from the coastal river (McPhail and Lindsey, 1986) or origin and dispersal from the Willamette River (Minckley et. al, 1986).

Phylogenies of two coastal genera *Catostomus* (Catostomidae) and *Ptychocheilus* (Cyprinidae) were analyzed using DIVA (Dispersal Vicariance Analysis) to test these alternative hypotheses. In both phylogenies, Columbia taxa were sister to Oregon Coastal Subprovince taxa. In the *Catostomus* phylogeny, *C. sp. A* (Coquille River) was basal to both, a pattern that was not present in *Ptychocheilus*. If both groups shared a common history, two vicariant events would be responsible for the basic patterns. The more ancient vicariance was a Columbia-Sacramento event and the more recent, within the Columbia, was a Columbia Subprovince – Oregon Coastal Subprovince. The sucker phylogeny also suggested an intervening event when the Coquille taxon separated prior to the separation of the Oregon Coastal Subprovince. Neither phylogeny required dispersal to explain the pattern and the divergence times among the major nodes were approximately supported by the geological evidence.

Introduction

The diversity of primary freshwater fishes in Oregon coastal river systems has a peculiar pattern. Most coastal rivers and streams have a low diversity of primary freshwater fishes (one to three species) but three areas have relatively high diversity: the Columbia subprovince (23 species), the Oregon Coastal Subprovince (Siuslaw-Umpqua-Coos rivers) (7 species) and the Klamath Subprovince (10 species) (McPhail and Lindsey, 1986; Minckley et al., 1986; Snyder, 1908). The high diversity in the Columbia Subprovince and Klamath Subprovince are expected and related to their size and stability, and past connections with other large rivers such as the Snake River (Smith, 1975; Wheeler and Cook, 1954; Taylor and Smith, 1981; Repenning et al., 1995). The relatively high diversity of primary freshwater fishes in the small to moderate drainages of the Siuslaw-Umpqua-Coos in the Oregon Coastal Subprovince and the low diversity in the moderate sized Rogue River in the Klamath Subprovince are not so easily explained.

The Oregon Coastal Subprovince (from the Miami River in the north to the Sixes River in the South) shares five genera of primary freshwater fishes with the Columbia system (*Catostomus*, *Oregonichthys, Ptychocheilus, Richardsonius* and *Rhinichthys*) and two with the Klamath (*Catostomus* and *Rhinichthys*) (Minckley et al., 1986; McPhail and Lindsey, 1986; Markle et al., 1991). The seven species in the subprovince are *Catostomus tsiltcoosensis*, *Oregonichthys kalawatseti, Ptychocheilus umpquae*, *Richardsonius siuslawi*, *Rhinichthys cataractae*, *Rh. evermanni* and *Rh. osculus*.

Some inferences about phylogenetic relationships of these species suggest the connection with Columbia Subprovince rather than the Klamath subprovince connections. Three genera, *Oregonichthys, Ptychocheilus* and *Richardsonius* are not

found in the Klamath River (Markle et al., 1991) nor is the *Rh. evermanni* - *Rh. cataractae* clade (Woodman, 1992). *Rhinichthys osculus* and *Catostomus* are found throughout the Columbia and Klamath rivers and the Oregon Coastal Subprovince.

There is also some suggestion of taxon differentiation within the Oregon Coastal Subprovince. Within *Rhinichthys*, the subprovince contains two taxa, *Rh. evermanni* and a disjunct *Rh. cataractae* in the Coos River that is morphologically distinct from other *Rh. cataractae* (Bisson and Reimers, 1977). Mayden et al (1991) found differences within *P. umpquae* between the Umpqua and Siuslaw rivers. They did not include *P. umpquae* (Siuslaw) in their phylogenetic analysis. Their data suggested placement of *P. umpquae* (Siuslaw) at the deepest node in the genus. In Chapter 2, I found two forms of *Catostomus*; one restricted to the Coquille River and the other widespread between the Coos and Siuslaw rivers.

Two theories attempt to explain the pattern of Oregon Coastal subprovince fishes through directional dispersal. Minckley et al (1986) and Bond (in Mayden et al, 1991) proposed isolation from the Willamette while McPhail and Lindsey (1986) suggested fishes in the Oregon coastal Subprovince were ancient and recently invaded the Willamette. Geological evidence of capture of the Long Tom River, a former Siuslaw River tributary, by the Willamette River in the Late Pleistocene (Baldwin and Howell, 1949) provides a mechanism supporting McPhail and Lindsey. In contrast, most of the mainstem of the Umpqua River was hypothesized to be a Willamette River tributary that was captured by a westward flowing stream between the Pliocene and the Pleistocene (Diller, 1915; Baldwin, 1959), providing a transfer mechanism from the Willamette to the Oregon Coastal Subprovince in support of Minckley et al (1986).

Phylogenetic relationships have been used in the study of biogeography. Hennig (1966) was the first person who used phylogenetic relationship in the study of biogeography. He suggested that there is a close relationship between taxa and areas they occupied. Humphries and Parenti (1999) explained the chorological method used by Hennig as follows: "The chorological method considered a group to be monophyletic if supported from synapomorphies and the shapes of the cladogram topologies followed clear dispersal patterns from 'center of origin' or exhibited sequential vicariance patterns". Humphries and Parenti (1999) further interpreted the property of chorological species (Hennig, 1966) as each species having a unique dispersal pattern and each species having and an independent history. Brudin (1966 and 1972) used Hennig's method to study biogeography of chironomid midges. Ross (1974) used Hennig's method to study caddisfly biogeography. Regardless of an attempt by Hennig, Brudin and Ross to superimpose the areas on to the phylogenies and assumed least dispersals for each group, their method relies on an *ad hoc* assumption that members of groups disperse from a 'center of origin'. Instead of assuming that certain areas are empty and later colonized by taxa from other area, Nelson, Platnick and Rosen included an alternative vicariance explanation (Platnick and Nelson, 1978; Rosen, 1976) which explained disjunction by the occurrence of barriers fragmenting ancestral species range.

Primary freshwater fishes are restricted to freshwater and therefore, they are a prime candidate for paleohydrology studies. Phylogenetic relationships of the primary freshwater fishes have been used to address dispersal and related paleohydrology issues (Smith et al., 2002; Oakey et al., 2004). Distribution data alone is not sufficient to decisively determine the process that resulted in current distribution (Humphries and

Parenti, 1999). What is important is the conformation of the distribution data with the generalized pattern shown by the relationship of other groups. The corroboration of one pattern by another suggests that biota share history (Platnick and Nelson, 1978). For the Oregon Coastal Subprovince, *Catostomus* allows biogeographical tests with the Klamath and Columbia systems and *Ptychocheilus* with the Columbia. Because these genera represent the diversity of distribution patterns within the subprovince, results from this study should be generalizable to other taxa.

Catostomids are classified in three subfamilies: the Ictiobinae, the Cycleptinae and the Catostominae. Ferris and White (1978) supported monophyly of the subfamilies and placed Ictiobinae sister to Cycleptinae-Catostominae. Within Catostominae, they recognized *Erimyzon* as a separate tribe (Erimyzonini) from Catostomini and Moxostomatini. When developmental characters were used to construct a phylogeny, monophyly of Ictiobinae, Catostominae, Catostomini and Moxostomatini was not supported (Fuiman, 1985). In his tree, *Erimyzon oblongus* was embedded with Ictiobine. Paraphyletic Ictiobinae was basal to Cycleptinae, which was sister to a paraphyletic Catostominae. Smith (1992) used 157 morphological characters, life history, and biochemical characters in a phylogeny that support monophyly of each subfamily. Harris and Mayden (2001) inferred catostomid phylogeny based on mitochondrial SSU and LSU rDNA. They suggested a new subfamily, Myxocyprininae for the Chinese sucker (*Myxocyprinus asiaticus*), obtaining conflicting results for monophyly of Cycleptinae supported monophyly of Catostomini, and suggesting new tribe Erimyzonini. Sun et al (2007) inferred catostomid phylogeny based on mitochondrial cytochrome b and nuclear 18S-ITS1-5.8S DNA sequences and did not support monophyly of Catostominae,

Catostomini, or Moxostomatini.

The relationships of *Ptychocheilus* have been hindered by a rooting problem (Carney and Page, 1990; Mayden et al, 1991 and Smith et al, 2002). Carney and Page (1990) noted that if the tree was rooted with a lower meristic count outgroup, a morphological based phylogeny *P. oregonensis* and *P. umpquae* will be basal groups while a higher meristic count outgroup, places *P. lucius* and *P. grandis* as basal groups. When *Mylopharodon conocephalus* was in the ingroup and a morphologically-based tree rooted with *Hesperoleucus symmetricus*, *P. oregonensis* and *P. umpquae* were again a basal pair (Mayden et al 1991). A cytochrome b phylogeny of Great Basin cyprinids suggested that *Ptychocheilus* was polyphyletic, and with fossil evidence, the possibility that genus is paraphyletic (Smith et al 2002).

The purpose of my study was 1) to investigate the relationship of *Catostomus* and *Ptychocheilus* in the Oregon Coastal Subprovince using cytochrome b sequence data, and 2) to address competing theories to explain their distribution patterns.

Materials and Methods

DNA extraction and amplification

I examined specimens from five Oregon coastal river systems: the Columbia-Willamette River, the Siuslaw River, the Umpqua River, the Coos River, and the Coquille River (Figure 3.1). I examined specimens from Woahink Lake, a coastal freshwater Lake located between the Siuslaw River and the Umpqua River. I also examined specimens from two drainages outside of the Oregon Coastal Subprovince: the Rogue River and the Klamath River (Figure 3.1). The entire cytochrome b sequence was sequenced for all specimens listed (Table 3.1). Some tissues from the OSU fish tissue

collection have no carcass deposited in the OSU fish collection. Additional sequences were complied from Gen Bank (*Catostomus tahoensis* (AF454874), *C. catostomus* (AF454871), *C. occidentalis* (AF454873), *Cyprinus carpio* (AY347295.1), *Deltistes luxatus* (AF454870), *Moxostoma anisurum* (AF454881), and *Mylocheilus caurinus* (AF117169)).

DNA was extracted from ethanol preserved specimens by using a Qiagen DNeasy Tissue Kit (Catalog No. 69504). The mitochondrial cytochrome b gene was amplified from genomic DNA with primers L14724 (5′-GTGACTTGAAAAACCACCGTTG-3′; Schmidt and Gold, 1993) and H15915 (5′-AACTGCAGTCATCTCCGGTTTACAAGA C-3′; Irwin et al., 1991).

PCR reaction consisted of 0.5 µg genomic DNA; 5 µL 10x buffer (0.1 M tris-HCL pH 8.5, 0.015 M MgCl2, 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, and deionized water added for a final volume of 50 µL. 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. The annealing temperature was 45° C for *Ptychocheilus* and 50° C for *Catostomus*.

Double stranded DNA was purified with a Qiagen QIAquick PCR purification kit (Catalog number 28106) and sent to Macrogen Inc (Korea) for sequencing. Double strand catostomid DNA was sequenced with primers trimL14724 (5′-GTGACTTGAAAAACCAC-3′; modified from Schmidt and Gold, 1993), trimH15919 (5′-AACTGCAGTCATCTCCGGTTTAC-3′; Irwin et al., 1991), L15424 (5′-ATTTCTTTCCACCCATACTTTTC -3′; modified from Edward et al. 1991 and H15149

(5′-AAACTGCAGCCCCTCAGAAATATTTGTCCTCA -3′;(Kocher et al., 1989). Double strand cyprinid DNA was sequenced with primers trimL14724, trimH15919, L479496 (5′-TTGTYCAATGAATCTGAG-3′), H600615 (5′- TCGATCCGGTTTCGTG -3′), L531546 (5′- ATTCTTCGCCTTCCAC -3′) and H636652 (5′- TTTTATCCGCATCAGAG -3′).

Phylogenetic analysis

DNA sequences were assembled and edited in SeqEd v1.0.3 (Applied Biosystems, Inc., Forest City, USA), and aligned by eye in PAUP*(Swofford, 1998). Phylogenetic relationships were estimated using maximum parsimony (MP) and maximum likelihood (ML) using PAUP* (Swofford, 1998). The heuristic method (1000 random additional sequences with tree bisection reconstruction for MP and 100 for ML) was used to generate trees for both MP and ML. Non parametric bootstrap analysis with 1000 pseudoreplicates and 100 random additional sequences were conducted for MP. ML bootstrap analysis used 200 pseudoreplicates and 100 random additional sequences. If there was more than one parsimonious tree, the consensus topology of the most parsimonious tree was used as a result for MP.

