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Molecular systematics and phylogeography of the genus Richardsonius

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MOLECULAR SYSTEMATICS AND PHYLOGEOGRAPHY OF THE GENUS *RICHARDSONIUS*

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ABSTRACT

Molecular Systematics and Phylogeography of the Genus *Richardsonius*

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The complex geological and climatic events that significantly altered the landscape throughout the Cenozoic Era impacted the diversification of many North American taxa, including freshwater fishes. Here, I employ an array of phylogenetic analyses using a multiple gene tree approach to address several questions regarding the phylogenetic relationships of the North American cyprinid genus *Richardsonius* and two other closely related genera, *Clinostomus* and *Iotichthys*. I also use divergence time estimates generated using fossil calibrations to qualitatively assess the phylogeographic implications of evolution within and among these three genera. Mitochondrial and nuclear DNA sequences show a sister relationship between *Iotichthys* and *Richardsonius*, with *Clinostomus* being sister to an *Iotichthys – Richardsonius* clade. Therefore, the currently recognized sister relationship between *Clinostomus* and *Richardsonius* is not supported by my analyses. The genera *Clinostomus, Iotichthys,* and *Richardsonius* appear to be monophyletic lineages, and the two species within *Richardsonius, R. balteatus* and *R. egregius*, appear to be reciprocally monophyletic sister species. *Richardsonius balteatus* and *R. egregius* both exhibit phylogeographic structure, and I examined phylogeographic structure within *R. egregius* using molecular phylogenetic and population genetic methods. Divergence time estimates between genera and species are

Miocene and Pliocene in age respectively, and divergence between phylogroups within *R. balteatus* and *R. egregius* occurred in the late Pliocene to Pleistocene. Splits between genera and species coincide with documented geological and climatic events, and the deepest divergence within *R. egregius* appears to have been influenced more by Pliocene or early Pleistocene events than by more recent drainage patterns associated with the latest glacial maxima of the Pleistocene Epoch. Contemporary migration does not appear to occur between eastern and western Lahontan Basin populations.

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CHAPTER 1

THE EVOLUTION OF THE WESTERN NORTH AMERICAN LANDSCAPE: A REVIEW OF LATE CENOZOIC PROCESSES THAT MAY HAVE INFLUENCED THE EVOLUTION OF CYPRINID FISHES IN THE GREAT BASIN AND ITS SURROUNDING DRAINAGES

Introduction

A major goal of biogeography is to investigate the influence of geological and climatic changes on the divergence and distribution of populations, species, and higher taxa. A reasonable expectation in biogeographic studies is that episodes of isolation, and hence, opportunities for divergence, may be more common on topographically complex landscapes (Vrba, 1992). Few regions of the world have a more complex landscape than western North America, which has been altered dramatically throughout the Cenozoic by a variety of geological processes (Lutgens and Tarbuck, 2006). The changing landscape would have frequently created barriers to dispersal within widespread taxa, potentially disrupting gene flow among populations. Indeed, the changing landscape appears to have caused deep divergences within terrestrial vertebrates (e.g., Riddle, 1995; Nielson et al., 2001; Carstens et al., 2004; Steele et al., 2005), plants (Brunsfeld et al., 2001; Calsbeek et al., 2003), aquatic invertebrates (Taylor, 1985; Hershler and Sada, 2002; Liu et al., 2003), and freshwater fishes (Smith, 1981; Minckley et al., 1986; Smith et al., 2002).

In addition to alterations made to the landscape by geophysical processes (Raymo and Ruddiman, 1992; Coblentz and Riitters, 2004; Kohn and Fremd, 2008), the dramatic climate oscillations that were the hallmark of the Pleistocene Epoch have further

influenced the evolution of temperate biotas by forcing the ranges of organisms to contract, expand, or shift (Hewitt, 1996; 2004). While it would be expected that individual taxa have responded to Pleistocene glacial cycles according to their own specific ecological requirements and life history strategies (Graham, 1986; Graham et al., 1996; Jackson, 2004; Gagnon and Angers, 2006; Burridge et al., 2008; Stewart et al., 2009), a range of phylogeographic studies have indicated that shared biogeographic patterns, likely associated with glacial cycles, exist in western North America (e.g., Soltis et al., 1997; Brunsfeld et al., 2001; Carstens et al., 2005; Topp and Winker, 2008). Shared biogeographic patterns occur when the same geological or climatic events have influenced genetic structure of multiple taxa within an area in a similar fashion. Hence, when shared biogeographic patterns are observed, inferences can be made regarding the extent to which episodes of Earth history have influenced the evolution of those taxa (Riddle et al., 2008).

