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The Phylogeography of the Mountain Sucker

[*Catostomus* (*Pantosteus*) *platyrhynchus*]

Nina Johanna Laitinen

A thesis submitted to the faculty of
Brigham Young University
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Master of Science

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ABSTRACT

The Phylogeography of the Mountain Sucker [*Catostomus* (*Pantosteus*) *platyrhynchus*]

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Pantosteus, a subgenus of *Catostomus*, includes the mountain sucker (*Catostomus playthyrnchus*), whose speculated older origins in the Miocene/Pliocene can provide insight into the ancient geographical events of western North America. We believe that major geologic events influencing the diversification of mountain suckers include the rise of the Colorado Plateau, the connections between the ancient Snake River system and the Lahontan system and subsequently the connection of the Snake River system to the Columbia Basin, dispersal of mountain suckers across the continental divide, as well as the Pleistocene Bonneville flood. If this is true, we should see evidence of geologic separation and timing through studying the phylogenetics of the mountain sucker. In order to clarify relationships of the mountain sucker with respect to other *Pantosteus* species, we examined cytochrome *b* (cyt *b*) sequences for 144 mountain suckers, 24 other *Pantosteus* species, and ten outgroup species. Phylogenetic relationships among haplotypes were constructed based on maximum likelihood and Bayesian criterion. In an effort to provide better resolution at some nodes, we also sequenced additional mitochondrial genes (ND1, ND2, ATP*ase*, ND4L, ND4, ND5, ND6, and cyt *b*) for a subset of 44 individuals taken from the major clades obtained from the cyt *b* phylogentic analyses. Trees from this data set were also constructed under maximum likelihood and Bayesian criterion. All phylogenetic analysis revealed that mountain sucker are paraphyletic, with two major clades of mountain suckers separated by other members of the subgenus *Pantosteus*. One clade included two sub-clades, one from the upper Snake River drainage/northern Bonneville/Green River drainage Basins and the other from the southern Bonneville Basin. The other major clade included sub-clades from the Lahontan Basin, Columbia River Basin, and Upper Missouri River Basin. Molecular clock analysis revealed that *Pantosteus* likely split from *Catostomus* during the Miocene and that major speciation events within *Pantosteus* occurred during the Pliocene and Pleistocene. Genetic structuring and gene flow estimates for mountain sucker populations, with groupings based on major drainage basins, were calculated with AMOVA and Fst estimates in Arlequin and revealed that most of the genetic structuring was explained by variation among drainage basins with limited gene flow occurring between drainage basins. Based on this study, the role of the Colorado Plateau's geologic history in the evolution of the mountain sucker remains unclear. However, all other geologic events as discussed in this study seem to have played a significant role in the evolution of the mountain sucker.

Keywords: *Catostomus platyrhynchus*, mountain sucker, *Pantosteus*, phylogeography, mtDNA cytochrome *b*, molecular dating

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INTRODUCTION

The biogeography of freshwater fishes often relates to the historical dynamics of the drainage basins they inhabit. Drainage basin isolation allows the development of endemic species, while between-basin connections have allowed the inter-basin dispersal of fish and other aquatic organisms. Because of these dynamics, fish populations within drainage basins are often discrete entities; however, their relationships with populations in nearby basins may correspond to past hydrographic events that have allowed dispersal or gene flow (Blackwelder 1933; Hubbs and Miller 1948; Smith and Dowling 2008). This suggests that modern fish distributions and geological history can provide complementary explanations and hypotheses regarding phylogeographic patterns.

Western North America has undergone a series of relatively recent (Oligocene-present) tectonic-induced changes including uplift and both the creation and destruction of vast, interior mountain ranges (Horton et al. 2004; Sonder and Jones 1999; Wallace et al. 2008). Associated with these tectonic activities have been the formation, separation, and coalescence of multiple interior basins, as well as climate shifts associated with topographical changes. As the geological history of Western North America becomes better understood, the relative timing of these events becomes more refined. Thus the Western North American landscape provides an excellent system for gaining understanding of the evolution and dispersal of native fishes.

Although dispersal and isolation of terrestrial organisms in North America have often been associated with Pleistocene events (Soltis et al 1997; Schafer et al. 2010), modern fish families within western North America have fossil records extending into the Pliocene or Miocene, which indicates that key dispersal events could be much older than the Pleistocene (Smith 1975, Smith et al 1982, 2000, 2002; Carstens et al 2005; Smith 1981; Taylor and Smith

1981; Uyeno and Miller 1965; Smith & Cossel 2002). These changes, and their associated effects on climate, have significantly influenced the distributions of modern fishes (Smith et al. 2002).

The catostomids (Pisces: Catostomidae), a family of freshwater fishes commonly known as suckers, are widespread throughout North America and, display a general biogeographic pattern consistent with those of other North American fresh-water fishes (Wiley and Mayden, 1985; Sun et al. 2007; Harris and Mayden 2001). In Western North America a single discrete lineage, the tribe Catostomini, occurs, being found in most major drainage basins. Based on fossil evidence, catostomids present in western North America do not appear until the late Oligocene and early Miocene (C. 25 mya; Sun et al. 2007). The widespread distribution of the tribe Catostomini throughout Western North America and a fossil history extending into the Oligocene and Miocene make it a good candidate for investigating the role of geological changes in shaping the genetic diversity of western North American taxa.

We are interested in the phylogeny and phylogeography of the mountain sucker (*Catostomus Pantosteus platyrhynchus*), a species within the *Catostomus* subgenus *Pantosteus.* The subgenus *Pantosteus* contains six recognized species (*C. platyrhynchus, C. discobolus, C. clarkii, C. santaanae, C. plebeius, C. columbianus*; Smith 1966) and mountain suckers are the most widely distributed species. Phylogenetic relationships within the subgenus *Pantosteus* are unclear because of incomplete species representation in the generation of molecular phylogenies (Doosey et al. 2010; Sun et al. 2007; Bart et al. 2010) or in the use of morphological-based characters alone (Smith 1966). More recent studies have rendered *C. columbianus* paraphyletic with respect to *Pantosteus* (Doosey et al. 2010) and have included *C. nebuliferus* in *Pantosteus* (Miller, 2005). In addition, the mountain sucker, *C. P. platyrhynchus*, occurs in a number of separate major drainage basins: the upper Missouri River Basin, the Columbia River Basin, the

Lahontan Basin, the Bonneville Basin, and the Colorado River Basin. Some of these basins have not been connected since the Pliocene. We felt it important to examine the phylogenetic relationships of the mountain sucker within the entire subgenus *Pantosteus*. Given the hypothesized older origins (Miocene and/or Pliocene) of the subgenus *Pantosteus* (Smith 1966), a molecular phylogeny may define alternate associations within the mountain suckers and give new insights into the hydrogeographic history of western North America.

Mountain suckers distributed within the Colorado River Basin, Bonneville Basin, and upper Snake River Basin, have shared several ancient hydrogeographic connections and may share a common evolutionary history. It has been postulated that the Colorado Plateau (i.e. Upper Colorado River Basin), covering parts of modern-day Utah, Colorado, Arizona, and New Mexico (See Figure 1), began to uplift in the Miocene (Hunt 1956) and likely was a key factor in the evolution of the subgenus *Pantosteus*, which are specialized benthic grazers that scrape algae from solid surfaces (Smith 1966). The Colorado Plateau uplift increased river gradients, which increased the abundance of rocky substrates in the rapidly flowing rivers and streams. While some members of the subgenus remain isolated on the Colorado Plateau, others dispersed, into the Rio Grande, upper Snake River Basin, Bonneville Basin, and Colorado River Basin and from there to the Lahontan, Columbia River, and Santa Ana River basins (Smith 1966). If this is the case, we would expect that the mountain sucker, *Catostomus Pantosteus platyrhynchus*, within the Colorado River Basin will be ancestral to *C. platyrhynchus* lineages in the Lahontan, Upper Snake River, Columbia River, and Missouri River basins.

The ancient Snake River system has also been shown to play a major role in the dispersal of western fishes in the Lahontan and Columbia River Basins (Smith et al 2002; Billman et al. 2010; Houston et al 2010a, b). While the path of the Upper and Middle Snake River is unclear prior to about 10 mya, once Lake Idaho developed in western Idaho, the river had connections with the Sacramento River of California. The ancient Snake River may have entered ancient Lake Idaho and exited through Oregon, ultimately merging with a Lahontan Basin river and flowing to the Klamath/Pit-Sacramento system (Miller 1965; Taylor 1985; Minckley et al. 1986; Smith et al. 2000). Alternatively the Snake River may have flowed south of Lake Idaho, through the Lahontan Basin to the Pit River system (See Figure 1; Repenning et al. 1995; Link et al. 2002; Billman et al. 2010). In the latter scenario, Lake Idaho, at least periodically, would have drained southeast to the Snake River. Approximately 3.5 mya Lake Idaho was captured by the lower Snake River of the Columbia River Basin. This should have created a dispersal route for mountain suckers from the western periphery of the Colorado Plateau, to the Columbia River system. Moreover, because the Snake River capture event severed the connection between the middle and upper Snake River from the Lahontan Basin, thus isolating the Lahontan Basin mountain suckers, we expect that Lahontan Basin mountain suckers will contain a phylogenetic signature unique from that of the mountain suckers in the Columbia River system.