There are currently five main methods for finding whether molecular sequences contain phylogenetic signal: 1) randomization or permutation tests (Archie, 1989 and Faith, 1991); 2) use of the g1 statistic for measuring skewness of tree lengths of alternative trees (Swofford, 1998); 3) relative apparent synapomorphy analysis (RASA), in which a measure of the rate of increase of cladistic similarity among pairs of taxa as a function of phenetic similarity is tested relative to a null equiprobable rate of increase (Lyons-Weiler et al., 1996); 4) a frequency-dependent significant test; and 5) an index of

substitution saturation, based on the notion of entropy theory. Methods 1 -3 suffer from the problem that, as long as we have two closely related species, the tests will lead us to conclude the presence of significant phylogenetic signal even if all the other sequences have experienced full substitution saturation (Xia et al., 2003). Method 4 is computationally clumsy with more than four taxa, associates specifically with the parsimony method, and has not been developed further since its introduction (Xia et al., 2003). In this study, an index of substitution saturation (Iss; Xia et al., 2003) was calculated in DAMBE (Xia and Xia, 2001) to assess phylogenetic signal.

Modeltest 3.7 (Posada and Crandall, 1998) was used to estimate the model of DNA substitution most appropriate for the data set under maximum likelihood criteria. The selected model for the catostomid data set was general time reversible with some sites assumed to be invariable. Variable sites followed a gamma distribution (GTR+I+G). Maximum likelihood settings were: nucleotide frequencies, A = 0.2925, C = 0.3018, G = 0.1213 and T = 0.2844; rate matrix, A-C= 0.8754, A-G = 68.2602, A-T = 0.4872, C-G = 2.4331, C-T = 11.0539, G-T = 1.0000; Proportion of invariable sites (I) = 0.5694; Gamma distribution shape parameter = 1.1263.

The selected model for cyprinid data was Tamura-Nei with some sites assumed to be invariable (TrN+I). Maximum likelihood settings were: nucleotide frequencies, A = 0.2918, C = 0.3058, G = 0.1281 and T = 0.2743; rate matrix, A-C= 1.0000, A-G = 23.3055, A-T = 1.0000, C-G = 1.0000, C-T = 10.5666, G-T = 1.0000; Proportion of invariable sites (I) = 0.6628; Gamma distribution shape parameter = equal rates for all sites.

The ingroup for *Catostomus* analysis was *Catostomus macrocheilus*, *C. tsiltcoosensis*, *C. sp. A*, *C. catostomus, C. occidentalis, C. tahoensis, C. snyderi, C. rimiculus, C. sp. B, Chasmistes brevirostris* and *Deltistes luxatus.* The outgroup was *Moxostoma anisurum* and *Cyprinus carpio*. The ingroup for the *Ptychocheilus* analysis was *Ptychocheilus oregonensis*, *P. umpquae*, *P. lucius* and *P. grandis* and the outgroup was *Mylocheilus caurinus* and *Cyprinus carpio*.

Estimating divergent time

Divergence time was estimated using nonparametric rate smoothing (NPRS) and a molecular clock calibration for cytochrome b. For NPRS maximum likelihood phylograms were imported into TreeEdit v1.0 (Rambaut and Charleston, 2002) and branch lengths transformed based on nonparametric rate smoothing (Sanderson, 1997). Ages at each node were superimposed by fixing the age at the deepest node based on the oldest catostomid fossil (Paleocene, 62 million years ago (Ma) (Wilson, 1980)) or *Ptychocheilus* fossil (Miocene, 15 Ma (Smith et al., 2000; Smith, 1981)).

Some heterogenitity in evolutionary rates is attributed to body size and habitat temperature (Estabrook, 2007). Smith et al. (2008) reported that the evolutionary rate by Smith et al (2002) were calculated from larger body northern cyprinids which could underestimate the evolutionary rates of smaller body southern fishes. The evolutionary rate for cyprinids suggested by Smith et al. (2002) are suitable for the fishes (larger body northern fish) used in this study. A molecular clock calibration of 1% per million years (Smith et al, 2002) was applied to pairwise sequence divergence data calculated using PAUP* (Swofford, 1998) and the model selected by Modeltest 3.7 in the likelihood analysis.

Biogeography study

To evaluate the historical biogeography, I optimized the known distribution of each species on to the shortest tree using DIVA 1.1 (Ronquist, 1997). DIVA infers ancestral distribution using a cost matrix derived from a simple biogeographic model. It does not require a hypothesis of area relationships. Catostomids were coded for presence and absence for the following areas: Columbia + Willamette, Umpqua + Siuslaw + Coos, Coquille, Rogue, Klamath, Sacramento and Lahontan. Cyprinids were coded for presence and absence for the following areas: Columbia + Willamette, Umpqua + Siuslaw, Sacramento and Colorado. DIVA can only optimize a fully resolved tree. Therefore, unresolved topologies were modified because DIVA requires fully resolved topologies.

Results

Catostomus phylogenetic analysis

A total of 1042 base positions were included in the analysis. Of these, 199 were informative, 148 were parsimony uninformative and 695 were constant. Of the 199 informative characters, 20 were first position and four were second position. The index of substitution saturation (Iss = 0.154) was significantly lower ($P < 0.0001$) than the critical index of substitution saturation Iss.c for symmetrical trees (IssSym = 0.749) or for asymmetrical trees (IssAsym = 0.449). This suggested that the sequence contain significant phylogenetic information and there was little saturation in the sequences. MP analysis yielded 18 trees with 602 steps, consistency index (CI) = 0.7027, retention index (RI) = 0.9523 and rescaled consistency index (RC) = 0.6692. The consensus topology of the MP analysis was 605 steps, CI = 0.6992, RI = 0.9515 and RC = 0.6653. The

relationship from the maximum likelihood analysis has the likelihood score of 4143.1037. The consensus topology of the 18 MP trees and the topology from the ML analysis were similar (Figures 3.2-3.3). Different topologies were seen in relationships among *C. tahoensis*, *C. columbianus*, *C. tsiltcoosensis, C. sp. A* and *C. macrocheilus*. In the MP tree, *C. sp. A* was sister to *C. tahoensis*, *C. columbianus, C. tsiltcoosensis and C. macrocheilus. Catostomus tahoensis* and *C. columbianus* were sister to *C. macrocheilus* and *C. tsiltcoosensis* (Figure 3.2). In the ML tree, relationships among *C. sp A.*, *C. tahoensis-C. columbianus* and *C. macrocheilus-C. tsiltcoosensis* was unresolved (Figure 3.2 and Figure 3.3). From now on the discussion is based on the MP tree because it provided more resolution than ML tree to the discussion. As currently recognized, *C. macrocheilus* (*sensu lato*) is paraphyletic since *C. tahoensis* and *C. columbianus* were contained within *C. macrocheilus* (*sensu lato*) clade (Figure 3.2).

Each drainage formed its own monophyletic group with strong support (94-100% bootstrap support) in MP analysis (Figure 3.2). The topology of the MP tree consisted of two major clades: a northern clade and a southern clade. The northern clade consisted of Coquille *C. sp A.*, Siuslaw-Umpqua-Coos *C. tsiltcoosensis*, Willamette-Columbia *C. macrocheilus* and *C. columbianus*, and Lahontan *C. tahoensis*. Coquille *C. sp A.* was sister to *C. tsiltcoosensis*, *C. macrocheilus*, *C. tahoensis* and *C. columbianus*. *Catostomus tahoensis* and *C. columbianus* were sister to *C. macrocheilus* and *C. tsiltcoosensis* in the MP analysis (Figure 3.2). *Catostomus macrocheilus* (Columbia River and Willamette River) was sister to *C. tsiltcoosensis* (Siuslaw River, Woahink Lake, Umpqua River, Coos River and Millicoma River). Siuslaw *C. tsiltcoosensis* was sister to Coos-Millicoma River *C. tsiltcoosensis* and Umpqua River *C. tsiltcoosensis*. The southern

clade consisted of northern California *C. occidentalis*, Rogue *C. sp. B* and Klamath's four species of suckers. Northern California *C. occidentalis* was basal to Rogue *C. sp B* and Klamath suckers. Rogue *C. sp B* was sister to Klamath suckers (Figure 3.2).

Catostomus divergence time estimation

The divergence times estimated by NPRS were older than the molecular clock calibration (Table 3.2 and Figure 3.4). Percent divergence estimated from PAUP ranged from 1.25 (Rogue vs. Klamath) to 17.21 (Coquille vs. Klamath). Average percent sequence divergence in the catostomid analysis was 10.32%. Differences between the estimates of divergence times from the two methods were generally greater at shallow nodes. Depending on method, divergence time for the Oregon Coastal Subprovince was Miocene to Pleistocene, 0.68 Ma to 8.57 Ma. Divergence time between Coquille and other areas was also Miocene to Pleistocene, 1.31 Ma to 9.44 Ma. Divergence time between the northern and southern clades was Miocene, 6.07 Ma to 12.90 Ma. Divergence time between Northern California and Rogue-Klamath was Early Miocene to Pliocene, 2.79 Ma to 5.34 Ma. The divergence time between Rogue and Klamath ranged from 0.62 Ma to 2.90 Ma (Middle Pliocene to Middle Pleistocene).

Catostomus biogeographic analysis

A parsimony tree with major nodes resolved was used in DIVA and suggested the common ancestor of catostomids had a wide distribution. Except for the cladogenesis between the common ancestor of *C. tsiltcoosensis* and *C. macrocheilus* and the common ancestor of *C. columbianus* and *C. tahoensis*, cladogenesis was always the result of

vicariance. DIVA could not resolve whether the former event was dispersal from the Columbia to the Lahontan or the opposite (Figure 3.5).

Ptychocheilus phylogenetic analysis

A total of 1041 base positions were included in the analysis. Of these, 137 were parsimony informative characters, 751 were constant and 153 were parsimony uninformative. Of the 137 parsimony informative characters, 23 were first position and four were second position. The index of substitution saturation (Iss = 0.051) was significantly lower ($P < 0.0001$) than the critical index of substitution saturation Iss.c for symmetrical trees (IssSym = 0.748) or for asymmetrical trees (IssAsym = 0.449). This suggested that the sequence contain significant phylogenetic information and there was little saturation in the sequences. MP analysis yielded a single tree with 394 steps. The parsimony tree has CI = 0.8503, RI=0.8778, and RC=0.7464. The relationship from the maximum likelihood analysis has the likelihood score of 3194.2646. The topologies of the relationship yielded by the maximum parsimony and maximum likelihood were identical (Figure 3.6 and Figure 3.7).

Each species of *P. umpquae* and *P. oregonensis* was monophyletic (bootstrap support 98-100% in MP analysis and 87%-93% in ML analysis). Siuslaw *P. umpquae* did not form a separate group from Umpqua *P. umpquae*. *Ptychocheilus umpquae* was sister to *P. oregonensis*. *P. lucius* was sister to *P. umpquae* and *P. oregonensis*. *P. grandis* was sister *P. lucius*, *P. oregonensis* and *P. umpquae*. The support for the placement of *P. lucius* and *P. grandis* was weak (59% for *P. lucius* and 53% for *P. grandis* in MP analysis and less than 65% for *P. lucius* and 69% for *P. grandis* in ML analysis) (Figure 3.6 and Figure 3.7).

Ptychocheilus divergence time estimation

Divergence times estimated by NPRS method were again older than molecular clock calibration (Table 3.3and Figure 3.8). The percent pair wise sequence divergence estimated from PAUP ranged from 1.73 (Siuslaw-Umpqua vs. Willamette) to 11.38% (Willamette vs. Northern California). Average percent sequence divergence in the *Ptychocheilus* analysis was 8.73%. Differences between the estimates of divergence times between the two methods were generally greater at the shallow nodes. Depending on methods divergence time between Siuslaw-Umpqua and Willamette ranges was Miocene to Pleistocene, 0.86 Ma to 5.43 Ma. Divergence time between Siuslaw-Umpqua- Willamette and Colorado was Miocene to Pliocene, 4.58 Ma to 11.07 Ma. Divergence time between Siuslaw-Umpqua-Willamette-Colorado and Northern California ranges was Miocene to Pliocene, 5.08 Ma to 13.71Ma (Table 3.3 and Figure 3.8).

Ptychocheilus biogeographic analysis

DIVA suggested that the *Ptychocheilus* ancestor had a wide distribution. All of the cladogenesis in the Ptychocheilus analysis were caused by vicariant events (Figure 3.9).