If, as seems likely, the evolution of many western North American taxa has been influenced by temporally deeper geological changes to the landscape as well as by more recent changes in global climate (Riddle, 1996), those taxa should exhibit shallow genetic structure superimposed on deep divergences. Such hierarchical structure has been demonstrated for various taxa in western North America (e.g., Demboski and Cook, 2001; Wilke and Duncan, 2004; Alexander and Riddle, 2005; Carstens et al., 2005a; Steele et al., 2005). Because their reduced vagility in comparison to terrestrial organisms yields fewer opportunities for dispersal and gene flow, aquatic taxa should be more likely to maintain intact signatures of the influences of both older geological processes and more recent climate changes (Bernatchez and Wilson, 1998). Freshwater fishes in

particular have retained evidence of isolation and divergence resulting from geological processes in many parts of the world (e.g., Bermingham and Avise, 1986; McGuigan et al., 2000; Unmack, 2001; Smith and Bermingham, 2005; Swartz et al., 2007; Burridge et al., 2008; Zemlak et al., 2008). In western North America, the evolution of freshwater fishes has been characterized by allopatric speciation resulting from the disruption of hydrological connections by episodes of block faulting, volcanism, and uplift (Smith, 1981; Minckley at al., 1986). However, sporadic inter-basin dispersal has occurred during events such as floods or stream-captures (geomorphological events where a stream or river changes its course and begins to flow into a neighboring stream or drainage basin) which, in some cases, have been facilitated by climatic changes (e.g., Taylor and Smith, 1981; Minckley et al., 1986; Johnson, 2002; Smith et al., 2002; Hershler and Liu, 2004; Mock et al., 2006).

The following chapters assess phylogeographic patterns within the genus *Richardsonius*, which is widespread throughout western North America, by testing hypotheses that reflect aspects of the geologic and climatic history of the region. There are two species within the genus *Richarsonius*: the redside shiner *Richardsonius balteatus* (Richardson) and the Lahontan redside shiner *Richardsonius egregius* (Girard). *Richardsonius* is of biogeographic interest in part because of its wide distribution throughout several of the drainage systems discussed in this chapter, and thus genetic structure within the genus was likely influenced by many of the late Cenozoic drainage connections outlined below.

The phylogenetic relationships between *Richardsonius* and other North American cyprinids are uncertain. Those relationships are examined more closely in Chapter 2

using more extensive taxonomic sampling than has been used previously, along with DNA sequence data from mitochondrial and nuclear genes. Molecular data are also used to infer possible geological or climatic events that have helped shape genetic structure within and among the two closely related species, including a qualitative look at phylogeographic structure within both species of *Richardsonius*. In Chapter 3, mitochondrial DNA sequence data are used to investigate phylogeographic structure among *R. egregius* populations within the Lahontan Basin.

Richardsonius balteatus has a broad distribution (Figure 1.1). The species is comprised of two subspecies: *R. b. balteatus* and *R. b. hydrophlox*, defined by morphological differences and geographic distribution (LaRivers, 1994; Minckley et al., 1986; Smith et al., 2002). One subspecies, *R. b. balteatus*, also exhibits morphological variation between coastal and inland populations (McPhail and Lindsey, 1986; Minckley et al., 1986). While such differences in morphology may result from phenotypic plasticity and responses to different selective pressures in different environments, they may also be indicative of an underlying genetic structure. The other subspecies, *R. b. hydrophlox*, exhibits genetic differences in somatic growth rates between upper Snake River and Bonneville Basin populations (Houston and Belk, 2006), which suggests that this taxon also exhibits phylogeographic structure.

Richardsonius egregius has a much more limited range than *R. balteatus*, being restricted to drainages within the Lahontan Basin (Figure 1.1). Given the degree of isolation that the Lahontan Basin has experienced (see below), *R. egregius* has likely been on a unique evolutionary trajectory at least since the end of the Pliocene. Populations within the basin may have experienced varying degrees of gene flow

between them as pluvial lake levels fluctuated and affected drainage connections during the Pleistocene.

Prior to engaging in a detailed discussion about the phylogenetic relationships and phylogeographic patterns I have discovered within the genus *Richardsonius*, I find it necessary to provide a short, but relevant review of some important geological and climatic processes. Specifically, I provide a brief overview of cyprinid systematics and biogeography, and then I review geologic and climatic processes and events that occurred within the geographic ranges of the two *Richardsonius* species, and thus likely influenced their genetic structure.