At some point, mountain suckers dispersed through a transfer event across the continental divide into the Upper Missouri Basin. Fish transfers across the Continental Divide have occurred numerous times. The westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, occurs in headwaters of the Missouri River Basin, but clearly originated in the Columbia River Basin and the Yellowstone Cutthroat trout, *O. c. bouvieri*, crossed from the upper Snake River system into the headwaters of the Yellowstone River of the Missouri River Basin (Behnke 1992, 2002). It has been hypothesized that, following rejuvenation of streams in the late Pliocene-early Pleistocene, *C. platyrhynchus* spread eastward from western Wyoming (Colorado River Basin) to the Big Horn Mountains of Wyoming and the Black Hills of South Dakota (Smith 1966; Love et al. 1963; Belika 2006; Campbell 1992) or that a form of mountain sucker in the upper Snake River (not the present-day upper Snake River mountain sucker) invaded across the continental divide in the upper Snake River of Eastern Idaho (G. R. Smith personal communication). It is also possible that the mountain sucker crossed the continental divide from the Columbia River Basin. If the latter transfer event occurred, the timing would likely be linked to Lake Idaho's capture by the lower Snake River of the Columbia River Basin approximately 3.5 mya. If this is the case, we suspect a close relationship of Upper Missouri Basin mountain suckers to the Columbia Basin mountain suckers will exist.

Several studies have documented phylogenetic divergences within species in the Northern Bonneville/upper Snake River drainage basins and the Southern Bonneville Basin (Martin et al. 1985; Johnson 2002; Mock et al. 2006). This is generally attributed to the Late Pleistocene transfer of the Bear River into the Bonneville Basin (approx. 35,000 ya; Bright 1963; Hubbs and Miller 1948; Taylor and Bright 1987), which allowed exchange of fishes between the upper Snake River Basin and the northern Bonneville Basin. Smith (1966) also reported morphological differentiation between the northern and southern Bonneville Basin mountain suckers. Phylogenetic analysis may reveal similar patterns of genetic divergence within the mountain suckers.

We first examined mitochondrial DNA sequence data to reconstruct evolutionary relationships among mountain sucker populations relative to the subgenus *Pantosteus*. We hypothesize that the four major geologic events described above have had a significant influence on the evolution of mountain suckers. In addition to phylogenetic construction and its correlation to biogeographic events, we also used DNA sequence data to evaluate genetic structuring and gene flow using various population genetic methods among mountain suckers populations in major drainage basins. We predict that genetic structuring will be due to variation among drainage basins and gene flow estimates will not show gene flow occurring between drainage basins. We also constructed a molecular clock analysis in order to determine if the timing of major transfer events correlates with geologic events as described above.

MATERIALS AND METHODS

Sampling

Specimens representing six recognized *Pantosteus* species were collected from each major drainage basin where mountain suckers occur (See Figure 1) via electrofishing, with extra focus on sampling multiple populations of mountain sucker for the phylogeographic portion of this study. In addition, *C. nebuliferus* was also included as a recent addition to *Pantosteus* (Miller, 2005). Whole fish were preserved in 90% ethanol and transported on ice to Brigham Young University (BYU) for later DNA extraction (See Figure 2). Specimens were then archived in the Monte L. Bean Life Science Museum (MLBM) at BYU and accession numbers are listed in Table 1. Some mountain sucker and other *Pantosteus* species tissue samples were already available in the MLBM tissue collection, and some tissues from each of the *Pantosteus* species were provided by other researchers. Hence, the resulting ingroup included all members of *Pantosteus* in an attempt to clarify the phylogenetic relationships of the mountain sucker in regards to other members of *Pantosteus*. Given the additional focus on sampling mountain suckers, the ingroup included a total of 144 mountain suckers representing 38 localities (see Table 1). An additional nine species from the genera *Catostomus* and *Moxostoma* were included as outgroup taxa (Table 1). DNA sequences for three of the outgroup species were obtained through GenBank (accession numbers GI|97293319, GI|28201355 and GI|347949601|.)

Molecular Methods

Whole genomic DNA was extracted from fin clips or muscle tissue using PureGene isolation kits (Gentra Systems Minneapolis Minnesota). The mitochondrial DNA cytochrome *b* gene (cyt *b*) was amplified by polymerase chain reaction (PCR) for 176 fish with approximately five fish from 37 populations (see Table 1) using primers Glu31 and Pan.Thr.52 (see Table 2 for primer sequences). With the exception of Glu31 (Unmack et al. 2009), all primers used were designed by us specifically for *Pantosteus* (Table 2). When external primers failed to amplify the cyt *b* gene, we used internal primer sets Glu31-Pan.636.HD and Pan.494F-Pan.Thr.52 (this study; see Table 2). PCR reactions had a 25.0 µl total reaction volume. DNA template (1-2 µl) was added to each reaction tube along with 13 µl sterilized distilled water, primers (10 pM each), deoxyribonucleotides (0.125 mM each), GoTaq buffer (10mM Tris-HCl, 1.5 mM MgCl2, 25 mM KCl), and GoTaq polymerase (0.1 units) (Promega). We ran a cycling program with initial denaturing at 95.0˚ for 2 minutes, denaturing occurring at 95.0˚ C for 30 seconds, annealing at 48.0˚ C for 30 seconds, and elongation at 72.0˚ C for 1.5 minutes for 35 cycles. PCR products were tested for amplification using gel electrophoresis on a 1% agorose gel and viewing the samples under ultraviolet light. Purification of amplified PCR products was done using the GeneClean III DNA purification protocol (Bio 101, Inc.,Vista, California).

Cycle sequencing of purified PCR product was performed using ABI Big Dye terminator protocol (Applied Biosystems, Inc., Foster City, CA) with a thermal profile consisting of 35 cycles with 10 seconds at 96.0˚ C, 5 seconds at 52.0˚ C, and 4 minutes at 60.0˚ C. The Big Dye product was cleaned with Sephadex G-50 medium (Sigma-Aldrich Co., St. Louis, MO). Following the removal of excess Big Dye, samples were submitted to the Brigham Young

University DNA Sequencing Center where they were sequenced on an ABI 3730xl automated sequencer.

Data Analysis

Following phylogenetic analysis of cyt *b*, we selected a subset of 44 individuals based on major clades and sequenced mitochondrial genes ND1, ND2, ATP*ase*, ND4L, ND4, ND5, and ND6 (this study). The primers developed for these genes are listed in Table 2. Sequencing multiple genes can often resolve relationships and provide more informative characters for phylogenetic analyses (Peng et al. 2006; Pratt et al. 2008; Li et al. 2007). Because branches in the cytochrome *b* analysis were not well supported at deeper nodes (see Figure 2), we sequenced a greater number of mitochondrial genes in order to provide resolution at these unresolved nodes.

Sequences were imported into Sequencher 4.8 (GeneCodes Co., Ann Arbor, MI) and aligned and trimmed. We sequenced 176 fish and collapsed these to 92 common haplotypes using MacClade version 4.8 (Maddison and Maddison 2005). We had haplotypes for 60 mountain suckers, eight bluehead suckers, five Rio Grande suckers (*C. plebeius*), four Santa Ana suckers (*C. santaanae*), four desert suckers (*C. clarkii*), one Nazas sucker (*C. nebuliferous*), one bridgelip sucker (*C. columbianus*; included in outgroup species), and 9 other outgroup species (See Table 1).

ND1, ND2, ATP*ase*, ND4L, ND4, ND5, and ND6 sequence data were imported into Sequencher 4.8 (GeneCodes Co., Ann Arbor, MI) and aligned and trimmed using the same procedures outlined above. Because these genes are mtDNA that is inherited as a single unit, and because all of them evolve under similar models of nucleotide evolution (see below), these genes plus cyt *b* were concatenated for a total length of 8055 base pairs.

Phylogenetic Analysis

Phylogenetic relationships were reconstructed for the two data sets using maximum likelihood (ML) and Bayesian optimality criterion. For ML analysis, we analyzed the data set in jModelTest and under the AIC obtained a GTR + I + G model of evolution (Posada 2008). A ML tree was then constructed in RaxML, which assumes a GTR model of evolution, with 1000 bootstrap replicates to estimate nodal support (Stamatakis 2004). For Bayesian analyses, appropriate models of sequence evolution were selected by jModeltest (Posada 1998) which subsequently selected the GTR + I + G model of evolution. In the case of the second data set (consisting of cyt *b*, ND1, ND2, ATP*ase*, ND4L, ND4, ND5, and ND6), we partitioned by gene and selected the appropriate model for each data partition using jModeltest (Posada 1998). AIC under each partition selected a GTR + I + G or in the case of ND4L selected a GTR + I model of evolution. Both data sets were analyzed using MrBayesv.3.1.2 (Huelsenbeck and Ronquist 2001) with 20,000,000 generations and sampling every 1000 generations. We discarded the first 1,000,000 generations as burn-in. Ten species from *Catostomus* and *Moxostoma* were used as outgroup species because calibration points for the molecular clock analysis were located within *Catostomus.* Outgroups selected were comprised of *M. poecilurum*, *C. wigginsi*, *C. occidentalis*, *C. insignis*, *C. commersonii*, *C. ardens*, *C. catostomus, C. machrocheilus*, *C. tahoensis,* and *C. columbianus* (See Table 1).