Discussion

The biogeography of primary freshwater fishes in the Oregon Coastal Subprovince could have separate or shared histories. If these taxa have separate histories, DIVA suggested that the biogeography of *Catostomus macrocheilus* in the Oregon Coastal Subprovince could be explained by vicariance. There are seven major

cladogenetic events: northern clade vs. southern clade, *Catostomus occidentalis* vs. *C. sp. B - C. rimiculus*, *Catostomus sp. B* vs. *C. rimiculus*, *Catostomus sp A. vs. C. columbianus – C. tahoensis - C. tsiltcoosensis - C. macrocheilus*, *C. columbianus – C. tahoensis* vs. *C. tsiltcoosensis - C. macrocheilus - C. columbianus* vs. *C. tahoensis* and *C. macrocheilus* vs. *C. tsiltcoosensis*.

If two taxa have separate history, DIVA suggested that the biogeography of *Ptychocheilus* could be explained by vicariance. There are four major cladogenetic events: *Ptychocheilus grandis* vs. *P. lucius - P. oregonensis - P. umpquae*, *P. lucius* vs. *P. umpquae – P. oregonensis* and *P. umpquae* vs. *P. oregonensis*.

If *Catostomus* and *Ptychocheilus* shared part of the history, vicariance theory also explained the pattern found in both groups.

Northern clade vs. Southern clade

This is the oldest cladogenetic event in this study. A similar ancient break also occurred in other organisms (Smith et al., 2002). The connection between the northern clade and southern clade was explained by the course of the former Snake River that was sequentially disconnected with Willamette-Columbia system and with Klamath system and Sacramento River system.

Previous investigators hypothesized that the Snake River had connections with the Klamath River and the Sacramento River based on the similarity of the composition of the living fauna and fossils records (Hershler and Liu 2004; Hubbs and Miller, 1948; Repenning et al., 1995; Smith et al, 2002; Smith et al., 2000; Taylor, 1960; Taylor, 1985; Taylor and Smith, 1981). Taylor and Smith (1981) and Smith (1985) suggested a course

that the former Snake River took to Northern California from Lake Idaho through Southern Oregon to the Sacramento River via the Pit River.

Repenning (1985) and Wagner et al. (1997) proposed an alternate route from the one used by Taylor and Smith (1981) and Smith (1975). They suggested that the former Snake River started from Twin Falls went across northeastern Nevada to the general area of the Humboldt River, which is west to the Black Rock desert of western Nevada. Then it went through Alturas Lake Basin and merged with the Pit River in Pit River meadow. Repenning (1985) and Wagner et al (1997) proposed this alternative route because they believed that it was unlikely that the former Snake River could go through southern Oregon. According to them, southern Oregon has been a high land since 15 Ma. The connection between the former Snake River, the Klamath River and the Sacramento River could occur as late as Pliocene and disappeared in early Pleistocene (Wagner et al, 1997). This alternative route was support by gastropod phylogeny (Hershler and Liu, 2004).

The disconnection among the former Snake River, the Klamath River and the Sacramento River was caused by the extrusion of large volumes of basalt of the Snake River plain and the Modoc plateau in the Pliocene (4.8 Ma). This extrusion created a topographic high, which when coupled with the uplift of the Klamath Mountains prevented the former Snake River to flow westward across the northwestern Nevada into Sacramento River (Wagner et al, 1997). Furthermore, Snake River was captured by the Columbia River in Late Pliocene (2.8 Ma) (Smith et al., 2000). The river capture event resulted in the disruption of the course of the former Snake River, which ultimately resulted in the disconnection among the Klamath River, the Sacramento River and the

former Snake River. The relative time in which the three drainages (Sacramento River, Klamath River and former Snake River) disconnected from each other was unclear. Based on the similarity of fish fauna (both fossil and living), Smith (1975) suggested the connection between the Sacramento River and the former Snake River was older than the connection between the Klamath River and the former Snake River. He suggested that the establishment of the connection between the Klamath River and the former Snake River was the same age as Deer Butte formation (Miocene to Pliocene).

In this study, *Catostomus occidentalis* (Sacramento River) was sister to *C. sp. B* (Rogue River) and *C. rimiculus* (Klamath River). This topology suggested that the three systems (Klamath River, Sacramento River and Rogue River) were once connected. The relationship of the catostomids suggested that Sacramento River was the first to disconnect from the Rogue River and the Klamath River; followed by the disconnection between the Rogue River and the Klamath River. The geological evidence suggested that the former Snake River was once connected to both the Klamath River and the Sacramento River. Due to the limited interpretation from a three taxa statement, this study did not provide the clarification of the relative disconnection time between Sacramento River-former Snake River and Sacramento River-Klamath River. This study provided a confirmation of the close relationship between Sacramento River and Klamath River.

The divergence time of the Northern clade vs. the Southern clade from the relationship of *Catostomus* ranges from 6.07 Ma to 12.9 Ma (middle to late Miocene). Smith (1975) suggested that drainage connection between the former Snake River and the Klamath River was prior to 8 Ma. My results also suggested that the connection between

the two drainages existed more than 8 Ma. The estimated time for the vicariance event that separated the former Snake River from the Sacramento River and the Klamath River from this study was older than previously reported by Repenning (1985), Wagner et al. (1997) and Smith et al. (2000). DIVA suggested that the separation of northern clade and southern clade was a vicariant event in the late-middle Miocene not in the late Pliocene like it was reported in previous studies (Repenning, 1985; Wagner et al., 1997 and Smith et al., 2000).

Catostomus occidentalis vs. *C. sp. B - C. rimiculus*

The next cladogenetic event in the southern clade was *C. occidentalis* vs. the common ancestor of *C. sp. B* and *C. rimiculus*. *Catostomus occidentalis* are found in the Sacramento River drainage. *Catostomus sp. B* are found in the Rogue River and *C. rimiculus* are found in the Klamath River (Figure 3.10). Robins and Miller (1957) suggested that Klamath River was connected to Pit River. Then it was later disconnectted from the Pit River prior to the establishment of the connection between the Pit River and the Sacramento River. They postulated that the headwater erosion of the Pit River was the cause of the connection between the Pit River and the Sacramento River. However, they did not provide any geological evidence of the vicariance event that separated the Klamath River from the Pit River. Wagner et al (1997) also suggested that the Upper Klamath Lake was connected to the Pit River (in Wagner et al (1997)'s figure 3). The disconnection between the Klamath River and the Pit River (tie to Sacramento system) was probably caused by crustal stretching (Orr and Orr, 2000), the uplift of the Klamath Mountain (Wagner et al., 1997) and the Basalt extrusion of the Modoc plateau (Wagner et al., 1997) in the time frame between Miocene to Pliocene. The results from this study

supported the close relationship of the Sacramento River and Klamath River. In this study, *Catostomus occidentalis* was sister to *C. sp. B* and *C. rimiculus*. DIVA suggested that the Sacramento River was disconnected from the Klamath River by a vicariant event around 2.79 Ma to 5.34 Ma (Pliocene to late Miocene), which was in a similar time frame suggested by Wagner et al. (1997). The divergence time in this study supported the probable geological evidence responsible for this vicariance event.

Catostomus sp. B vs. *C. rimiculus*

Catostomus sp. B is sister to *C. rimiculus*. DIVA suggested that a vicariant event in the middle Pleistocene to late Pliocene (0.62 Ma to 2.9 Ma) was responsible for the separation between the Rogue River and Klamath River. The paleohydrology connection between the Klamath River and the Rogue River has not been well studied. One possible past connection between the Rogue River and the Klamath River could occur in Late Eocene (37-48 Ma). Sand from Idaho was transported by some kind of well-developed river system to the Pacific Ocean during the Eocene (Orr and Orr, 200). Proto-Klamath – Rogue - Snake River could be a single system that transported such sand (Tyee formation) from Idaho to the Pacific Ocean during the Eocene (37-48 Ma). The Rogue River probably separated out from the proto-Snake - Klamath due to the uplift of the Klamath Mountain (Wagner et al., 1997) and the Basalt extrusion of the Modoc plateau (Wagner et al., 1997) in the time frame between Miocene to early Pliocene. The divergence time from this study suggested that the separation was younger than the hypothesized geological evidence suggested.

Catostomus sp. A. vs. *C. columbianus – C. tahoensis* vs. *C. tsiltcoosensis* - *C. macrocheilus*

The common ancestor of *C. sp. A, C. columbianus*, *C. tahoensis*, *C. tsiltcoosensis* and *C. macrocheilus* had a wide distribution. *Catostomus sp. A* was probably isolated in the Coquille River by the uplifting and tilting of the terrain between the Cape Arago and Cape Blanco in the Pleistocene (Orr and Orr, 2000). The split between the Coquille and Columbia-Willamette-Siuslaw-Umpqua-Coos was estimated by molecular clock to be around 1.31 Ma to 9.44 Ma (Late Miocene to Pleistocene). In this study, the estimated time of the vicariance event that responsible for the split between Coquille and Columbia-Willamette-Siuslaw-Umpqua-Coos was congruent with the time frame of the Pleistocene uplifting of the area between Cape Arago and Cape Blanco. Sixes suckers is morphologically similar to *C. sp. A*. Due to the lack of genetic material of this sucker, morphological features of these suckers suggested that they are the same species as *C. sp. A*. Therefore, the biogeography of Sixes sucker would be similar to *C. sp. A*. The biogeography of Sixes River suckers has one additional vicariant event that separated the Sixes River from the Coquille River. The vicariance event that probably occurred after Coquille River was isolated from the other rivers in Oregon Coastal Subprovince and Willamette-Columbia Rivers.

Catostomus tahoensis - *C. columbianus* vs. *C. macrocheilus – C. tsiltcoosensis*

DIVA suggested that the split between the common ancestor of *C. macrocheilus* and *C. tsiltcoosensis* and the common ancestor of *C. tahoensis* and *C. columbianus* could be explained by vicariance speciation followed by a dispersal. The common ancestor of *C. macrocheilus*, *C. tsiltcoosensis*, *C. tahoensis* and *C. columbianus* had a wide

distribution. Then the common ancestor had allopatric speciation by a vicariant event followed by subsequent dispersal of one of the descendent back to the habitat of another descendent. The dispersal could have been caused by the disappearance of the structure that cause vicariant event. DIVA suggested that there were two possibilities of the direction of the dispersal: from the Columbia to the Lahontan and from the Lahontan to the Columbia. The molecular clock estimated the divergence time of this cladogenetic event to be around 1.03 Ma to 9.44 Ma, which was in the period between Late Miocene and Pleistocene.

Catostomus columbianus and *C. tahoensis*

Cope (1883) suggested a zoogeographic connection between Lake Lahontan and Oregon Lakes region. The close relationship of *C. columbianus* and *C. tahoensis* supported Cope's suggestion. The common ancestor of the *Catostomus columbianus* and *C. tahoensis* had a distribution in the Columbia drainage and the Lahontan drainage. The common ancestor of the *Catostomus columbianus* and *C. tahoensis* had a vicariance speciation. The allopatry probably took place between southeast Oregon and northeast Nevada.

Hubbs and Miller (1948) hypothesize a connection between Alvord basin (southeastern Oregon) and Lahontan Basin. Reheis and Morrison (1997) and Reheis (1999) presented geological evidence of the Connection between Alvord basin of Oregon and Lahontan basin. During the Pliocene and Pleistocene, the large lakes in southern Oregon and Lahontan basin were filled with water. During the Pleistocene, Alvord Lake was much larger than the present size. It was located near Pleistocene pluvial Coyote Lake, which was connected to Owyhee River via several small streams. There are also

several intermittent streams that are located between the Cayote Lake and Alvord Lake. Therefore, there were connections between Owyhee River and Alvord Lake. During the period of maximum lake elevation, the surface water of some of these Pleistocene lakes was interconnected. The surface water of the Lake Alvord and Lake Lahontan was interconnected in the area of the present Black Rock desert (Reheis and Morrison, 1997; Reheis, 1999). This Pleistocene interconnection between Alvord Lake and the Lake Lahontan was the last connection that the Columbia system had with the Lahontan system. Later in the Pleistocene, these lakes dried up (Benson et al., 1992). This probably caused the allopatry of the common ancestor of *C. tahoensis* and *C. columbianus*. The extinction of the suckers in the Alvord basin probably occurred after allopatry. DIVA suggested a vicariant event isolated the common ancestor of *C. columbianus* and *C. tahoensis*. The desiccation of the Pleistocene lakes was probably the vicariant event. In this study, the divergent time between *C. tahoensis* and *C. columbianus* was 1.71-7.65 Ma (Late Miocene to Early Pleistocene). The divergence time between *C. tahoensis* and *C. columbianus* support the desiccation of the lakes in southeastern Oregon and Lahontan basin in Late Pliocene and early Pleistocene.