The Cyprinidae: An Evolutionary and Biogeographic Synopsis

With approximately 220 genera containing 2420 recognized species, the Cyprinidae is the largest family of freshwater fishes in the world (Nelson, 2006; Mesquita et al., 2007). The family is postulated to have originated in southeastern Asia during the Eocene (Nelson, 2006), and its members now occur throughout Asia, Europe, northern Africa, and North America (Mayden, 1991; Simons et al., 2003). Movement of cyprinids from Asia into North America is hypothesized to have occurred across the Bering land bridge. Migration of cyprinids across Beringia was initially hypothesized to have occurred during the Pleistocene (Uyeno, 1961), but is now postulated to have occurred much earlier, during the early to mid-Oligocene (Briggs, 1979; Simons and Mayden, 1998; Briggs, 2005). The oldest cyprinid fossils from North America come from three different locations in the Pacific Northwest and are Oligocene (~30 Ma) in age

(Cavender, 1991). Most extant North American cyprinid genera had already evolved by the end of the Miocene, 5 Ma (Smith, 1981).

There are a number of subfamilies within the Cyprinidae (Cunha et al., 2002; Nelson, 2006), but all North American taxa belong to the subfamily Leuciscinae, and all but one species belong to the Phoxinins, which is one of two distinct lineages within the subfamily Leuciscinae (Cavender and Coburn, 1992; Simons et al., 2003). The single exception is the golden shiner *Notemigonus chrysoleucas*, which is postulated to have a different biogeographic history than all other North American cyprinids, having migrated from Europe across the North Atlantic land bridge instead of across Beringia. The North American Phoxinins consist of three major clades: the western clade, the creek chub-plagopteran clade, and the open posterior myodome (OPM) clade (Mayden, 1989; Simons and Mayden, 1999; Simons et al., 2003). The western and creek chub-plagopterin clades are primarily comprised of species distributed west of the Rocky Mountains, whereas most of the species in the OPM Clade are distributed east of the Rocky Mountains, although there are some exceptions (Table 1.1). One genus from the western clade (*Phoxinus*) occurs in eastern North America, as do two species within the genus *Gila* (Table 1.1). Three genera from the creek chub-plagopterin clade (*Hemitremea, Margariscus,* and *Semotilus*) are distributed east of the Rocky Mountains, and at least one species from this clade (*Couesius plumbeus*) can be found on both sides of the Continental Divide (Table 1.1). Similarly, the majority of taxa from the speciose OPM clade are distributed east of the Rocky Mountains, although some genera from the OPM clade are native to western North America (Table 1.1). Even with such exceptions, Simons et al. (2003) call attention to the persistence of a generalized view of separate

eastern and western faunas within the North American cyprinids, but it appears that at least some cyprinids traversed the Continental Divide to attain their current distributions.

Geological Transformation of Portions of the Western North American Landscape

Eocene

The Rocky Mountains began to form in the late Cretaceous (~75 Ma) and reached their maximum height during the Eocene (Saleeby, 2003; English and Johnston, 2004; Spencer et al., 2008), and thus were in place by the time cyprinids are thought to have immigrated to North America. The uplift of the Rocky Mountains was followed by extensive uplift and westward extension of western North America beginning in the Eocene, continuing episodically through the Miocene, and is still underway (Sonder and Jones, 1999; Horton et al., 2004). The Great Basin formed as a result of the extension of western North America (Stokes, 1988; Kohn and Fremd, 2008). These drastic changes to the landscape presented aquatic taxa with formidable barriers to dispersal, and appear to have played a role in the extinction of many western North American fish species by the early Miocene (Smith, 1978; 1981).

As cyprinids immigrated into North America, their dispersal occurred both east and west of the crest of the Western Cordillera, a series of mountain ranges along the Pacific Coast of Canada (Smith et al., 2000). Geomorphological reconstructions indicate that there are times during the Paleogene when the transfer of aquatic taxa could have occurred across the Continental Divide. One possible aquatic connection was established across the Continental Divide when the headwaters of the Columbia River flowed eastward to the Hudson Bay area prior to reversing their flow during the Eocene (Lemke

et al., 1965; McMillian, 1973; Minckley et al., 1986). This connection may have allowed for the transfer of aquatic taxa, but likely pre-dated the arrival of the Cyprinidae to North America (see Cavender, 1991). Instead, cyprinid dispersal from western to eastern North America (or vice versa) may have been possible when present day tributaries to the Missouri River in western Montana were connected to the Snake River in southern Idaho during the late Miocene and Pliocene, a connection that is postulated to have involved the transfer of fishes (Smith, 1981; Smith et al., 2000) and spring snails (Hershler and Gustafson, 2001; Hershler et al., 2008). This connection, and others like it, provide a plausible mechanism for cyprinid dispersal across the Continental Divide, and may explain how some North American cyprinids (see Table 1.1) attained current distributions that are not geographically concordant with many of their closest relatives.