Molecular Clock

We implemented BEAST version 1.4.9 (Drummond and Rambaut 2007) to estimate divergence times between taxa. A molecular clock analysis allowed us to estimate if division of major clades within the subgenus correlates with historic geologic events (Drummond & Rambaut 2007). In order to reduce computing time and achieve appropriate levels of mixing between chains, a subset of 23 cyt *b* haplotypes were selected for this analysis, with each individual representing a unique evolutionary lineage as determined through our phylogenetic analyses (see results). The appropriate model of sequence evolution for the reduced data set was selected using jModeltest version 3.7 (Posada 2008), and the GTR+I+G model was selected. This model was then used in establishing the tree prior for the analysis. The only recognized fossil of *Pantosteus* comes from fossils from the fluvial sediments of the Glenns Ferry formation of southern Idaho, the lower section being dated as over three million years old (K:A determinations, Evernden et al. 1964; Smith 1966; Neville et al. 1979; Kimmel 1982). However, because *Pantosteus* species are fluvial fish they do not fossilize well and are predicted to be much older (Miocene and/or Pliocene) than fossils dating to the Glenns Ferry formation according to Smith (1966). Therefore, we used a fossil date available within *Catostomus* to calibrate the tree instead. Based on *Catostomus* fossils from specimens that are not included in *Pantosteus*, yet are found in the Glenns Ferry quarry, we can approximate the node that gives rise to *C. machrocheilus* at roughly 4 mya and a prior at this branch was set (see Figure 4). Fossils of C. tahoensis, which were found in the Mopung Hills can be traced back to the Pliocene approximately between 5.4 and 2.4 mya, were also included (Taylor 1981; Smith et al. 2002). A relaxed molecular clock was calibrated with GTR + I + G model of evolution and Tree Prior for Speciation (Yule Process) using these dates to calibrate nodes. The BEAST analysis was run with MCMC length of run for 10,000,000 with Log Parameters and Echo State every 1000. The first 1,000,000 steps (10%) were discarded as burn-in.

AMOVA and Fst

We examined genetic differentiation within and among populations of mountain suckers and population structuring using analysis of molecular variance (AMOVA; Excoffier et al. 1995) using Arlequin 2.00 (Excoffier et al. 2005). Data were partitioned into seven groups of mountain suckers representing geographically separated populations based on drainage basin dynamics. These groups are as follows: (1) the northern Bonneville Basin, (2) the southern Bonneville Basin, (3) upper Snake River drainage, (4) the Lahontan Basin, (5) the Columbia River Basin/lower Snake River, and (6) the Missouri River Basin. Based on the predicted genetic divergence between the Lahontan Basin and Columbia River Basin fish due to geographic isolation, we conducted AMOVA between groups 4 (Lahontan Basin) and 5 (Columbia River Basin/lower Snake River) in order to determine how much of the variation of mountain sucker populations between these two drainage basins is due to geography versus variation within populations. Populations of mountain suckers on either side of the continental divide in the Columbia River and Missouri Basins were also tested by comparison of groups 5 (Columbia River Basin/lower Snake River and 6 (the Missouri River Basin). Based on the late Pleistocene transfer of the Bear River into the Bonneville Basin, we combined groups 1 and 3 (upper Snake River drainage and northern Bonneville Basin) in comparison to group 2 (southern Bonneville). Fst values with Bonferroni corrections were also calculated in Arlequin 2.000 (Excoffier et al. 2005) to measure gene flow and effective migration rates between the basins/sub-basins.

RESULTS

Of the total of 1140 base pairs of cyt *b* sequenced, 394 characters were variable and 304 of those characters were parsimony informative. Maximum likelihood and Bayesian analyses yielded similar topologies for cyt *b* and the concatenated mtDNA set. Most of the discrepancies occurred either at the extreme tips of the trees or at nodes with low bootstrap values. As lineages were congruent in all analyses, only the Bayesian trees are shown for each data set with corresponding supporting values listed at each node (Figures 2 & 3). ML converged on a single tree with a log-likelihood score (-lnL) of 7,264.60. The Bayesian analysis resulted in a tree with a likelihood score of 1766.54. For the concatenated mtDNA data set, ML converged on a single tree with a log-likelihood score (-lnL) of 58,695.13. The models of sequence evolution revealed by jModelTest for Bayesian partitions in the mtDNA data set were all GTR + I + G and GTR + I for ND4L. The Bayesian analysis for the mtDNA data set resulted in a tree with a likelihood score of 58,349.92. Deeper nodes within the cyt *b* analysis had weak bootstrap support. The concatenated mtDNA phylogeny in no way contradicted the one produced by cyt *b* analysis but did reveal stronger support at many of the deeper nodes.

Based on mtDNA, mountain suckers (*C. platyrhynchus*) are paraphyletic, with one major clade representing mountain suckers from the Bonneville Basin, upper Snake River drainage, and Green River drainage and the other major clade consisting of populations from the Lahontan Basin, Columbia River Basin, and upper Missouri River Basin (see Figure 2). These clades were rendered paraphyletic by all *Pantosteus* species (*C. discobolus, C. clarkii, C. santaanae, C. plebeius,* and *C. nebuliferus*) except for *C. columbianus* which is located within the outgroup. Divisions of mountain sucker clades (labeled here as by number as clades I, II, III, IV, and V) correspond to geographic regions (i.e. drainage basins). The majority of sampled locations had only one species of *Pantosteus* present. However, samples collected from the White River (UT) and Gooseberry Creek (UT), both of which are in the upper Price River Basin at the western edge of the Colorado Plateau (see Figure 1), contained both mountain sucker and bluehead sucker haplotypes. Samples containing both mountain suckers and bluehead suckers were also collected from the Weber River (UT), Twin Creek (UT), TinCup Stream (ID), and Hayden Fork (UT).

Clade I and II were sister to each other, clade I being from the upper Snake River Basin (Columbia Basin), Green River drainage (Colorado River Basin) and northern Bonneville being (Great Basin) and clade II being from the southern Bonneville (Great Basin) as well as a few individuals from the western edge of the Colorado River drainage. Clades III, IV, and V consisted exclusively of mountain suckers containing individuals from the Columbia River Basin (VI), Lahontan Basin (VII), and upper Missouri River Basin (VIII). *C. platyrhynchus* found within the Colorado River Basin (Rock Creek, WY; White River, UT, Gooseberry Creek, UT; See Table 1) contained similar haplotypes to those within the Bonneville Basin Clades (I and II).

Estimated time to the most recent common ancestor of *Pantosteus* dates back to the Miocene at approximately 15.9 mya and the divergence between *Pantosteus* and *Catostomus* dates to 24.38 mya. Divergence of clades I and II are dated to the early Pleistocene at 1.96 mya. Divergence between bluehead suckers and mountain suckers from the Bonneville Basin and upper Snake River drainage Basin is dated at 2.81 mya. Clades III, IV, and V diverged in the late Pliocene at roughly 4.00 mya. Estimates of divergence times, being based on a single mitochondrial marker, was surrounded by wide confidence intervals (See Figure 4).

AMOVA testing among the Lahontan and Columbia River drainage basins indicates that most of the variation (79.32%) is explained by differences among the drainage basins (Table 3; AMOVA No. 1). AMOVA between the Columbia River Basin and the Upper Missouri River Basin indicates that most of the genetic structuring is due to variation among localities (84.77%) within all drainage basins included (Table 3; AMOVA No.2). AMOVA between the upper Snake River Basin/northern Bonneville Basin and the southern Bonneville Basin indicates that most of the variation (91.88%) is explained by differences among the upper Snake River/northern Bonneville and the southern Bonneville drainage basins (Table 3; AMOVA No. 3). Fst values for populations within all major drainage basins indicate that little or no gene flow is occurring between drainage basins (Tables 4-5).

DISCUSSION

Within the subgenus *Pantosteus*, the mountain sucker exhibits a large amount of phylogeographic structure that can be tied to known hydrographic events. In addition, divergence time estimates from molecular clock analyses coincide with changes in drainage patterns that have been shown to involve the transfer of other aquatic taxa (e.g. Smith et al. 2002; Houston et al. 2010b). One major phylogenetic group includes those mountain suckers of the Bonneville Basin, upper Snake River Basin, and Green River Basin. This group is subdivided into a clade containing the upper Bonneville Basin, upper Snake River Basin, and Green River Basin (clade I) and a clade made up of the southern Bonneville Basin (clade II). The other *C. platyrhynchus* phylogenetic group includes three genetic lineages representing the Columbia River Basin (clade III), Lahontan Basin (clade IV), and upper Missouri River Basin (V). These mtDNA-based subdivisions are previously unrecognized in mountain suckers and give us insight into our phylogeographic hypotheses.