Catostomus macrocheilus vs. *C. tsiltcoosensis*

The common ancestor of *C. macrocheilus* in the Columbia Subprovince and *C. tsiltcoosensis* in the Oregon Coastal Subprovince could have separated when the upper Umpqua River, a former tributary of the Willamette River (Diller 1915) was captured by a westward flowing stream between Pliocene and Pleistocene and became the current Umpqua River (Baldwin (1959). Alternatively, the ancestor could have been an Oregon Coastal Subprovince endemic and moved to the Columbia Subprovince in late

Pleistocene when the Long Tom River, a tributary of the Siuslaw River, was captured by the Willamette (Baldwin and Howell, 1949). In this study, *Catostomus tsiltcoosensis* was sister *C. macrocheilus*. DIVA suggested that the common ancestor of *C. macrocheilus* and *C. tsiltcoosensis* had wide distribution and a vicariant event was responsible for the speciation of *C. macrocheilus* and *C. tsiltcoosensis*. The molecular clock estimate suggested that the vicariance event occurred 0.68 Ma to 8.57 Ma (late Miocene to early Pleistocene). This vicariance occurred prior to the Willamette-Siuslaw connection.

Catostomus macrocheilus is also found in the Nehalem River. *Catostomus macrocheilus* could have entered the Nehalem River by the stream captured of an old Columbia tributary by the upper Nehalem (Reimers and Bond, 1967). Another unlikely route can be explained by dispersal from the Columbia River and the Nehalem River to the rest of the coastal system. *Catostomus macrocheilus* was observed in the Columbia River estuary by Reimers and Bond (1967). This observation suggests they have some degree of salt tolerance. However, it is unlikely that they dispersed by using the coastal route because the salinity in the open ocean is greater than the salinity in the estuary area. In addition, the fact that *Mylocheilus caurinus* which has higher salt tolerance than *C. macrocheilus* are not present in the Nehalem (Reimers and Bond, 1967) and the fact that there are no suckers present between the Siuslaw River and the Nehalem River suggested that *C. macrocheilus* entered the Nehalem River via the head water stream capture rather than dispersal to the coastal route (Minckley et al., 1986). This strongly supports vicariance at the junction between Willamette system and Siuslaw-Umpqua system instead of dispersal from the Columbia River down through the coastal route. It is not parsimonious to think that *Catostomus tsiltcoosensis* was the result of the isolation of the

C. macrocheilus that dispersed down via the coastal route from the Columbia River-Nehalem River followed by the extinction of *Catostomus macrocheilus* in the Miami River to Alsea River.

The fauna exchange among the Siuslaw River, Umpqua River and Coos-Millicoma River could have been the result from low land conjunction. Several perennial freshwater lakes located in sand dunes between the Siuslaw and Coos rivers are evidences of low land conjunction and low sea level (Orr and Orr, 2000). Due to the unstable nature of the structure of these lakes, they are likely more susceptible to geological changes such as landslide resulting water movement from one lake to other. During the Pleistocene, the sea level was about 130 m below the current sea level (Fleming et al., 1998) and created more coastal areas where sand dune freshwater lakes could occur. Because *C. macrocheilus* can withstand low salinity, lowland conjunction among unstable freshwater lakes and meandering river mouths is likely responsible for faunal exchange among *C. tsiltcoosensis* in coastal systems.

Ptychocheilus grandis vs. *P. lucius – P. oregonensis – P. umpquae*

Rooting has been a problem in the past studies of *Ptychocheilus* phylogeny (Carney and Page, 1990; Mayden et al., 1991 and Smith et al., 2002). The phylogenetic relationship of *Ptychocheilus* based on morphological characters was studied by (Carney and Page, 1990) and (Mayden et al., 1991). The inclusion of *Mylocheilus caurinus* as a closely related outgroup and *Cyprinus carpio* as a distance outgroup in this study made outgroup comparison more robust. The congruence between the geological evidence and the divergence time (in the case of the clock 1% per My) among *Ptychocheilus* also strengthened support of the topology. The *Ptychocheilus* topology was similar result to

Carney and Page (1990) where *P. lucius* and *P. grandis* were more basal to *P. oregonensis* and *P. umpquae*. In this study, *P. grandis* was the basal taxon in *Ptychocheilus*. *Ptychocheilus grandis* is found in Sacramento River and its tributaries (Figure 3.11). The oldest cladogenesis in *Ptychocheilus* phylogeny was *P. grandis* and the common ancestor of *P. lucius, P. oregonensis*, and *P. umpquae*. This is supported by the fact that *P. grandis* fossil has the oldest (Pleistocene) fossil record among the living *Ptychocheilus* (Smith, 1981). DIVA suggested that the common ancestor of *Ptychocheilus* had wide distribution and a vicariant event was responsible for the isolation of *P. grandis*. Similar to catostomids, the vicariant event that caused cladogenesis between *Ptychocheilus grandis* and the common ancestor of *P. lucius, P. oregonensis*, and *P. umpquae* involved the former Snake River and Pit River. It is difficult to explain why *Ptychocheilus* are not present in the Klamath drainage because Klamath drainage is between the two systems that contain *Ptychocheilus*. Based on the age of the Deer butte formation where *P. arciferus* was found, Kimmel (1975) suggested that the connection between the former Snake River and the Klamath Basin happened between Miocene and Late Pliocene. Based on the fact that *Ptychocheilus* are not present in Klamath system, Carney and Page (1990) suggested that *Ptychocheilus* were not present in the former Snake River until Pliocene or they are extinct in the Klamath drainage. Both explanations are possible. The explanation that *Ptychocheilus* entering the Snake during the Pliocene seems logical based on two reasons: 1) *Ptychocheilus arciferus* are found in former Snake River in the Miocene-Pliocene bed (Smith, 1975) and 2) *Ptychocheilus* are not found in the upper Snake River (McPhail and Lindsey, 1987). Extinction in the Klamath drainage is also a possible explanation. Smith et al (2002)

suggested that extinction is more prominent than speciation in the Great Basin because of the periods of aridity and insularity during the Holocene. Furthermore, small isolated population has higher extinction rate than larger population (Frankham et al., 2002). The common ancestor of *P. lucius*, *P. oregonensis* and *P. umpquae* probably had the distribution that included Klamath drainage. The geological events that responsible for the vicariance were the capture of the former Snake River by the Columbia River, the Snake River plain and the Modoc plateau basalt extrusion and the uplift of the Klamath Mountain. The divergence time between *Ptychocheilus grandis* and the common ancestor of *P. lucius, P. oregonensis* and *P. umpquae* suggested that Sacramento drainage was separated from the rest of the systems by a vicariance event around 5.08 Ma to 13.71 Ma (middle Miocene to early Pliocene). The relationship of *Ptychocheilus* suggested that a vicariance in late Miocene to early Pliocene was responsible for the separation of the Sacramento drainage from the former Snake River. This is older than previously reported by Smith (1975) and Wagner et al., (1997).

Ptychocheilus lucius vs. *P. oregonensis – P. umpquae*

The next cladogenesis is *Ptychocheilus lucius* and the common ancestor of *P. oregonensis* and *P. umpquae*. *Ptychocheilus lucius* had adjacent distribution to *P. oregonensis* around the area of Green River, Wyoming. Fauna in Green River has similarity to both Snake River and Bear River. This led Miller(1958) and Taylor (1985) to suspect past connection between the two systems. Taylor (1985) suggested two possible points of fauna transfer by the mean of glacial diversion water between Snake River and Green River in the Pleistocene. The result from this study suggested a vicariance event separated Green River from Snake River around 4.58 Ma – 11.07 Ma,

which was in the early Pliocene. This divergence time from this study was older than time of the possible fauna transfer presented by Taylor (1985).

Ptychocheilus oregonensis and *P. umpquae*

The biogeography of *P. oregonensis* and *P. umpquae* is similar to the biogeography of *Catostomus macrocheilus* and *C. tsiltcoosensis*. Isolation of the common ancestor of *P. umpquae* and *P. oregonensis* could occur via two possible separate geological events (Siuslaw connection (Baldwin and Howell, 1949) and Umpqua connection (Baldwin, 1959; Diller, 1915)). The two geological events differed in the direction of dispersal. In this study, *Ptychocheilus umpquae* was sister to *P. oregonensis*. DIVA suggested that a vicariance event was responsible for cladogenesis of the two sister species (*P. oregonensis* and *P. umpquae*). This study suggested that vicariance event that was responsible for speciation of *P. umpquae* and *P. oregonensis* occurred 0.86 Ma to 5.43 Ma, which occurred between Late Miocene to early Pleistocene. The divergence time of *P. umpquae* and *P. oregonensis* covered the time frame of both geological events (Pliocene to Pleistocene). Fauna exchange between Siuslaw River and Umpqua River probably occurred via lowland conjunction along the coastal perennial freshwater lakes.

An alternative view of the biogeography of the primary freshwater fishes in the Oregon Coastal Subprovince is *Catostomus* and *Ptychocheilus* shared history. Corroboration of the pattern of area cladograms of one group of taxa with area cladogram shown by the relationship of the other groups suggests that biota shares history (Platnick and Nelson, 1978). Only the relationship of the areas, which both groups occur, can be generalized. *Ptychocheilus* are not present in the Klamath basin. If *Catostomus* and *Ptychocheilus* shared history, it is likely that *Ptychocheilus* are extinct in Klamath Basin

or *Ptychocheilus* is not a monophyletic group (Smith et al., 2002) with some cyprinids in the Klamath Basin that belongs to *Ptychocheilus*. Based on the topology of the phylogeny of *Catostomus* and *Ptychocheilus*, only vicariance can explain the co-occurrence of the topologies found in both group. There are two common nodes in the *Catostomus*' area cladogram and the *Ptychocheilus*' area cladogram: Sacramento (Northern California) vs. Willamette-Umpqua-Siuslaw and Willamette vs. Umpqua-Siuslaw (Figure 3.12). The relationship between the common areas where both taxa occurred suggested that Willamette-Columbia system was more closely related to Siuslaw-Umpqua system than to Sacramento system (Northern California) was to Siuslaw-Umpqua and vicariance events were responsible for this share pattern.

Northern California vs. Willamette-Umpqua-Siuslaw

The relationship of catostomids and *Ptychocheilus* suggests a close relationship of the Sacramento River (Northern California) and the Willamette-Columbia-Siuslaw-Umpqua. Based on the area cladogram of the relationship of the catostomids, the Sacramento River (Northern California) was sister to the Willamette-Siuslaw-Umpqua. This was also true with the area cladogram of the relationship of *Ptychocheilus*. As discussed in the cladogenesis event of northern clade vs. southern clade in the biogeography of catostomid and the cladogenesis event of *P. grandis* vs. *P. lucius – P. oregonensis – P. umpquae*, the former Snake River was the former connection among Sacramento River, Klamath River and Columbia River. The divergence time of Sacramento River (Northern California) vs. Willamette-Columbia-Siuslaw-Umpqua from the relationship of *Catostomus* ranges from 6.87 Ma to 7.84 Ma and the divergence time of Sacramento River (Northern California) vs. Willamette-Columbia-Siuslaw-Umpqua

from the relationship of *Ptychocheilus* ranges from 5.08 Ma to 5.69 Ma. Based on the divergence time of both groups, the divergence time of Sacramento River (Northern California) vs. Willamette-Columbia-Siuslaw-Umpqua ranges from 5.08 Ma to 7.84 Ma (late Miocene to early Pliocene). The divergence time from this study was older than what was reported by Wagner et al. (1997) (subsequent to early Pliocene) and Smith et al. (2000) (Pliocene). The results from my study suggest that the separation of the Sacramento River from the Willamette-Columbia-Umpqua-Siuslaw was the result of a vicariance event in the late Miocene to early Pliocene, which was older than reported by Wagner et al. (1997) and Smith et al. (2000).

Willamette-Columbia vs. Siuslaw-Umpqua

The relationship of catostomids and *Ptychocheilus* suggests a close relationship of the Willamette-Columbia system and the Umpqua-Siuslaw system. The Willamette-Columbia was sister to the Umpqua-Siuslaw in area cladograms of both groups (Willamette-Columbia sister Siuslaw-Umpqua-Coos in *Catostomus* phylogeny and Willamette-Columbia sister to Siuslaw-Umpqua in *Ptychocheilus* phylogeny). As discussed in the cladogenesis of *C. macrocheilus* and *C. tsiltcoosensis* and the cladogenesis of *P. oregonensis* and *P. umpquae*, separation between Willamette River and Siuslaw-Umpqua River could occur via two possible separate geological events (Siuslaw connection (Baldwin and Howell, 1949) and Umpqua connection (Baldwin, 1959; Diller, 1915). The estimated divergence time of the Willamette-Columbia vs. Siuslaw-Umpqua from the relationship of *Catostomus* and *Ptychocheilus* ranged from 0.68 Ma to 8.57 Ma, which is in the time frame of late Miocene to Pleistocene. Based on *Catostomus* and *Ptychocheilus* relationships, DIVA suggested that a separation of the

Oregon Coastal Subprovince from the Willamette-Columbia Subprovince was the result from a vicariant event in the time frame between late Miocene and Pleistocene.