Miocene

The ancestral Snake River Plain began to form in the mid-Miocene when the Yellowstone Hotspot began erupting in the north-central Great Basin (Pierce and Morgan, 1992; Pierce et al., 2002), with subsequent shifts in drainage divides and reversal of flows associated with these processes (Pierce et al., 2002; Link et al., 2002; Beranek et al., 2006; Wallace et al., 2008). Such reversals are typically characterized by the patterns of reverse barbing observed in streams that have changed direction of flow, and these reversals appear to have influenced the movements of aquatic taxa within and between drainage basins (e.g., Smith, 1981; Taylor and Smith, 1981; Minckley et al., 1986; Taylor and Bright, 1987; Johnson, 2002; Smith et al., 2002; Hershler and Liu, 2004; Liu and Hershler, 2005).

Once formed, the Snake River drained westward and emptied directly into the Pacific Ocean for the duration of the Miocene (Smith et al., 2002), and is hypothesized to have been connected to the Sacramento River system during that time (Repenning et al., 1995; Link et al., 2002; Smith et al., 2002; Hershler and Liu, 2004). A Miocene Snake – Sacramento River connection is supported by biogeographic evidence. Several fishes which now occur in the Sacramento River system have ancestors that appear as fossils in sediments deposited by pre-historic Lake Idaho in southern Idaho (Miller and Smith, 1967; Kimmel, 1975; Smith, 1975; Smith et al., 1982; Smith and Cossel, 2002), suggesting a direct connection between the two rivers. The distributions of many freshwater mollusks also support such a connection (Taylor and Smith, 1981; Taylor, 1985), as does fossil evidence from at least one species of mammal (Repenning et al., 1995). Recent evidence suggests that the upper Snake River did not directly flow into the middle Snake River (and Lake Idaho) during the Miocene. Instead, detrital zircons suggest that the upper Snake River did not flow into Lake Idaho until after Lake Idaho was captured by the Columbia River system (see below) in the late Pliocene (Link et al., 2002; Link et al., 2005; Beranek et al., 2006). Rather, it is feasible that both the upper and middle Snake Rivers flowed into the Humboldt River system during the Miocene (Figure 1.2). Fossil muskrat distributions (Repenning et al. 1995) and current distributions of spring snails (Hershler and Liu, 2004) also provide evidence supporting the hypothesis that the Snake River flowed into the Humboldt system. Thus, a growing body of evidence suggests the course of the Snake River during the Miocene was indeed across northern Nevada via the Humboldt River system.

The Humboldt River began to form in the late Miocene (~9.8 Ma) when a number of separate drainages in northeastern Nevada began to coalesce (Wallace et al., 2008), thus constraining any connection between the Humboldt River and the upper Snake River to the late Miocene. The upper Snake River likely flowed into the Humboldt River in northern Nevada during the Miocene (Link et al., 2002; Link et al., 2005, Beranek et al., 2006), an aquatic connection that would account for pikeminnow fossils that have been found in the Lahontan Basin (Kelly, 1994), as well as for similarities of the Pliocene mollusks and fishes from the Snake River and western Great Basin (Taylor and Smith, 1981). Lake Idaho, of the Middle Snake River, also may have drained into the Humboldt River through northeastern Nevada during the Miocene (Figure 1.2). If this connection did exist, it likely would have cut across the northwestern corner of what is now the Bonneville Basin in Utah (Figure 1.2). While undocumented, a connection across the northwestern Bonneville Basin could have occurred via the Raft River in southern Idaho (Link et al., 2002), a river that exhibits the characteristic reverse barbing of a stream that has experienced a reversal in its direction of flow. Moreover, such a connection would explain the occurrence of pikeminnow fossils in the Bonneville Basin, where no pikeminnows currently exist (Smith et al., 2002), and is also consistent with the "westward drainage" of the northwestern Bonneville Basin as suggested by distributions of fossil mollusks (Taylor, 1985).