Fossil evidence indicates that in the Eocene to Oligocene suckers, having originated in Asia, crossed Beringia and began to evolve into the modern North American genera (Sun et al. 2007). In North America two major lines of catostomids formed one, the Catostomini, in Western North America and the other, the Moxostomatini, in Eastern North America (Smith 1992). By the late Eocene to the early to mid-Miocene (c. 25 mya) the modern genera developed. Since the Colorado Plateau uplift during the Miocene (Hunt 1956) is thought to have played a key role in the initial evolution of the subgenus *Pantosteus* (Smith 1966; Campbell 1992), ancestral *Pantosteus* lineages should occur on the Colorado Plateau (Smith 1966). The ancestral lineages to the modern species (*C. platyrhynchus, C. discobolus, C. plebeius,* and *C. clarki*) may then have dispersed to the adjacent drainage basins leading to their current-day distribution. By the middle Pliocene much of the differentiation of *Pantosteus* may have already occurred (Figure 4; Campbell 1992; Belika 2006). One species (*C. discobolus*) remains primarily on the Colorado Plateau itself (Smith 1966) while *C. platyrhynchus, C. plebeius,* and *C. clarki* occur on the periphery of the plateau. Our molecular clock dating indicates that *Pantosteus* split from *Catostomus* in the Miocene roughly 15.9 mya, which fits Smith's (1966) projections. However the phylogenetic relationships did not agree with our first hypothesis regarding ancestral *C. platyrhynchus* being in the Colorado River Basin. Neither Bonneville Basin nor Colorado River Basin mountain suckers (*C. platyrhynchus*) were basal to other members of that species, with those within the Colorado River Basin containing similar haplotypes to the Bonneville Basin lineages. In addition, *C. platyrhynchus* does not form a monophyletic clade. This implies that other geographic events such as major river captures, rather than the rise of the Colorado Plateau, may have been instrumental in the evolution of the mountain sucker mitochondrial genome.

The headwaters of the early Snake River are known to have had connections to the Lahontan Basin. The Snake River, as it flowed west from the rise of the Yellowstone hotspot, may have been connected to Pliocene Lake Idaho as early as 9 mya (Kimmel 1982). Some hypotheses regarding the exit route of the Snake River system suggest that it flowed into Lake Idaho and exited through eastern Oregon, eventually entering the Pit-Sacramento or Klamath River basins (See Figure 1; Miller 1965, Taylor 1985, Minckley et al. 1986, Smith et al. 2000) with secondary connections to the Lahontan Basin. However, fossil dating of mammals (Repenning et al. 1995) and phlyogenetic investigations of mollusks (Hershler and Li 2004) suggest that the Oregon connection may not have existed (Billman et al. 2010). Instead an alternate route for the pre-modern Snake River may have been southwest through the Lahontan Basin to the Pit River system (Repenning et al. 1995, Link et al 2005, Beranek et al. 2006, Billman et al. 2010). If this is the case, when the Snake River was captured into the Columbia River Basin by the draining of Lake Idaho, the Lahontan-Snake River connection would have been broken by 3 mya, (Beranek et al. 2006, Billman et al. 2010). Our data indicate that the Lahontan mountain suckers and Columbia Basin mountain suckers are monophyletic sister taxa, that are very distinct from the other mountain suckers as well as other members of the subgenus *Pantosteus*. Their association strongly supports the mountain suckers in the vicinity of Lake Idaho being geographically linked, likely during a Lahontan Basin connection with the upper Snake River. While this association does not directly negate the Oregon dispersal route, it does indicate that the draining of Lake Idaho and the transfer of the upper Snake River into the Columbia River Basin may have been a pivotal vicariant event separating the two western lineages of *C. platyrhynchus*.

Following the draining of Lake Idaho (3.5 mya), mountain suckers in the middle Snake River would be cut off from those of the Lahontan Basin and at the same time the transfer of the lower Snake River through Hell's Canyon would have created a connection with the Columbia River system (see Figure 1). Our dating, at roughly 4.00 mya, may reflect this event (Figure 4). Accordingly, our hypotheses regarding the phylogenetic patterns of the Lahontan and Columbia River Basin mountain suckers are correct. Lahontan Basin mountain suckers show a close phylogenetic tie to the Columbia River Basin mountain suckers and yet contain a unique genetic signature due to their long isolation. AMOVA supports this in that most of the genetic differences were due to variation between drainage basins (Table 3). Fst values for these two drainage basins indicate that little gene flow is occurring (Table 4). This is also to be expected, as the modern drainage basins remain isolated. However, it should be noted that Fst may not be the best indicator of gene flow estimates (see Pearse and Crandall 2004).

The positioning of this Lahontan-Columbia River clade as separate from other *Pantosteus* indicates that dispersal history is more complex than a derived mountain sucker dispersing from the Colorado Plateau to the Bonneville Basin and then to the Lahontan Basin. Rather, assuming that the origin of *Pantosteus* was indeed from the uplift of the Colorado Plateau, a basal form dispersed west, soon after the evolution of the subgenus, and independent of the mountain suckers (*C. platyrhynchus*) that would give rise to the Bonneville and Upper Snake River basin forms.

Our phylogeny indicates that the sister taxon for the Missouri River *C. platyrhynchus* is the Columbia Basin *C. platyrhynchus*. The Missouri River clade is also part of the monophyletic clade with the Lahontan Basin and Columbia River Basin mountain suckers. Divergence dating indicates that the Missouri River Basin, Columbia River Basin, and Lahontan Basin mountain suckers may have initially separated from the Bonneville and Colorado River Basin suckers as early as 15.94 mya in the Miocene, which predates a late, Pliocene cooling induced stream rejuvenation by well over 10 million years (Smith 1966). Thus, the spread of mountain suckers from the Columbia River Basin to the Missouri River Basin may be more reflective of hydro-geographic history. The close relationship of the Columbia River system mountain suckers with those of the upper Missouri River Basin may indicate that if mountain suckers dispersed from the Columbia River system to the upper Missouri River system, dispersal would have been a rapid event and likely occurred in the late Pliocene to early Pleistocene. We believe a possible transfer event from the Columbia River Basin to the Upper Missouri River Basin may have occurred near

Clark's Fork (see figure 1) in current-day Montana or possibly during the capture of the upper Salmon River by the lower Salmon River in Idaho.

We found no evidence for a transfer event of southern Bonneville Basin *C. platyrhynchus* across the continental divide into the Missouri River drainage via the upper Snake River nor for Green River *C. platyrhynchus* crossing the continental divide in Wyoming, as hypothesized by Smith (1966; figure 1). AMOVA indicates that most of the genetic structuring is due to variation between the Upper Missouri and Columbia River Basins drainage basins also suggesting isolation and Fst values show that little gene flow is occurring. Fst values indicate that gene flow is not occurring between the Missouri, Columbia, and Lahontan Basins (Table 4).

In addition to the transfer point across the continental divide leading to the divergence of Missouri River Basin mountain suckers, we found evidence of a more recent transfer event between the upper Snake River Basin and the Green River of the Colorado River Basin. Taylor (1985) reported evidence for a connection from the upper Snake River drainage to the Green River drainage in mollusks, and suggests that a possible diversion of glacial waters in the Snake River drainage into the Green River in what is now present-day Uinta County, Wyoming (see figure 1). It is possible that the same, or a geographically similar, transfer event may have occurred for the mountain sucker, allowing the population we identified to cross over into the Green River drainage.

Smith (1966) described three major subdivisions of the mountain suckers within the Great Basin; the Sevier River drainage (southern Bonneville Basin); the streams flowing west from the northern Wasatch Mountains (northern Bonneville Basin); and the Lahontan Basin, with inhabitants from the Sierra Nevada and the Humboldt River drainages (See Figure 2). Little morphological differentiation between these groups was thought to indicate slow evolutionary change in the species (Smith 1966, Campbell 1992, Belika 2006). Our analysis for the Bonneville Basin correlates with the morphological divisions found by Smith. We found two separate lineages within the Bonneville Basin—one lineage being in the southern Bonneville Basin and the other lineage being in the northern Bonneville Basin and upper Snake River drainage, directly north of the Bonneville Basin above Shoshone Falls. Diversification between the northern Bonneville/upper Snake River basins and the southern Bonneville Basin has been exhibited by trout (Martin et al. 1985), leatherside chub (Johnson 2002), and Utah sucker (Mock, et al. 2006). This separation is often attributed to the Late Pleistocene transfer of the Bear River into the Bonneville Basin (Hubbs and Miller 1949), which allowed the dispersal of fish from the upper Snake River Basin into the Bonneville Basin. The divergence dating at 1.96 mya (Figure 4), may reflect the initial separation of the populations in the upper Snake River and Bonneville Basins, likely by a vicariant event such as the severing of an earlier connection between the upper Snake River and the Bonneville Basin, or a short lived spillover from the Bonneville Basin to the Snake River much earlier than the final high stand of Lake Bonneville. Alternatively, the upper Bonneville Basin and southern Bonneville Basin may have been isolated by Lake Bonneville acting as an isolating barrier separating the northern and southern mountain sucker populations during pluvial times and saline lakes may have continued that isolation during interglacial periods. This has been seen in other pluvial lake systems (Houston et al 2011). AMOVA indicates that most of the genetic structuring is due to variation between drainage basins also suggesting isolation and Fst values show that little gene flow is occurring (Table 3, 5).

In this study we also examined *C. platyrhynchus* populations in the White River (UT) and Gooseberry Creek (UT), both of which are in the Price River Basin of the Colorado River Basin.

They contained haplotypes from both the northern and southern Bonneville Basin (see Figure 1). This may indicate a headwater transfer point southeast of Utah Lake at Soldier Summit or these haplotypes may be remnants of bait-bucket transfers. Some haplotypes from these two Colorado River Basin populations were identical to haplotypes found in both the northern and southern Bonneville Basin indicating that a bait-bucket transfer event is more likely.