Table 3.1 Accession number from sequences extracted from specimens used in this study. OS represents OSU fish collection catalog number. OSF represents tissue collection that did not have deposited carcass. "Tissue" represents tissue samples received as gift.

Species	Genbank Accession Number	Voucher	Drainage	Haplotype
C. catostomus	EU676808	Tissue	Columbia River	-
C. rimiculus	EU676816	OS13737A	Klamath River	CRK1
		OS17877 (G, I)		CRK1
	EU676817	OS17877 (A, B)		CRK2
		OS17880 (A)		CRK2
		OS17881		CRK2
		OS17882		CRK2
	EU676818	OS17877 C		CRK3
	EU676819	OS17877 (J)		CRK4
	EU676820	OS17877 (D, E)		CRK5
	EU676821	OS17877 (H)		CRK6
	EU676822	OS17879		CRK7
	EU676823	OS17880 (B)		CRK8
	EU676824	OS17880 C		CRK9
C. snyderi	EU676826	OS15904 (A)	Klamath River	-
C. sp. A	EU676843	OS17868 (B, D, E)	Coquille River	CO1
		OS17867 (B, C)		CO1
	EU676844	OS17868 (C, A)	Coquille River	CO2
	EU676845	OS17866		CO3
	EU676846	OS17864		CO4
	EU676847	OS17867 (A)		CO5
C. sp. B	EU676809	OS17875 (A, D)	Rogue River	CRR1
		OS17876		CRR1
	EU676810	OS17875 (B)		CRR2
		OS15913 (B, C, E, F, J)		CRR2
	EU676813	OS15913 (A)		CRR5
	EU676814	OS15913 (G)		CRR6
	EU676815	OS15913 (I, H)		CRR7
	EU676811	OS17875 C		CRR3

Table 3.1 Accession number from sequences extracted from specimens used in this study (continued).

Species	GenBank Assession Number	Voucher	Drainage	Haplotype
C. sp. B	EU676812	OS17883	Rogue River	CRR4
C. columbianus	EU676807	OS17857 (B)	Columbia River	-
C. macrocheilus	EU676833	OSF43	Willamette River	CMW1
	EU676834	OS17858 (B)	Columbia River	CMW8
	EU676835	OS17863 (A, D)	Willamette River	CMW2
		OS17873 C		CMW2
	EU676836	OS17863 (B, E)		CMW4
	EU676837	OS17863 C		CMW5
	EU676838	OS17860		CMW6
	EU676839	OS17873 (A)		CMW3
		OSF44		CMW3
	EU676840	OS17873 (B)		CMW7
		UW49018	Columbia River	CMW7
C. tsiltcoosensis	EU676827	OS13656 (A)	Woahink Lake	TS1
		OS13656C		TS1
		OS15461 (142, 143, 144)	Siuslaw River	TS1
	EU676828	OS15461 (SIU148)		TS3
	EU676829	OS15461 (SIU149)		TS2
		OS15461 (141, 145, 146, 150)		TS2
	EU676830	OS15427 (A)	Umpqua River	TU1
		OS17872 (H)		TU1
	EU676831	OS17871		TU2
		OS15427 (B, C, D)		TU2
		OS17872 (A, B, E, F, G)		TU2
	EU676832	OS17872 (I)		TU3
	EU676841	OS15442 (2)	Millicoma River	TMC1
		OS17859 (A, B, C)	Coos River	TMC1
		OS17861 (B)		TMC1
	EU676842	OS17861(A)	Coos River	TMC2
		OS15442 (3, 4, 7)	Millicoma River	TMC2
		OS17861(C)	Coos River	TMC2
Ch. brevirostris	EU676825	OS15956	Sprague River	-

Table 3.1 Accession number from sequences extracted from specimens used in this study (continued).

Species	GenBank Assession Number	Voucher	Drainage	Haplotype
M. caurinus	EU676869	Tissue	Willamette River	-
P. grandis	EU676867	OS17901	Kern River	-
P. lucius	EU676868	Tissue	Colorado River	-
P. oregonensis	EU676849	OS17884 (B, D)	Willamette River	W3
	EU676850	OS17884 (G)		W4
	EU676851	OS17884 (H)		W5
	EU676852	OS17884 (I)		W6
	EU676853	OS17884 C		W7
	EU676854	OS17885 (C, B)		W2
		OS17884 (F, J)		W2
	EU676855	OS17902 A		W8
	EU676856	OS17902 B		W1
		OS17884 (A, E)		W1
P. umpquae	EU676857	OS17899A	Umpqua River	U1
	EU676858	OS17900A		U3
	EU676859	OS17897A		U2
		OS17887		U2
		OS17896		U2
		OS17898 B		U2
		OS17899 (B, C. I, J, K)		U2
	EU676860	OS17899 F		U4
	EU676861	OS17899 G		U5
	EU676862	OS17888 A	Siuslaw River	S1
	EU676863	OS17888 (D, F)		S3
	EU676864	OS17886 B		S5
		OS17900 C	Umpqua River	S5
P. umpquae	EU676865	OS17886 (D, A. C, E, F, G)	Siuslaw River	S4
		OS17888 E		S4
		OS17889 B		S4
		OS17898 A	Umpqua River	S4
		OS17899 (D, E, H)		S4
		OS17900 B		S4
	EU676866	OS17886 H	Siuslaw River	S2
		OS17888 (B, C)		S2
		OS17889 (A, C)		S2

Table 3.2 Percent divergence and divergence time (Ma) at major nodes on catostomid trees. The divergence time was estimated by the NPRS method and by likelihood model GRT+I+G based on the rate 1% per million years

Comparison	% Sequence divergence	NPRS (Ma)	Calibration 1% / million years (Ma)
Coquille vs. Siuslaw-Umpqua-Coos-Willamette-Lahontan	2.63-6.46	9.44	1.31-3.23
Willamette vs. Siuslaw-Coos-Umpqua	1.36-2.47	8.57	0.68-1.23
Siuslaw vs. Umpqua-Coos	1.33-1.79	8.47	0.67-0.89
Umpqua vs. Coos	0.92-1.27	6.88	0.46-0.64
Northern clade vs. Southern clade	12.02-20.95	12.90	6.07-10.47
Rogue-Klamath vs. Northern California	5.57-6.40	5.34	2.79-3.20
Rogue vs. Klamath	1.25-2.09	2.90	0.62-1.04

Table 3.3 Percent sequence divergence and divergence time (Ma at major nodes on *Ptychocheilus* tree. The divergence time was estimated by the NPRS method and by likelihood model GRT+I+G based on the rate 1% per million years

Comparison	% Sequence Divergence	NPRS (Ma)	Calibration 1% / Million years (Ma)
Willamette vs. Siuslaw-Umpqua	1.73-2.64	5.43	0.862-1.318
Siuslaw-Umpqua-Willamette vs. Colorado	9.17-9.98	11.07	4.585-4.992
Siuslaw-Umpqua-Willamette-Colorado vs. Northern California	10.17-11.38	13.71	5.083-5.689

Figure 3.1 Map showing drainages in Oregon and California.

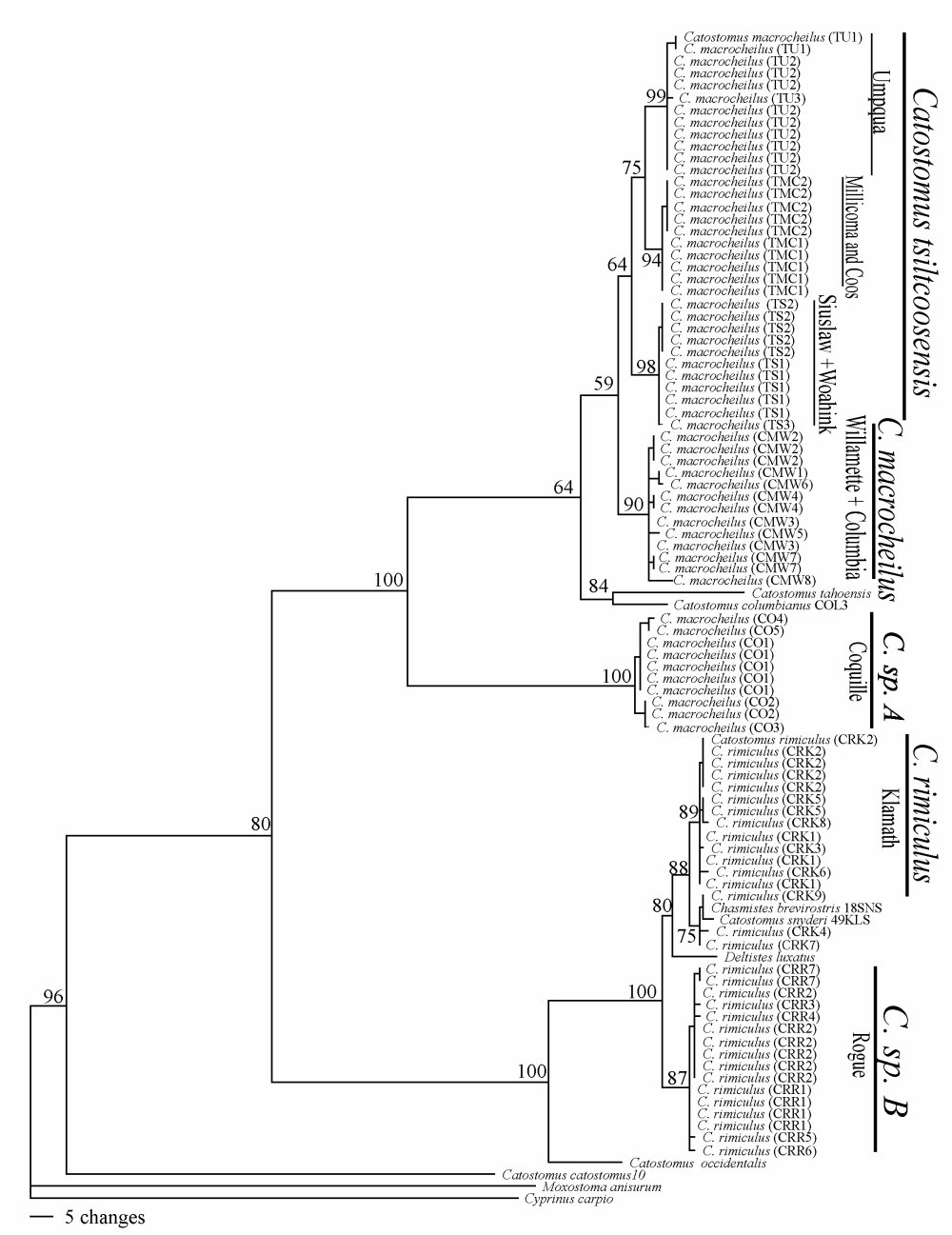

Figure 3.2 Strict maximum parsimony consensus tree of suckers in Oregon coastal river system from 18 trees (602 steps long with consistency index (CI) = 0.7027, retention index (RI) = 0.9523 and rescaled consistency index (RC) = 0.6692) base on cytochrome b sequence (1042 base pair with 200 parsimony informative characters) from parsimony algorithm. *Moxostoma anisurum* and *Cyprinus carpio* are outgroups. The tree is 605 steps long with CI =0.6992, RI= 0.9515 and RC= 0.6653. Branch length represented changes occur on each branch. The number indicates the bootstrap value at each node. Node that without a number had bootstrap values less than 50%. Scale indicates 5 base pairs changed. Haplotype for each sample is provided in the parenthesis.