Tectonic activity during the Miocene was instrumental in the continued evolution of the western North American landscape, and was a major factor in the formation of the Great Basin (Minckley et al., 1986). The Bonneville and the Lahontan Basins formed in the eastern and western edges of the Great Basin, respectively. Each of these basins

subsequently shared aquatic connections with surrounding drainages, as well as with each other. Biogeographic evidence supports a hydrological connection between the Snake River and the Colorado River via the Bonneville Basin (Figure 1.2), and Smith and Dowling (2008) state that any connection between the Bonneville Basin and the Colorado River drainage during that time must have been large enough to support big river fish species. The Colorado River itself was not a continuous system until the upper Colorado River was captured by the lower Colorado River at the end of the Miocene, 5-6 Ma (Howard and Bohannon, 2001; Faulds et al., 2001; Pederson, 2001; Powell, 2005; Pederson, 2008; Spencer et al., 2008), and a connection between the Snake River and the Colorado River is supported by the distributions of fossil mollusks (Taylor, 1983; Taylor, 1985), thus aquatic taxa were likely able to disperse between the Snake and Colorado rivers at that time. A number of freshwater fishes also indicate that dispersal of aquatic taxa through a Snake River – Bonneville Basin – upper Colorado River connection occurred (Smith, 1966; Johnson and Jordan, 2000; Dowling et al., 2002; Smith et al., 2002; McKell, 2003; Crowley, 2004; Johnson et al., 2004; Oakey et al., 2004; Smith and Dowling, 2008). It has been suggested that headwater transfers across drainage divides has occurred between the Bonneville and Colorado River basins (Hubbs and Miller, 1948; Miller, 1958; Smith, 1978), and these appear to have involved the transfer of some fish species (Miller, 1958; Dowling and DeMarais, 1993; Oakey et al., 2004), and may have involved the transfer of other aquatic taxa as well. Geological evidence for such a connection (or connections) is lacking (Powell, 2005; Pederson, 2008), but a connection through the Grand Wash Cliffs in southern Utah/northern Arizona is possible (Hunt, 1956; Pederson, 2008). Recent authors suggest that connections between the Snake

River, Bonneville Basin and Colorado River system may have occurred in the early Pliocene instead of the Miocene based on molecular clock estimates corrected to account for the effects of body size and water temperature on DNA mutation rates (Spencer et al., 2008; Smith and Dowling, 2008). Additional research is needed to evaluate these competing hypotheses regarding the timing (Miocene vs. Pliocene) of a Snake – Bonneville – Colorado connection, as well as to find the location of the ancient drainage, but the biogeographic evidence clearly shows that such a connection existed, and that it involved the transfer of multiple aquatic taxa.

Strong biogeographic evidence for aquatic connections can outweigh an overall lack of geological evidence supporting those connections. Instances in which biogeographic evidence supports ancient landscape features that lack geological evidence are not unique to the hypothesized drainage connections that are discussed above. Another example is in Baja California, where researchers have uncovered genetic evidence within several disparate taxa that is suggestive of a mid-peninsular seaway that is postulated to have existed during the mid-Pleistocene, approximately 1.6 Ma (Riddle et al., 2000; Bernardi and Lape, 2005; Riginos, 2006). There is little geological evidence for a mid-peninsular seaway in Baja California (Ledesma-Vásquez, 2002; Oskin and Stock, 2003; Bernardi and Lape, 2005), likely because any geomorphological features it formed were masked by subsequent volcanic activity in the region. Nevertheless, the genetic evidence strongly supports the existence of a mid-peninsular seaway, much like biogeographic evidence supports various drainage connections in the intermountain west. Once discovered, such information can be used to direct geologists in searching for physical evidence of connections.

Pliocene

The Snake River continued to flow through the Lahontan Basin on its way to the Pacific Ocean (as discussed above) for much of the Pliocene, but was captured by the Columbia River system 3.2 Ma (Link et al., 2002). The capture of the Snake River occurred when a tributary to the Salmon River captured Lake Idaho at the head of today's Hells Canyon and eroded the sill of the basin containing Lake Idaho (Wheeler and Cook, 1954; Minckley et al., 1986; Repenning et al., 1995; Link et al., 2002; Hershler and Liu, 2004; Figure 1.3). The resulting spill-over of Lake Idaho further deepened Hells Canyon (Malde, 1965; Wood, 1994), firmly embedding the waters of the Snake River into the new channel. Reduced flow into the Humboldt system as a result of the capture of the Snake River may have been responsible for severing the Humboldt River's connection with the Sacramento River system.