Conclusions

This study has shown the importance of hydrogeographic events in shaping the evolution of fishes. Our tree topology also indicates that while the subgenus *Pantosteus* is monophyletic, the species now classified as the mountain sucker (*C. P. platyrhynchus*) appears to be paraphyletic, indicating that taxonomic revision of this species is necessary. However, additional nuclear markers should be sequenced to ensure that any future taxonomic revision is done correctly. In addition, the mountain sucker shows close ties to the bluehead sucker and further examination of the bluehead sucker as well as other *Pantosteus* species will refine relationships of individuals in this group.

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Table 1: List of populations included in phylogenetic analysis. Clade numbers are identified for each locality. ID number corresponds to BYU identification number, GenBank number, or in the case of samples sent to us by the Tom Dowling lab, ID numbers begin with TD and are followed by the researchers' alpha-numerical code.

Clade	Locality	Basin	Sub-Basin	GPS	Species	ID number
I	Bear River, UT	Great Basin	Bonneville	40° 54' 40.20" N 110° 49' 50.10" W	P. platyrhynchus	114410, 114677
I	Big Creek, UT	Great Basin	Bonneville	41° 63' 29.80" N 111° 15' 46.40" W	P. platyrhynchus	114643, 45-48
I	Blackrock Creek, WY	Columbia	Upper Snake	43° 49' 48.00" N 110° 21' 52.00" W	P. platyrhynchus	145155-59
I	Current Creek, UT	Great Basin	Bonneville	39° 54' 05.60" N 111° 53' 36.60" W	P. platyrhynchus	111940-41, 43-45
I	Dipper Creek, WY	Great Basin	Bonneville	42° 25' 04.21" N 110° 59' 06.40" W	P. platyrhynchus	145288-92
I	Eccles Creek, UT	Great Basin	Bonneville	40° 58' 27.60" N 111° 25' 53.50" W	P. platyrhynchus	114615-19
I	Giraffe Creek, WY	Great Basin	Bonneville	42° 26' 00.00" N 111° 00' 34.00" W	P. platyrhynchus	112111-15
I	Gooseberry, UT	Colorado	Upper Colorado	38° 36' 55.00" N 111° 47' 41.00" W	P. platyrhynchus	227642, 45
I	Hayden Fork, ID	Great Basin	Bonneville	44° 42' 18.00" N 113° 45' 26.00" W	P. platyrhynchus	227398
I	Lone Springs, UT	Great Basin	Bonneville	41° 45' 53.69" N 112° 58' 39.68" W	P. platyrhynchus	79151, 60, 69, 78
I	Mill Creek, UT	Great Basin	Bonneville	40° 59' 43.50" N 110° 50' 41.20" W	P. platyrhynchus	114380-84
I	Rock Creek, WY	Colorado	Green	42° 19' 09.85" N 110° 25' 24.81" W	P. platyrhynchus	145285-87
I	Silver Creek, UT	Great Basin	Bonneville	40° 44' 36.35" N 111° 28' 27.56" W	P. platyrhynchus	78984-87
I	Soldier Creek, UT	Great Basin	Bonneville	39° 59' 40.13" N 111° 29' 25.95" W	P. platyrhynchus	114497-99, 501
I	TinCup Stream, ID	Columbia	Upper Snake	42° 58' 27.30" N 111° 16' 08.70" W	P. platyrhynchus	227220-24
I	Tuponce Creek, ID	Columbia	Upper Snake	42° 51' 05.30" N 112° 01' 06.20" W	P. platyrhynchus	227230-34
I	Weber River, UT	Great Basin	Bonneville	40° 54' 57.61" N 111° 24' 26.90" W	P. platyrhynchus	57108, 10-12
I	White River, UT	Colorado	Upper Colorado	39° 59' 07.40" N 111° 01' 44.10" W	P. platyrhynchus	111950, 54, 71
II	Mammoth Creek, UT	Great Basin	Bonneville	37° 37' 37.00" N 112° 27' 10.40" W	P. platyrhynchus	112179-83
II	Salina Creek, UT	Great Basin	Bonneville	38° 52' 52.00" N 111° 34' 39.70" W	P. platyrhynchus	111910-12, 15
II	San Pitch River, UT	Great Basin	Bonneville	39° 31' 20.50" N 111° 32' 35.30" W	P. platyrhynchus	112234-38
II	Sevier River, UT	Great Basin	Bonneville	38° 03' 14.80" N 111° 58' 21.40" W	P. platyrhynchus	112208-12
II	White River, UT	Colorado	Upper Colorado	39° 59' 07.40" N 111° 01' 44.10" W	P. platyrhynchus	111952, 57, 66
III	Payette, ID	Columbia	Mountain Snake	44° 101' 18.70" N 115° 57' 20.30" W	P. platyrhynchus	227954-57
III	Salmon Falls Cr., ID	Columbia	Lower Snake	41° 58' 25.00" N 114° 42' 08.70" W	P. platyrhynchus	227306-08
III	Similkameen R., WA	Columbia	Columbia Cascade	48° 53' 30.00" N 119° 25' 49.00" W	P. platyrhynchus	TD.simil.cb
III	Willamette, OR	Columbia	Columbia Plateau	44° 01' 23.00" N 123° 01' 25.00" W	P. platyrhynchus	227388-91
III	Wolf Creek, Canada	Columbia	Columbia Cascade	unknown	P. platyrhynchus	TD.wolf.cb
IV	Dog Valley Creek, NV	Great Basin	Lahonton	39° 32 '41.44" N 119° 59' 10.47" W	P. platyrhynchus	112284, 86-88
IV	E. Walker R. (Zanis), NV	Great Basin	Lahonton	38° 31' 09.80" N 118° 57' 43.10" W	P. platyrhynchus	112323-26
IV	Humboldt River, NV	Great Basin	Lahontan	41° 24' 00.00" N 117° 19' 09.00" W	P. platyrhynchus	H1, H2
IV	Lower Truckee R., NV	Great Basin	Lahonton	39° 30' 29.60" N 119° 39' 43.24" W	P. platyrhynchus	112258-59
IV	McDermitt Creek, WA	Great Basin	Lahontan	47° 99' 98.80" N 117° 92' 68.10" W	P. platyrhynchus	1300-33, 1300-34
IV	River Bend, NV	Great Basin	Lahonton	39° 29' 24.05" N 119° 59' 19.01" W	P. platyrhynchus	112302-06
V	Little Popo Agie R., WY	Missouri	Upper Missouri	42° 68' 25.73" N 108° 66' 52.37" W	P. platyrhynchus	227332-36
V	Tongue River, WY	Missouri	Upper Missouri	46° 24' 30.00" N 105° 52' 02.00" W	P. platyrhynchus	59571-72, 74-75
V	Whitewood Ck, SD	Missouri	Upper Missouri	44° 46' 00.20" N 103° 62' 00.20" W	P. platyrhynchus	227362-66
V	Willow Ck., Canada	Missouri	Upper Missouri	50° 01' 10.00" N 113° 34' 42.00" W	P. platyrhynchus	Can1, Can2
C. clarkii	Aravaipa Ck, AZ	Colorado	Lower Colorado	unknown	P. clarkii	TD.ARA13.cb
C. clarkii	Bill Williams Cr., AZ	Colorado	Lower Colorado	unknown	P. clarkii	TD.FRA1
C. clarkii	Virgin R., UT	Colorado	Lower Colorado	unknown	P. clarkii	56800
C. clarkii	White River, CO	Colorado	Lower Colorado	unknown	P. clarkii	TD.wr2
C. discobolus	Animas River, CO	Colorado	Upper Colorado	37° 11' 12.79" N 107° 52' 44.55" W	P. discobolus	179232-36
C. discobolus	Hayden Fork, ID	Great Basin	Bonneville	44° 42' 18.00" N 113° 45' 26.00" W	P. discobolus	227399, 401
C. discobolus	Irish Canyon, WY	Colorado	Green	42° 40' 19.00" N 109° 23' 14.00" W	P. discobolus	145269, 72-73, 75
C. discobolus	TinCup Stream, ID	Columbia	Upper Snake	42° 58' 27.30" N 111° 16' 08.70" W	P. discobolus	227225
C. discobolus	Havasu Creek, AZ	Colorado	Lower Colorado	36° 18' 28.00" N 112° 45' 43.00" W	P. discobolus	TD.hav2
C. discobolus	East Clear Ck, AZ	Colorado	Lower Colorado	34° 37' 25.00" N 111° 02' 22.00" W	P. discobolus	TD.EC1
C. discobolus	Raft River, UT	Columbia	Lower Snake	41° 58' 02.50" N 113° 39' 60.60" W	P. discobolus	58619-23
C. discobolus	Weber River, UT	Great Basin	Bonneville	40° 54' 51.00" N 111° 24' 24.00" W	P. discobolus	145125, 27, 29
C. nebuliferous	Rio Nazas, Mexico	Nazas	Nazas	unknown	P. nebuliferus	TD.CATO.78
C. plebeius	Rio Conchos, Mexico	Rio Grande	Conchos	unknown	P. plebeius	TD.CATO.45
C. plebeius	Rio Escalariado, Mexico	Rio Grande	Guzmán	unknown	P. plebeius	TD.CATO.11
C. plebeius	Rio Fuerte, Mexico	Rio Grande	Fuerte	unknown	P. plebeius	TD.CATO.63
C. plebeius	Rio Nutria, NM	Colorado	Lower Colorado	unknown	P. plebeius	TD.zbs.033
C. plebeius	Rio Santa Clara, Mexico	Rio Grande	Guzmán	unknown	P. plebeius	TD.CATO.17
C. plebeius	South Fork Palomas Ck., NM	Rio Grande	Palomas	unknown	P. plebeius	Rio1
C. plebeius	unknown	Rio Grande	unknown	unknown	P. plebeius	TD.CATO.87
C. santaanae	Big Tununga River, CA	Los Angeles	Tununga	unknown	P. santaanae	TD.psbt1
C. santaanae	San Gabriel River, CA	Los Angeles	San Gabriel	unknown	P. santaanae	TD.pssg1
C. santaanae	Santa Ana River, CA	Los Angeles	Santa Ana	unknown	P. santaanae	TD.pssa3
C. santaanae	unknown, CA	Los Angeles	unknown	unknown	P. santaanae	TD.pssg9