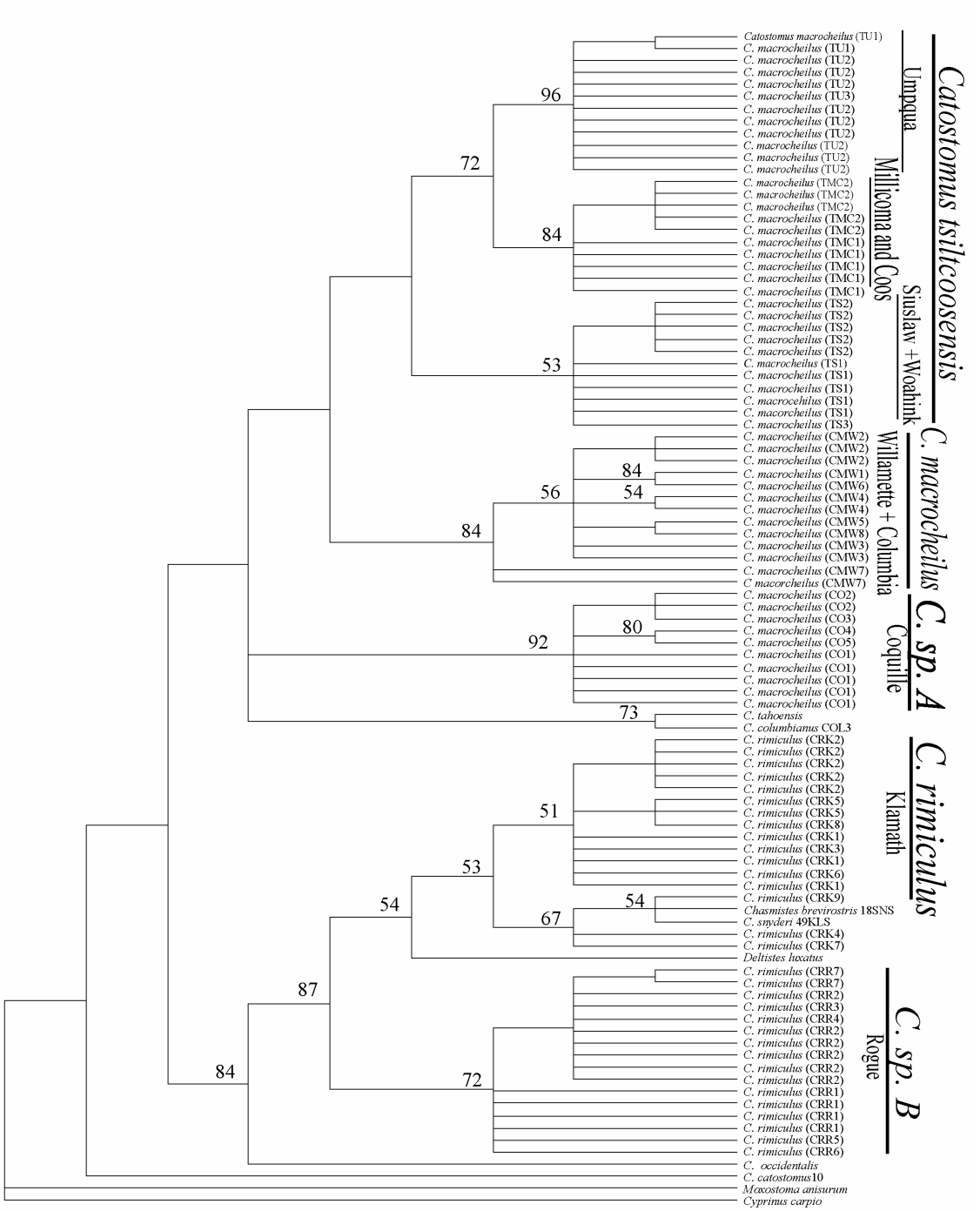

Figure 3.3 Cladogram from likelihood analysis based on the model GTR+I+G model of suckers from different coastal river system in Oregon. The likelihood score of the tree was 4143.10372. The number at each node represented bootstrap support. Nodes without a number had bootstrap values support less than 50%. Haplotype for each sample is provided in the parenthesis.

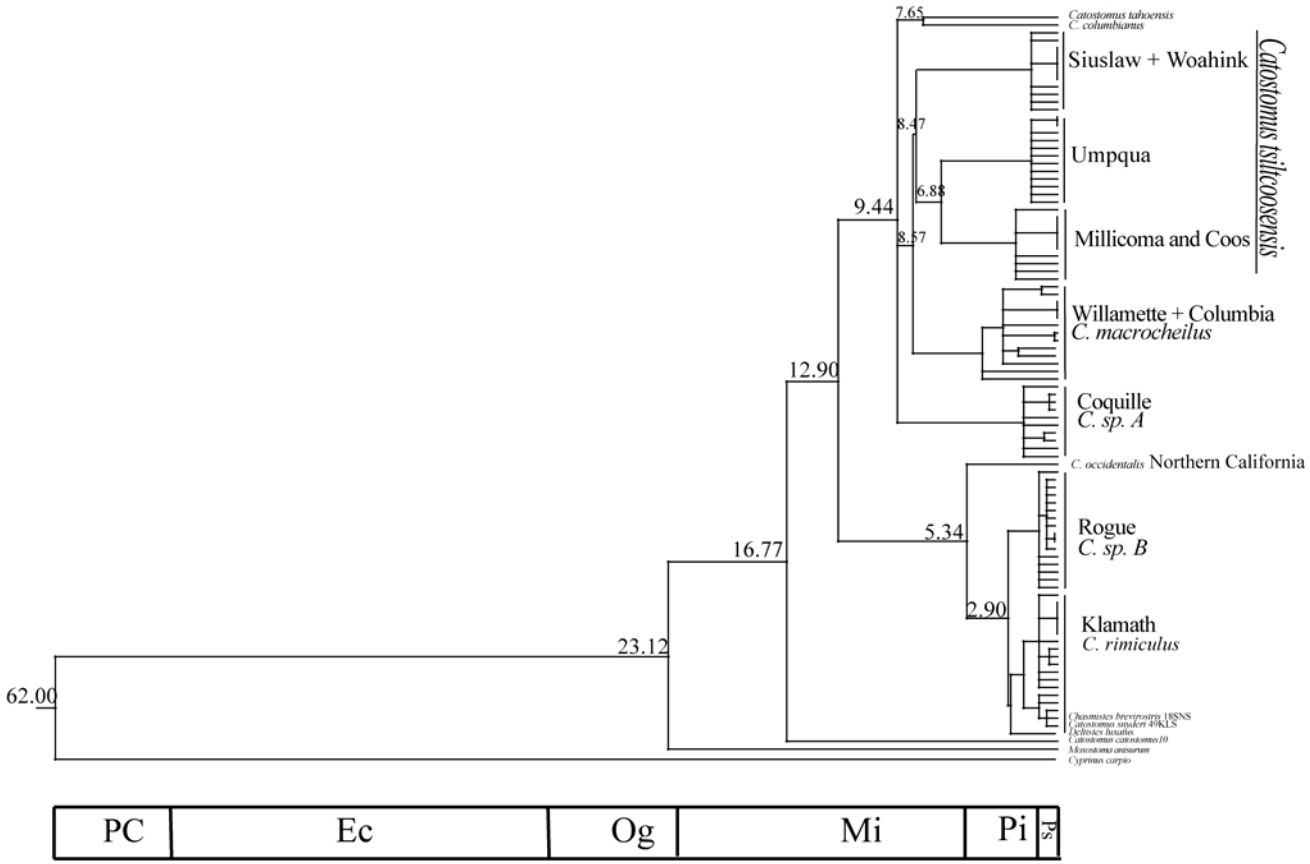

Figure 3.4 Age estimates for the representative nodes within Catostomid tree. Numbers on nodes indicate the node age (in million years ago = Ma). Taxon names were replaced by Areas (coding scheme as previously). Branch lengths were transformed from the original phylogram based on the nonparametric rate smoothing using the TreeEdit ver. 1.0a10. Age for the deepest node was fixed by the oldest catostomid fossil and time scale below the tree was superimposed based on the age estimate for the deepest node. Scale shows super impose geological time period (PC= Paleocene, EC = Eocene, Og = Oligocene, Mi = Miocene, Pi = Pliocene and Ps = Pleistocene)

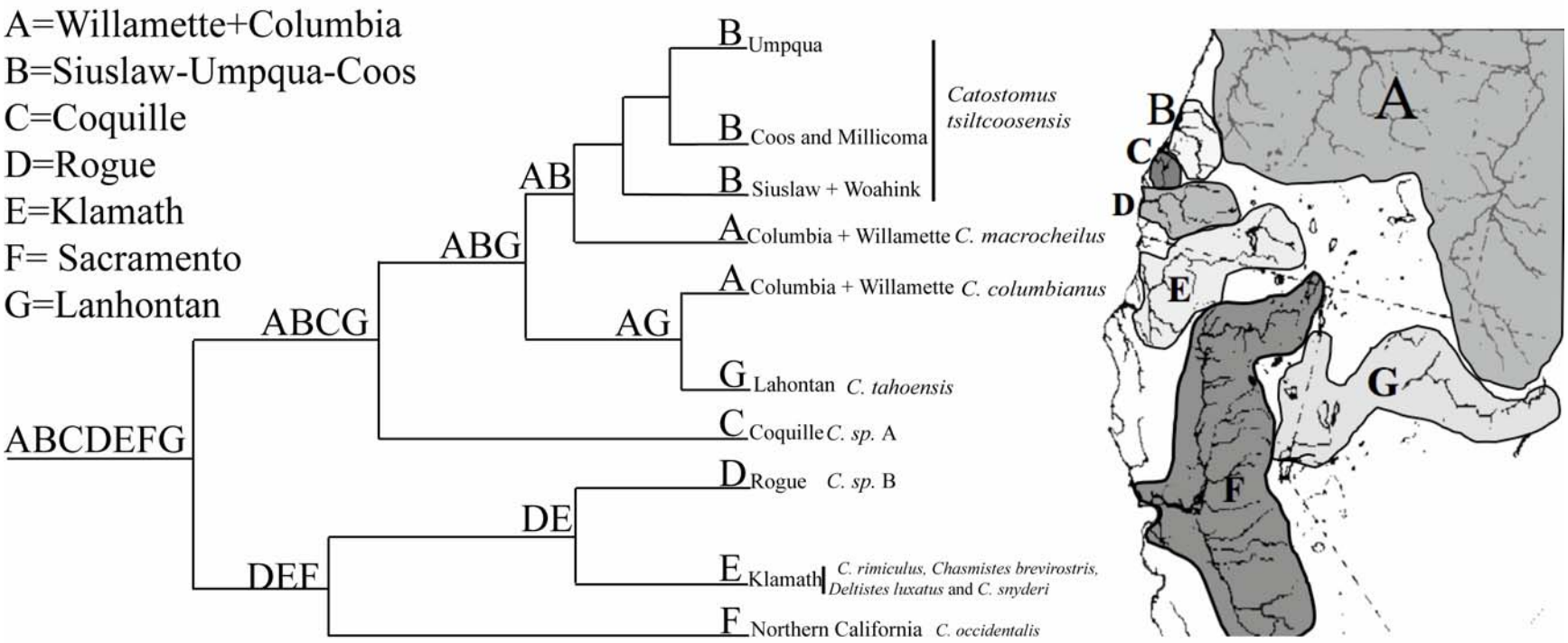

Figure 3.5 Simplified taxon-area cladogram of catostomids used for DIVA analysis and a map that shows the areas of endemism used in the analysis. Characters above branches indicate the results of the ancestral area reconstructions using DIVA. Two or more characters indicate that the ancestors were widespread across those areas.

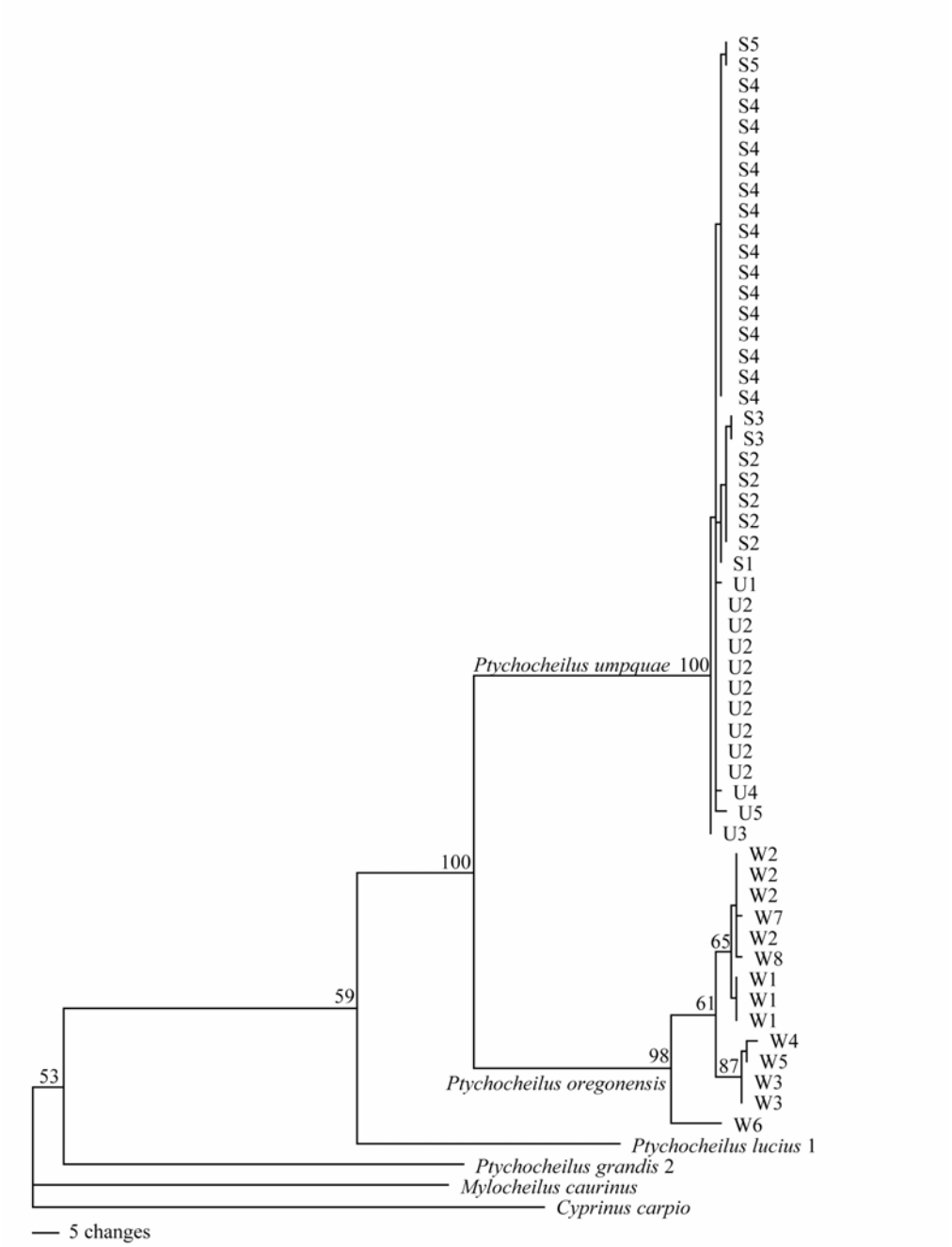

Figure 3.6 Relationship among 4 species of *Ptychocheilus* base on cytochrome b sequence (1041 base pair with 134 parsimony informative characters) from parsimony algorithm. *Mylocheilus caurinus* and *Cyprinus carpio* are the outgroup. The tree is 394 steps long with CI = 0.8503, RI=0.8778, and RC=0.7464. Haplotype for each sample is labled at the terminal branch for *P. oregonensis* and *P. umpquae*. Branch length represent changes occurred on each branch. The number represents the bootstrap value at each node. Nodes without a number had bootstrap values less than 50. Scale indicates 5 base pairs changed.

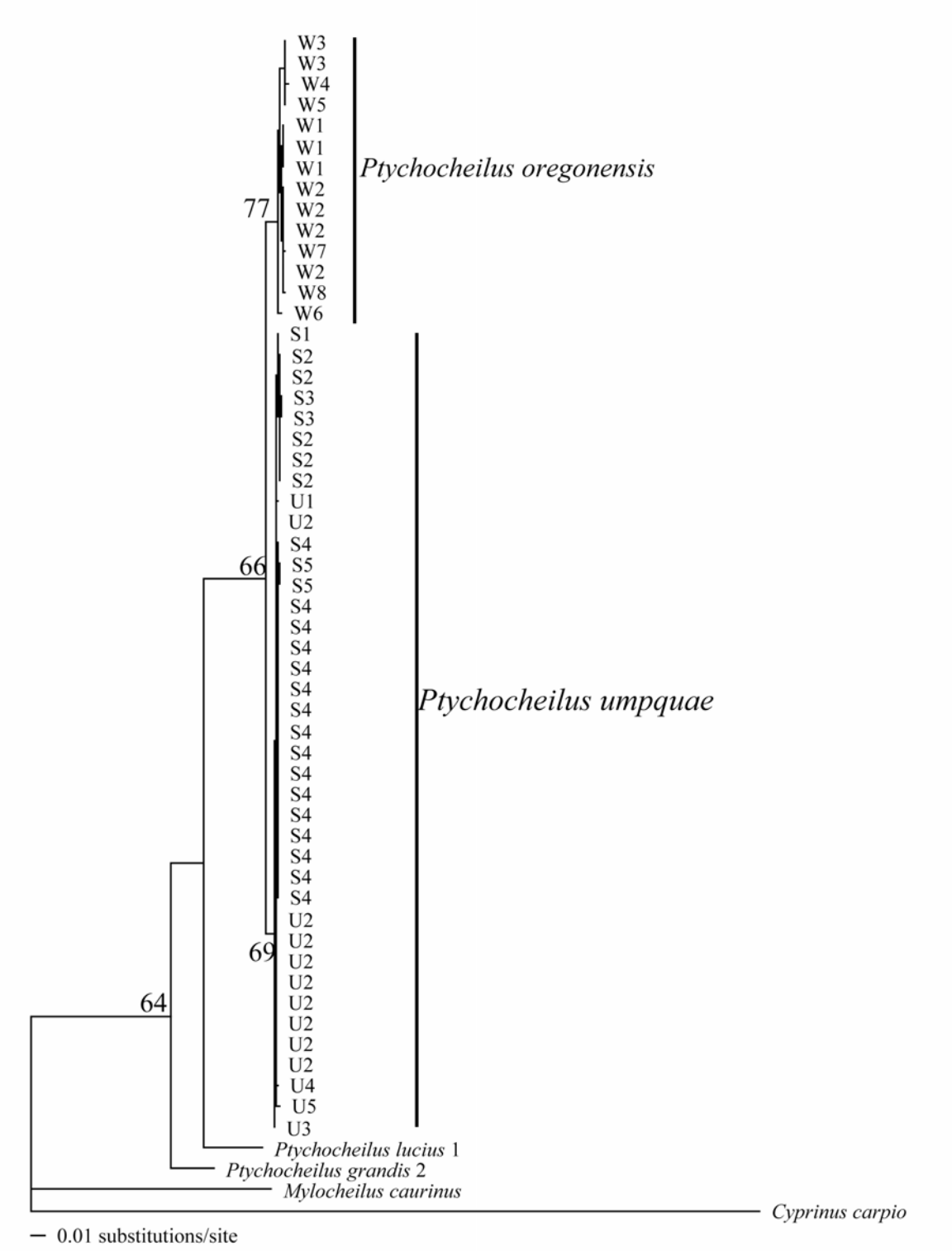

Figure 3.7 Phylogram of the relationship among 4 species of *Ptychocheilus* base on cytochrome b sequence from likelihood analysis based on TrN+I model. The likelihood score of the tree is 3194.26468. Haplotype for each sample is labled at the terminal branch for *P. oregonensis* and *P. umpquae*. Scale indicates 0.01 substitutions per site.

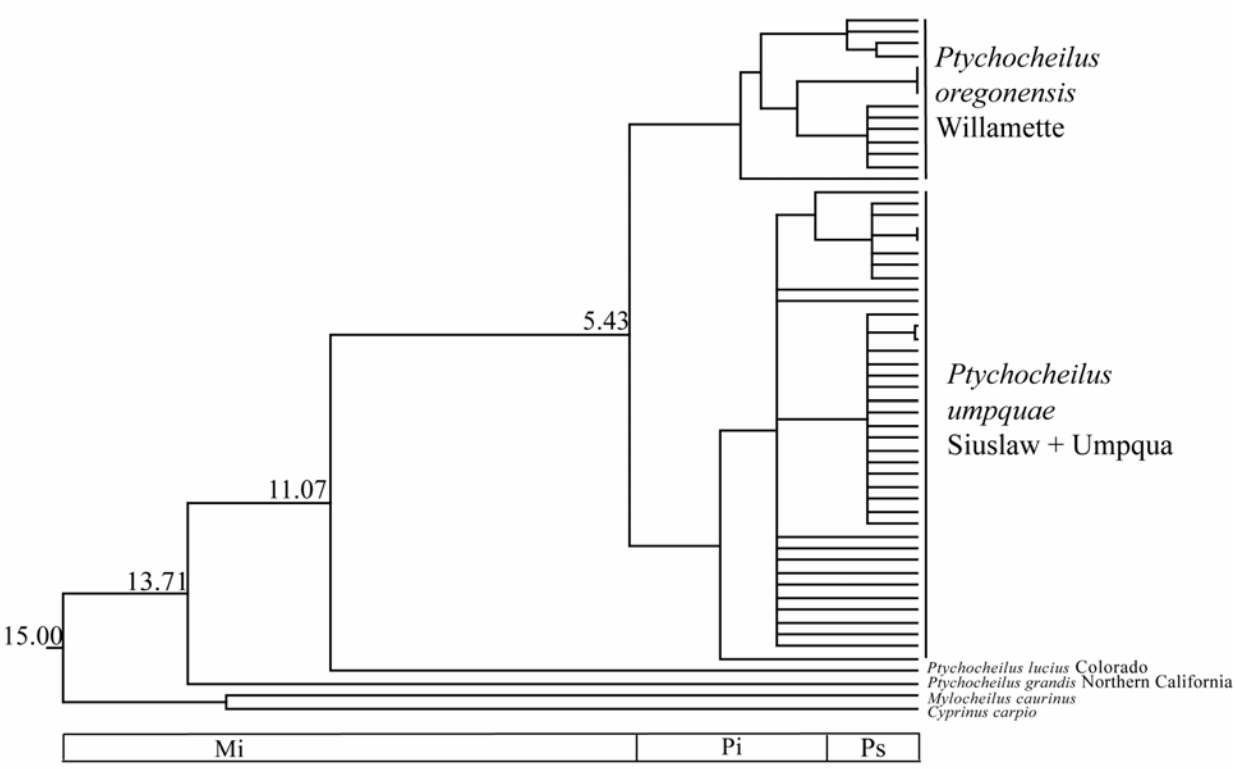

Figure 3.8 Age estimates for the representative nodes within *Ptychocheilus* tree. Numbers on nodes indicate the node age (in million years ago = Ma). Taxon names were replaced by Areas (coding scheme as previously). Branch lengths were transformed from the original phylogram based on the nonparametric rate smoothing using the TreeEdit ver. 1.0a10. Age for the deepest node was fixed by the oldest catostomid fossil and time scale below the tree was superimposed based on the age estimate for the deepest node. Scale shows super impose geological time period (Mi = Miocene, Pi = Pliocene and Ps = Pleistocene)

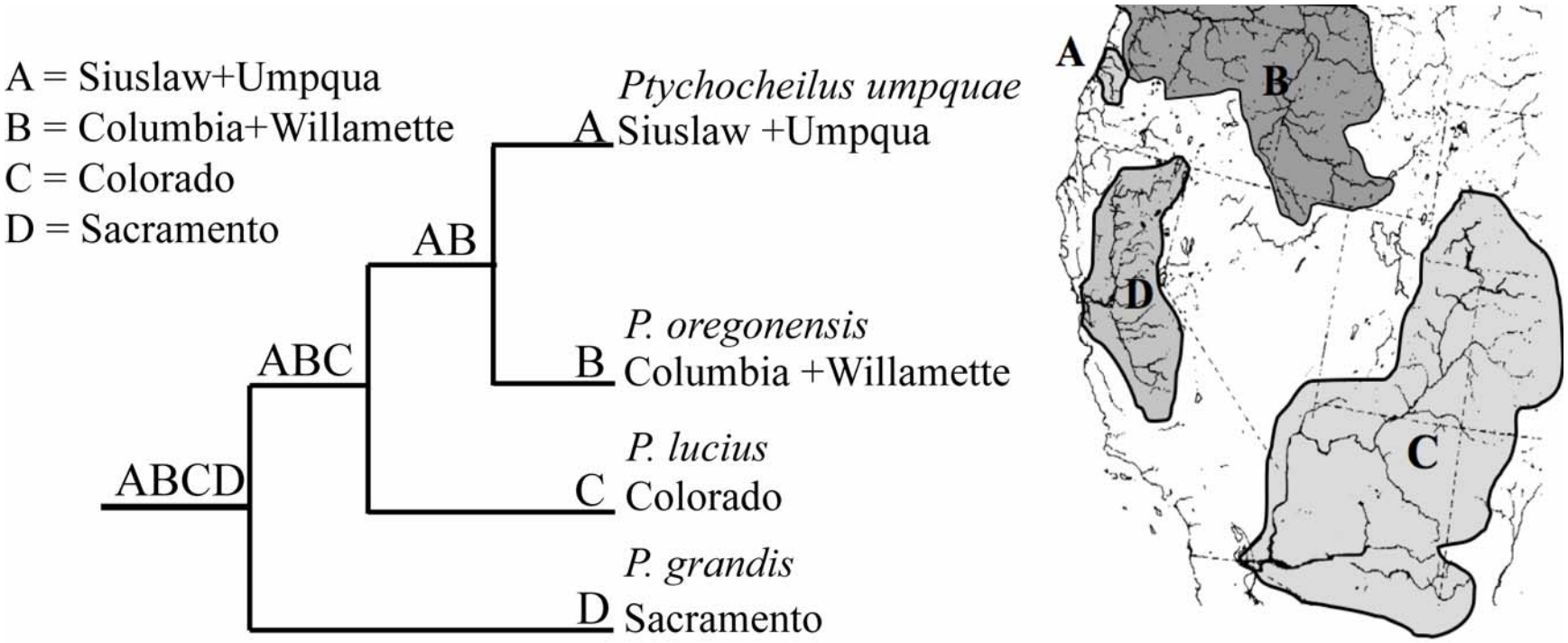

Figure 3.9 Simplified taxon-area cladogram of *Ptychocheilus* used for DIVA analysis. Characters above branches indicate the results of the ancestral area reconstructions using DIVA. Two or more characters indicate that the ancestors were widespread across those areas.