Table 1 (continued):

Clade	Locality	Basin	Sub-Basin	GPS	Species	ID number
Outgroup	N/A	N/A	N/A	N/A	C. ardens	TD.STR4CA
Outgroup	N/A	N/A	N/A	N/A	C. catostomus	TD.MINCCAT1
Outgroup	N/A	N/A	N/A	N/A	C. columbianus	227288
Outgroup	N/A	N/A	N/A	N/A	C. commersonii	TD.C.comm
Outgroup	N/A	N/A	N/A	N/A	C. insignis	TD.ecci1
Outgroup	N/A	N/A	N/A	N/A	C. machrocheilus	227304
Outgroup	N/A	N/A	N/A	N/A	C. occidentalis	GI|28201355|
Outgroup	N/A	N/A	N/A	N/A	C. tahoensis	112322
Outgroup	N/A	N/A	N/A	N/A	C. wigginsi	GI|197293319|
Outgroup	N/A	N/A	N/A	N/A	M. poecilurum	GI|347949601|

Table 2: List of primer sequences. With the exception of Glu31 (Unmack et al. 2009), all primers used in this study were designed for this research.

Gene	Primer	Nucleotide Sequence
Cyt*b*	Glu31	TGRCTTGAAAAACCACCGTTGT
Cyt*b*	Pan.Thr.52	GCTCTAGGGAGGAGTTTAACC
Cyt*b*	Pan.636.Hd	AAGAGATTTTGTCCGCATCCGAG
Cyt*b*	Pan.494F	GAGGTGGATTTTCAGTAGATAATGC
ND1	Leu3F	GCAGAGCCCGGTAATTGCGAGAG
ND1	Ile24R	CTATCAGCCACGCTATCAAGGTG
ND2	Gln56F	ACTACACCACTTTCTAGTAAGGTCAGC
ND2	Ala13R	GCATTCAGAAGATGTGGGATAAAGTC
ATP*ase*	Lys.22F	AAAGCGTTAGCCTTTTAAGC
ATP*ase*	Co3.cato.23R	GGCTTGGATCAACCATATGAT
Arg	Arg.cato.2F	CAAGACCTCTGATTTCGGCTC
ND4	ND4.cato.598R	TTACGAGGAAGGCAATTAAGCA
ND4	ND4.cato.369F	GAAGCCACTCTAATTCCAACCTTA
His	His.cato.R	CACAATCTAGTGTTTTAAGTTAAAC
LEU	LEUF	GTCTTAGGAACCAAAAACTCTTG
ND5	ND5.cato.907R	CTGGATGTAGAGAAGGCTACAAT
ND5	ND5.cat.739F	GCCCTACTCCACTCAAGCAC
ND5	ND5.cato.1636R	GGACAAACAATCGCCACCCA
ND5	ND5.cato.1522F	ACCCTAACTAACAAGCAATTTA
Glu	Glu.cato.2R	TAGTTGAATAACAACGGTGGTTC

Table 3: AMOVA results for Lahontan Basin vs. Columbia Basin mountain sucker populations (A). AMOVA results for the Columbia River Basin vs. Upper Missouri River Basin mountain suckers (B). AMOVA results for Upper Snake/northern Bonneville Basins vs. southern Bonneville Basin mountain suckers (C).

AMOVA No. 1 (Lahontan Basin vs. Columbia Basin mountain suckers)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Indices	P-values
Among groups	1	268.635	17.08480 (Va)	79.32	0.79316 (FCT)	0.00000+ - 0.00000
Among populations within groups	9	94.567	3.38313 (Vb)	15.71	0.75934 (FSC)	0.00000+- 0.00000
Within populations	21	22.517	1.07222 (Vc)	4.98	0.95022 (FST)	0.00098+- 0.00098
Total:	31	385.719	0.36617	100.00		

AMOVA No. 2 (Columbia Basin vs. Upper Missouri River Basin)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Indices	P-values
Among groups	1	570.062	17.58191 (Va)	84.77	0.84773 (FCT)	0.00000+- 0.00000
Among populations within groups	7	96.267	2.36071 (Vb)	11.38	0.74749 (FSC)	0.00000+- 0.00000
Within populations	19	26.317	0.79747 (Vc)	3.85	0.96155 (FST)	0.00000+- 0.00000
Total:	27	692.646	20.74010	100.00		

AMOVA No. 3 (Upper Snake/northern Bonneville Basins vs. southern Bonneville Basin)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Indices	P-values
Among groups	1	336.708	11.35751 (Va)	91.88	0.91879 (FCT)	0.00000+- 0.00000
Among populations within groups	18	27.213	0.15847 (Vb)	1.28	0.15786 (FSC)	0.00293+- 0.00164
Within populations	65	54.950	0.84538 (Vc)	6.84	0.93161 (FST)	0.00000+- 0.00000
Total:	84	418.871	12.36136	100.00		

Table 4: Pairwise Fst values (cyt b) for populations in the Lahontan Basin, Upper Missouri Basin, and the Columbia Basin populations of mountain suckers as reported by Arlequinn. Statistically significant values with Bonferroni corrections are shaded in blue.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Dog Valley C., NV	-														
2. East Walker R., NV	0.24	-													
3. Humboldt R., NV	0.11	0.21	-												
4. Lower Truckee, NV	0.25	0.48	0.50	-											
5. McDermitt C., OR	0.25	0.48	0.50	1.00	-										
6. River Bend, NV	-0.01	0.35	0.29	0.55	0.55	-									
7. Little Popo Agie, WY	0.18	0.25	0.14	0.42	0.42	0.30	-								
8. Tongue, WY	0.58	0.61	0.72	1.00	1.00	0.67	0.28	-							
9. Whitewood, SD	0.40	0.45	0.44	0.69	0.69	0.50	0.12	-0.05	-						
10. Willow C., Can.	0.11	0.21	0.00	0.50	0.50	0.29	-0.08	0.38	0.02	-					
11. Payette R., ID	0.17	0.24	0.11	0.42	0.42	0.29	0.18	0.58	0.40	0.11	-				
12. Salmon Falls, ID	0.24	0.31	0.21	0.57	0.57	0.37	0.26	0.72	0.50	0.20	0.24	-			
13. Similkameen, Can.	0.17	0.30	0.00	1.00	1.00	0.40	0.20	1.00	0.60	0.00	0.17	0.33	-		
14. Willamette R., OR	0.09	0.18	0.00	0.37	0.37	0.24	0.12	0.58	0.36	0.00	0.09	0.17	0.00	-	
15. Wolf C., Canada	0.17	0.30	0.00	1.00	1.00	0.40	0.20	1.00	0.60	0.00	0.17	0.33	0.00	0.00	-