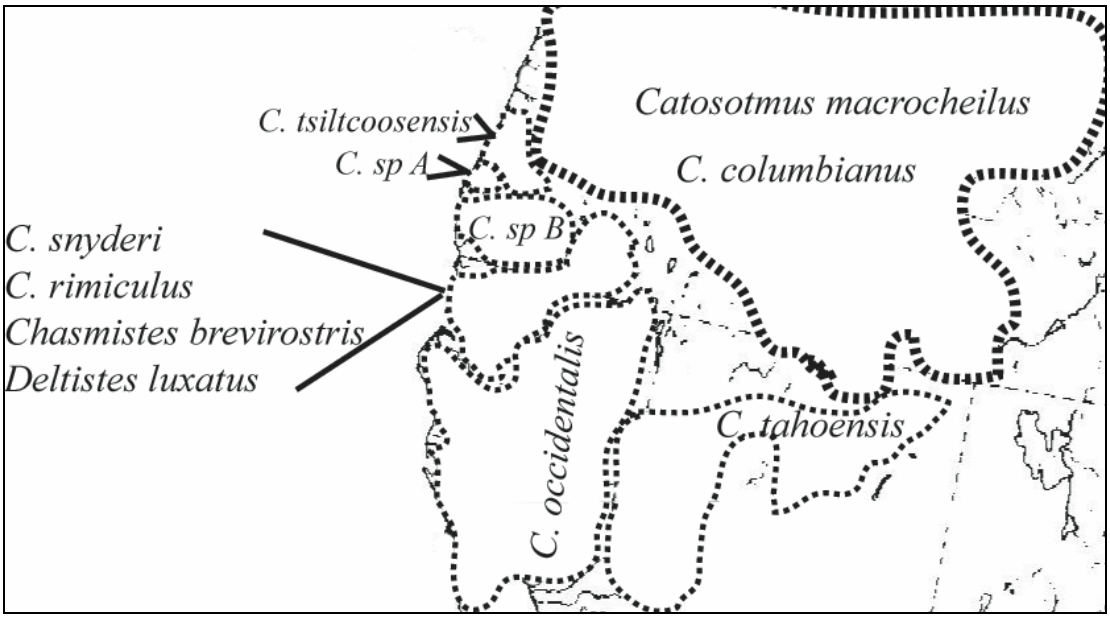

Figure 3.10 Distribution of catostomids in this study.

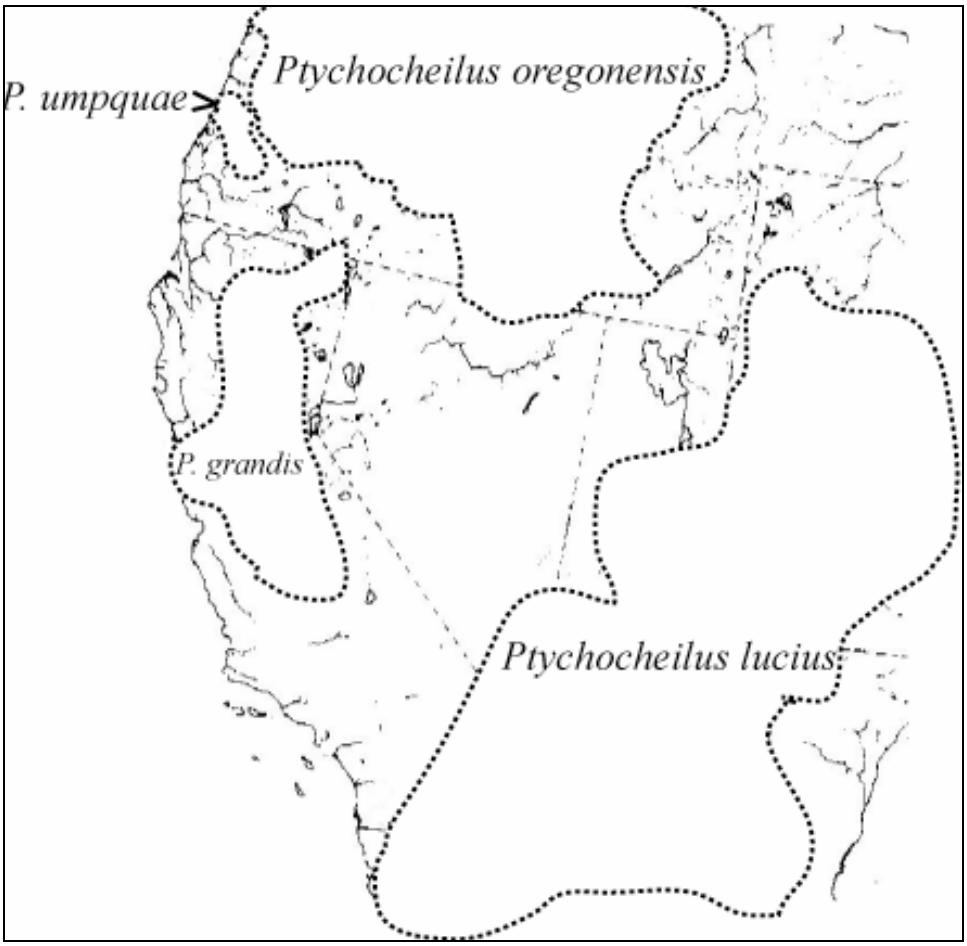

Figure 3.11 Distribution of fishes in the genus *Ptychocheilus*

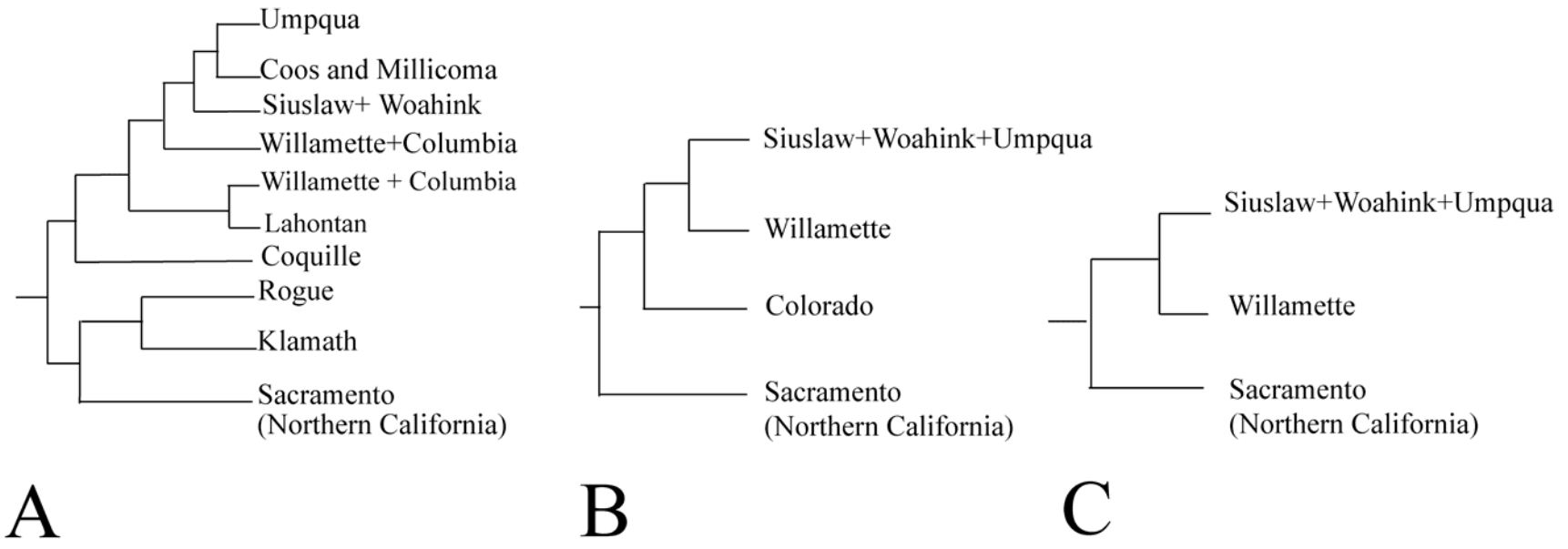

Figure 3.12 Areas cladograms of A) catostomids B) *Ptychocheilus* and C) consensus co-occurance area cladogram from catostomids area cladogram and *Ptychocheilus* area cladogram.

Chapter 4

General conclusion

Catostomus tsiltcoosensis was first described by Evermann and Meek (1898), but Snyder (1908) suggested that it was part of *C. macrocheilus (sensu lato)*. In this study *C. tsiltoosensis* was morphologically (infraorbital pores and dorsal fin rays) and molecularly (cytochrome b sequence) different from *C. macrocheilus (sensu stricto)* and they are considered allopatric sister taxa. *Catostomus sp A* (Coquille River) was also previously considered to be part of *C. macrocheilus (sensu lato),* but again is shown to be morphologically and molecularly distinct (Chapter 2). These results raise the diversity and endemism of primary freshwater fishes in the Oregon Coastal Subprovince to 8 species and 5 endemics (*Catostomus tsiltcoosensis*, *C. sp. A*, *Oregonichthys kalawatseti, Ptychocheilus umpquae* and *Rhinichthys evermanni*). In addition, *Rh. cataractae* and *Rh. osculus* in the Oregon Coastal Subprovince show significant morphological differences from the Willamette *Rh. cataractae* and *Rh. osculus* (Zirges, 1973; Bisson and Reimers, 1977) and *Richardsonius siuslawi* was at least recognized by Evermann and Meek (1898) as a different species from *R. balteatus*. If the Oregon Coastal Subprovince *Rh. cataractae*, *Rh. osc*ulus and *R. siuslawi* are also local endemics, the level of endemism of primary freshwater fishes in the Oregon Coastal Subprovince would be 100%. Endemism is a very important criterion for establishing biogeographic provinces and subprovinces (Brown and Lomolino, 1998) and is ample support for recognizing the Oregon Coastal Subprovince.

Most, and probably all, primary freshwater fishes in the Oregon Coastal

Subprovince have sister taxa in the Willamette-Columbia system (Carney and Page, 1990; Mayden et al., 1991; Markle et al., 1991). Oregon Coastal Subprovince together with the Columbia Subprovince constitutes the Cascadia Province, which is closely related to Klamath-Rogue-Northern California. Previous dispersal explanations for the existence of Oregon Coastal Subprovince (Minckley et al. (1986) and McPhail and Lindsey (1986)) were shown in phylogenetic studies of *Catostomus* and *Ptychocheilus* to be explained by vicariance instead. The first event, affecting *Catostomus* only, separated Coquille River from the Cascadia. The second event, affecting both genera, separated Columbia and Oregon Coastal Subprovinces (Chapter 3).

Allopatric species have an immense impact on the management plan of freshwater fishes. If allopatric populations of fishes are different from each other (molecularly or morphologically), they should be recognized at least as evolutionary significant units. My studies suggested that suckers and pikeminnows in the Oregon Coastal Subprovince are different species from suckers and pikeminnows in the Willamette-Columbia system. Therefore, the Oregon Coastal Subprovince deserves to be a separate management unit from the Columbia and Willamette basin. Cytochrome b sequence suggests that catostomids in each coastal drainage have unique mitochondrial DNA haplotypes. Therefore, it is important not to have fish transfers from one system to the others in order to maintain genetic distinctiveness of these fishes. Furthermore, *C. tsiltcoosensis* and *C. sp A* should be managed as different species from *C. macrocheilus*. *Catostomus sp. B* should be managed as a separate species from *C. rimiculus*.

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