Table 5: Pairwise F_{st} values (cyt *b*) for populations in the Upper Snake River Basin/northern Bonneville Basin and the southern Bonneville Basin populations of mountain suckers as reported by Arlequinn. Statistically significant values with Bonferroni are shaded in blue.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. Mammoth C., UT	-																				
2. Salina C., UT	-0.11	-																			
3. San Pitch, UT	0.25	0.07	-																		
4. Sevier R., UT	0.10	0.05	0.25	-																	
5. Bear R., UT	0.35	0.31	0.55	0.35	-																
6. Big C., WY	0.35	0.32	0.50	0.35	-0.29	-															
7. Blackrock C, WY	0.20	0.16	0.35	0.20	-0.16	-0.06	-														
8. Current C., UT	0.25	0.22	0.40	0.25	0.29	0.26	0.14	-													
9. Dipper C., WY	0.15	0.11	0.30	0.15	0.08	0.12	0.01	0.17	-												
10. Eccles C., UT	0.20	0.16	0.35	0.20	0.37	0.35	0.20	0.29	0.18	-											
11. Giraffe C., ID	0.35	0.33	0.50	0.35	-0.29	-0.11	-0.06	0.26	0.12	0.35	-										
12. Hayden Fork, ID	0.35	0.31	0.55	0.35	0.00	-0.29	-0.16	0.29	0.08	0.37	-0.29	-									
13. Lone Springs, UT	0.21	0.16	0.37	0.21	0.53	0.48	0.32	0.10	0.26	0.32	0.48	0.53	-								
14. Mill C., UT	0.35	0.33	0.50	0.35	-0.29	-0.11	-0.06	0.26	0.11	0.35	-0.11	-0.29	0.48	-							
15. Rock C., WY	0.44	0.4.	0.62	0.44	1.00	0.74	0.56	0.62	0.50	0.46	0.74	1.00	0.61	0.74	-						
16. Silver C., UT	0.13	0.08	0.29	0.13	-0.08	-0.01	-0.09	0.11	-0.02	-0.02	-0.01	-0.08	0.25	-0.01	0.52	-					
17. Soldier C., UT	0.13	0.08	0.29	0.13	0.25	0.25	0.10	0.21	0.09	0.12	0.25	0.25	0.25	0.25	0.52	0.05	-				
18. Tincup S., ID	0.55	0.55	0.70	0.55	0.00	-0.00	0.13	0.50	0.33	0.56	-0.00	0.00	0.71	-0.00	1.00	0.23	0.50	-			
19., Toponce S., ID	0.55	0.55	0.70	0.55	0.00	-0.00	0.13	0.50	0.33	0.56	-0.00	0.00	0.71	-0.00	1.00	0.23	0.50	0.00	-		
20. Twin C., WY	0.10	0.00	0.40	0.10	1.00	0.60	0.30	0.40	0.20	0.30	0.60	1.00	0.33	0.60	1.00	0.17	0.17	1.00	1.00	-	
21. Weber R., UT	0.13	0.08	0.29	0.13	-0.08	-0.01	-0.09	0.11	-0.02	0.15	-0.01	-0.08	0.25	-0.01	0.52	-0.11	0.05	0.23	0.23	0.17	-

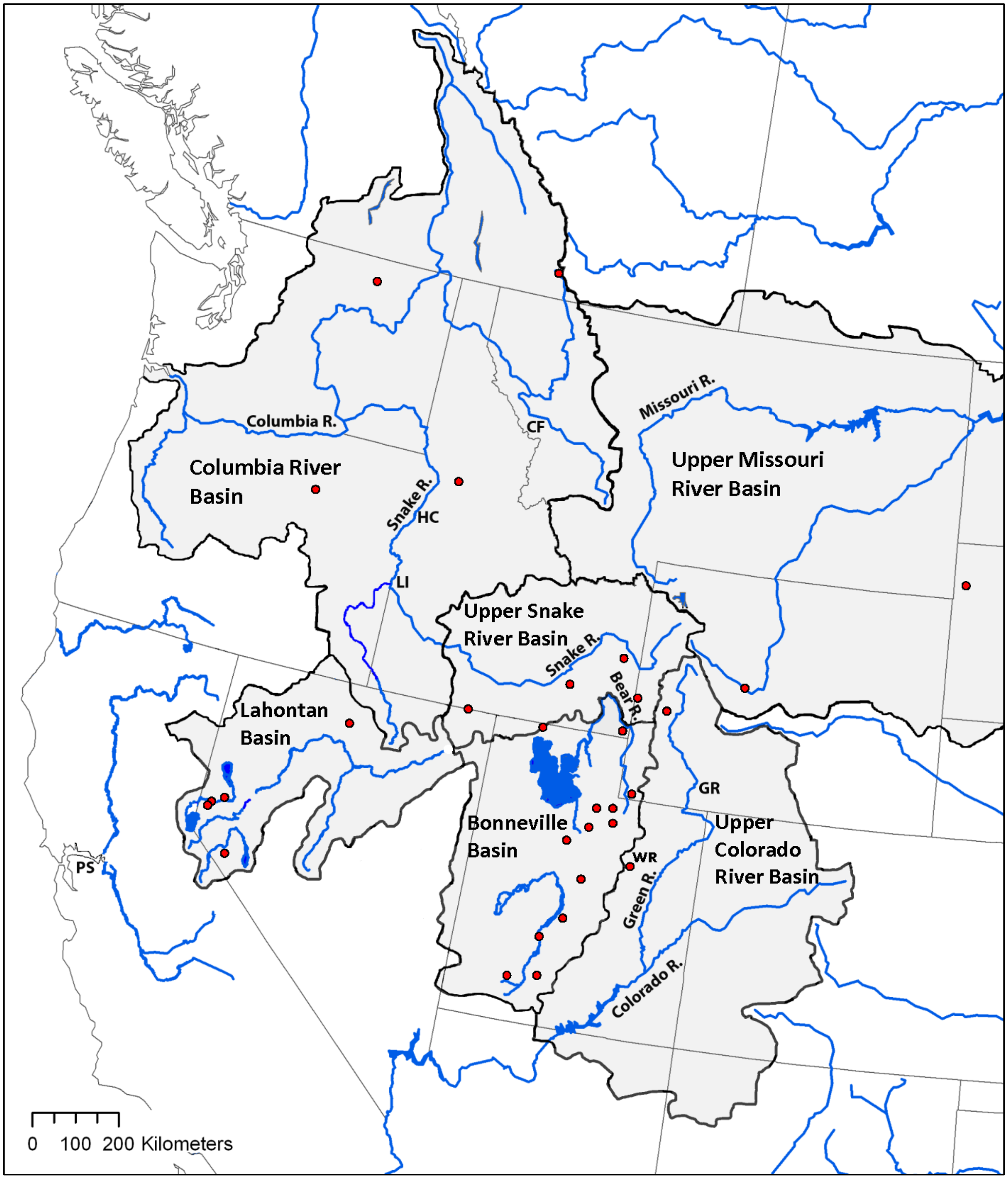

Figure 1: Collection sites for mountain suckers indicated in red within the Columbia River Basin, Upper Missouri River Basin, Upper Snake River Basin, Lahontan Basin, Bonneville Basin, and Upper Colorado River Basin. Certain localities as discussed in the paper are pointed out by the following code: LI=Lake Idaho, HC=Hell's Canyon, PS=Pit-Sacramento system, GR=hypothesized Green River transfer point, CF=Clark's Fork, WR=White River, Utah

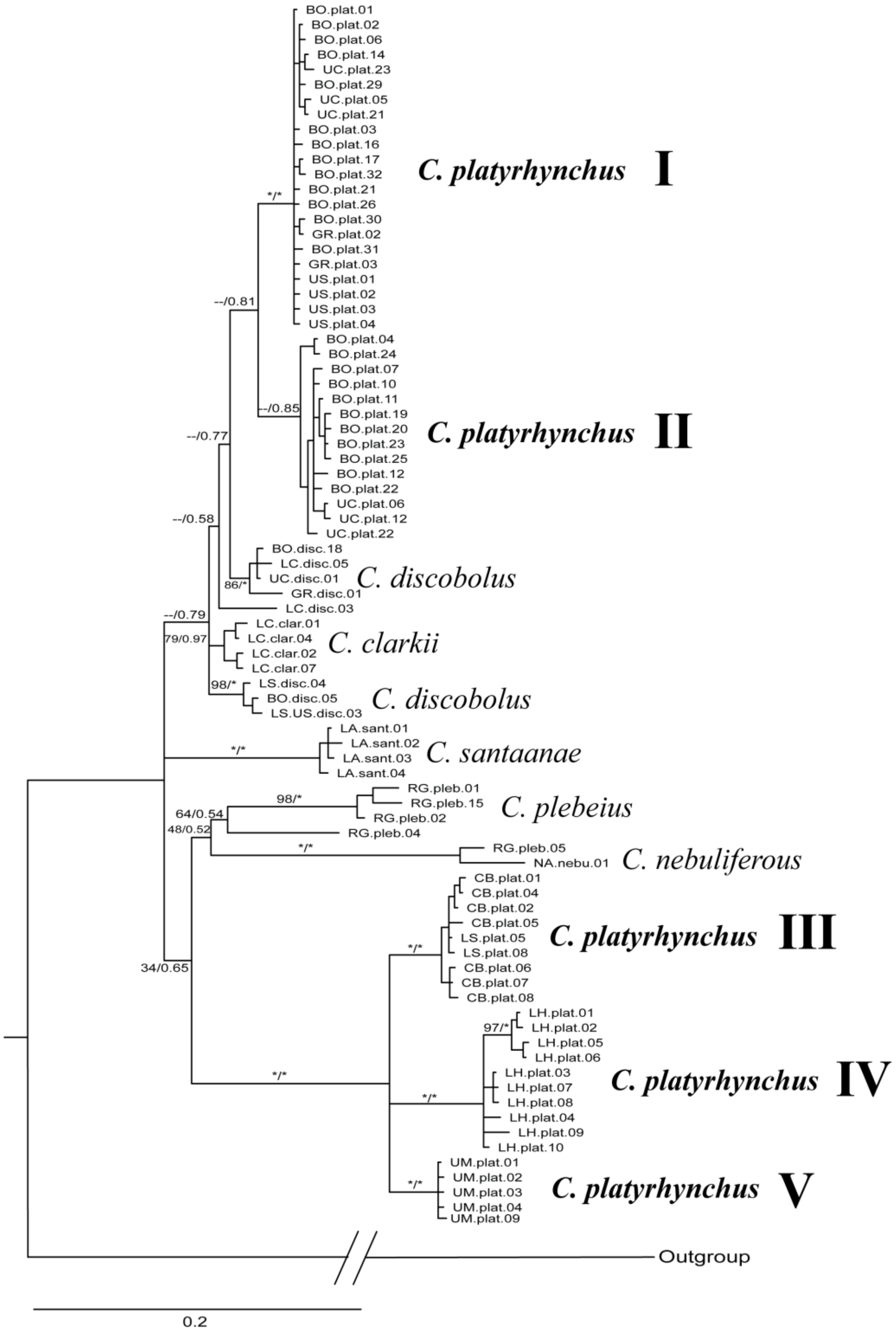

Figure 2: Bayesian tree generated for cytochrome *b* sequence data. Terminal taxa are grouped by species with corresponding clade reference numbers. Bootstrap values from maximum likelihood analysis and posterior probabilities from Bayesian analysis are listed at each branch respectively. Bootstrap values 99 or above and posterior probabilities of 1.0 are indicated by (*). Bootstrap values below 50 and posterior probabilities below 0.50 are not shown or indicated by (--). Some support values were also removed from terminal taxa within major clades.

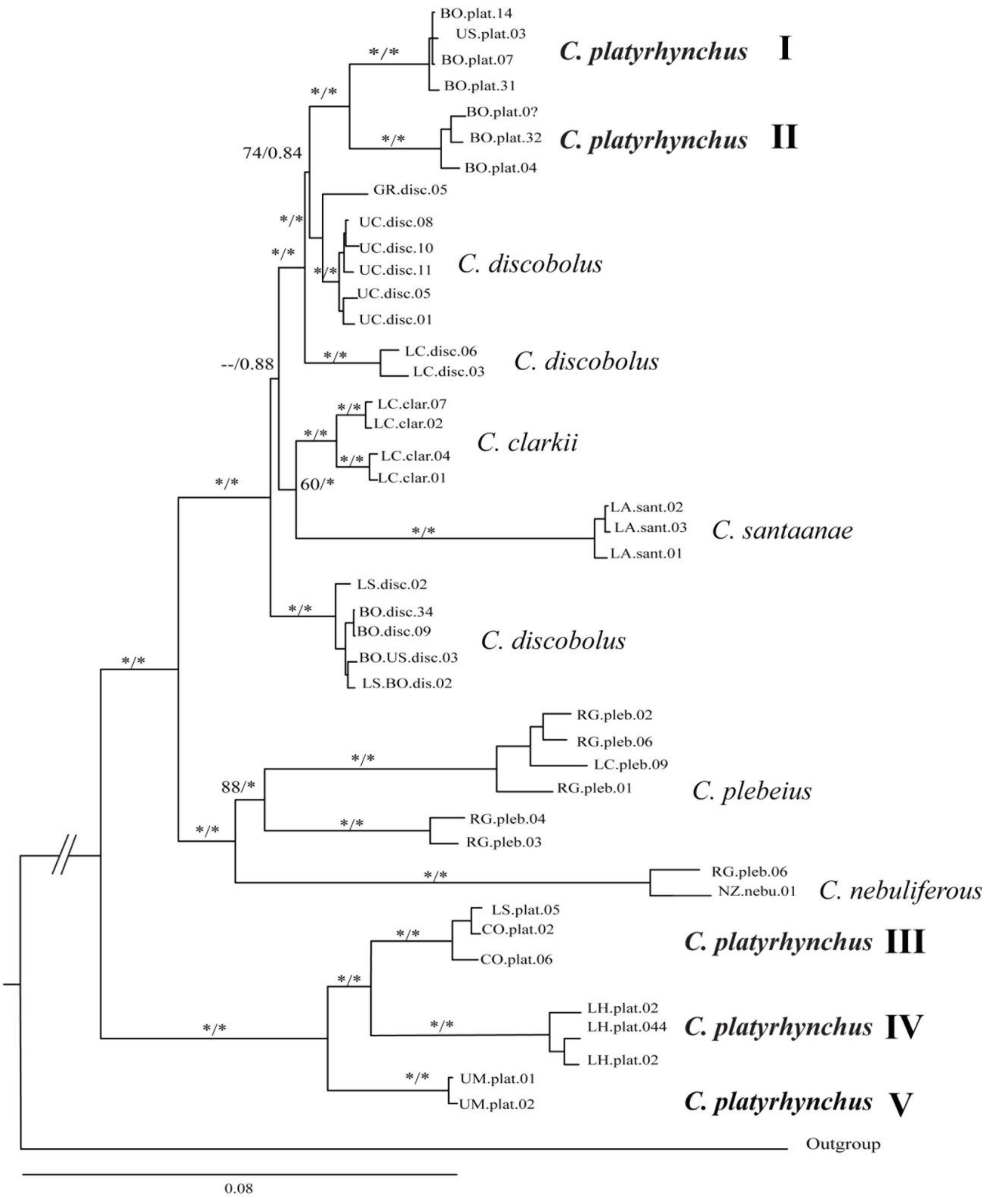

Figure 3: Bayesian tree generated for mtDNA sequence data. Terminal taxa are grouped by species with corresponding clade reference numbers. Bootstrap values from maximum likelihood analysis and posterior probabilities from Bayesian analysis are listed at each branch respectively. Bootstrap values 99 or above and posterior probabilities of 1.0 are indicated by (*). Bootstrap values below 50 and posterior probabilities below 0.75 are not shown. Some support values were also removed from terminal taxa within major clades.

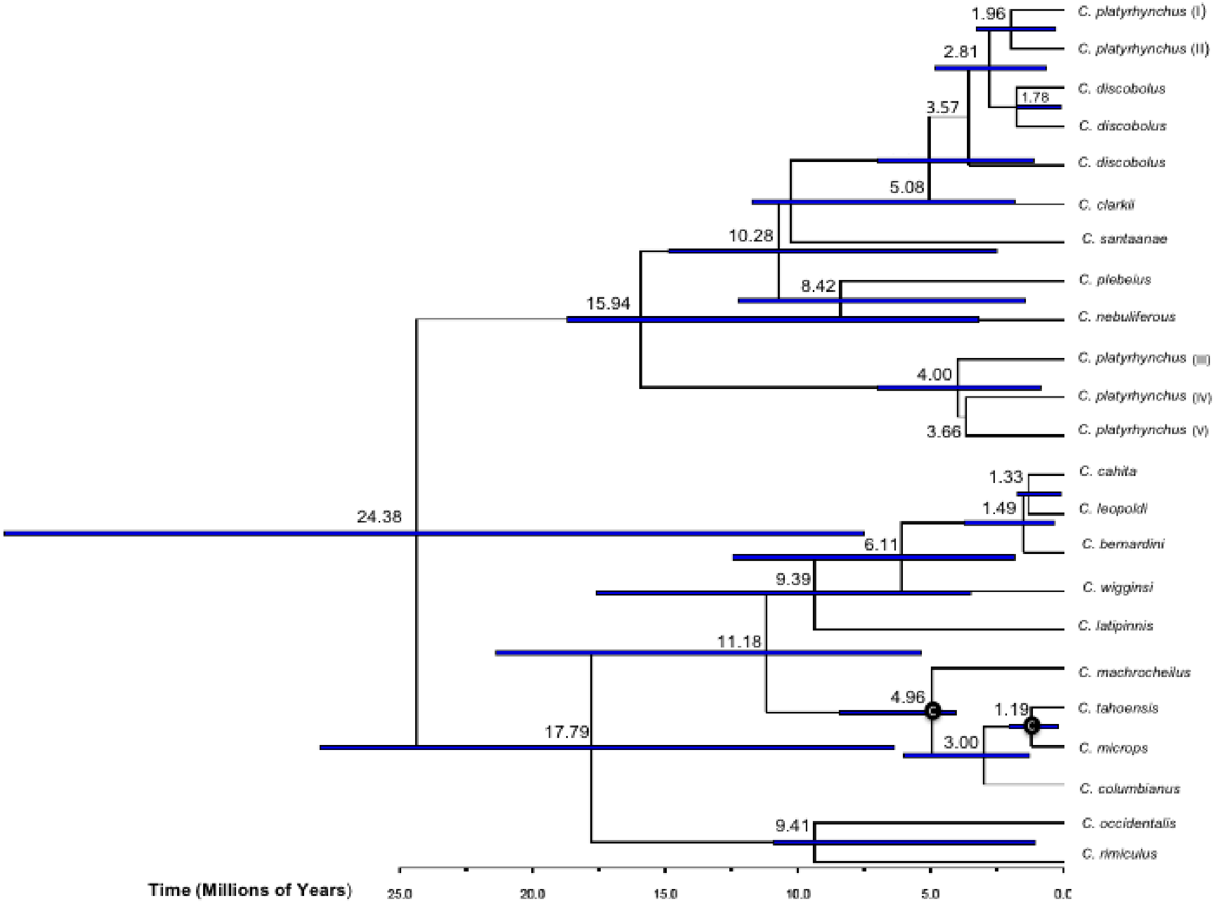

Figure 4: Molecular clock analysis showing divergence time estimates based on BEAST analysis. Mean divergence times, listed above the branch leading to corresponding nodes, are given in millions of years. 95% confidence intervals are shown on designated nodes. The calibration points are marked with a "C". Terminal taxa are labeled with scientific names and clade numbers from phylogenetic analysis are designated on corresponding lineages.