The Lahontan Basin has been connected to Mono Lake in California at various times during the Cenozoic (Smith et al., 2002), and has also shared aquatic connections with the Columbia drainage and the Bonneville Basin during the Pliocene. One such connection appears to have existed between the Alvord Basin in southeastern Oregon and northwestern Nevada (Reheis and Morrison, 1997; Smith et al., 2002; Figure 1.3). Additionally, rivers along the eastern side of the Sierra Nevada demonstrate the reverse barbing pattern that is characteristic of flow reversal (Minckley et al., 1986), also suggesting previous connections between the Lahontan and Columbia basins. Pliocene connections between the Lahontan and Bonneville Basins existed in eastern Nevada and western Utah (Minckley et al., 1986), and appear to have involved the transfer of multiple Great Basin fish species (Miller, 1958). Moreover, there were late Pliocene connections

between the Lahontan Basin and Mono Lake, and Mono Lake was later connected to the Death Valley system (Hubbs and Miller, 1948; Smith, 1978; Miller and Smith, 1981; Taylor, 1985; Reheis and Morrison, 1997; Figure 1.3). However, the Lahontan Basin appears to have been completely isolated from all of its surrounding drainages since the end of the Pliocene (Minckley et al., 1986; Repenning et al., 1995; Reheis and Morrison, 1997; Smith et al., 2002).

The majority of the coastal streams in the Pacific Northwest have been isolated from interior drainages since the early Pliocene (Baldwin, 1981), but some stream capture events have occurred. Geomorphic evidence from stream terraces show that the upper Umpqua River was captured from the Columbia drainage (via the Willamette River in western Oregon) by a westward flowing stream (Diller, 1915; Minckley et al., 1986; Mayden et al., 1991; Figure 1.3). While the timing of this capture event is unknown, it is postulated to have been relatively recent (end Pliocene or Pleistocene) and is hypothesized to have involved the transfer of aquatic taxa (Mayden et al., 1991). Geological activity clearly continued to influence drainage patterns in western North America throughout the Pliocene, and thus likely affected patterns of gene flow for numerous aquatic taxa.

Pleistocene

Climatic oscillations during the Pleistocene have played a significant role in the evolution of the western North American biota by forcing organisms through a series of range contractions and expansions as glacial ice sheets expanded and retracted (Hewitt, 1996; 2004). In western North America, refugia (Figure 1.4) are postulated to have occurred in Beringia (e.g., Hultén, 1937; Nadler et al., 1978; Hopkins and Smith, 1981; Elias et al., 1996; Pruet and Winker, 2008; Aubry et al., 2009), the Chehalis River Valley

(e.g., McPhail, 1967; Soltis et al., 1997; Redenbach and Taylor, 2002), the lower Columbia River (e.g., Bickham et al., 1995; McCusker et al., 2000; Haas and McPhail, 2001), the upper Columbia River drainage (e.g., Nielson et al., 2001; Carstens et al., 2004; Carstens et al., 2005b; Steele et al., 2005), Haida Gwaii (a.k.a., Queen Charlotte Islands: an archipelago off the Pacific Coast of British Columbia) (e.g., Pielou, 1991; Zink and Dittman, 1993; Topp and Winker, 2008), and the Klamath – Siskiyou region (e.g., Wake, 1997; Brunsfeld et al., 2001; Wilke and Duncan, 2004; Kuchta and Tan, 2005; Steele and Storfer, 2006). Some species appear to have survived in single refugia during glacial maxima and expanded their ranges post-glacially (e.g., Brown et al., 1992; Conroy and Cook, 2000a; Spellman and Klicka, 2006), whereas many others appear to have survived in multiple refugia. Several species of fish (McPhail and Lindsey, 1986; Taylor et al., 1999; Redenbach and Taylor, 2002), mammals (Byun et al., 1997; Conroy and Cook, 2000b, Stone and Cook, 2000; Good and Sullivan, 2001, Stone et al., 2002; Latch et al., 2009), and plants (Latta and Mitton, 1999; Mitton et al., 2000) exhibit population structure consistent with survival in western coastal and eastern inland refugia during the Pleistocene. Additionally, some fishes (Taylor et al., 1996; Redenbach and Taylor, 2002), plants (Soltis et al., 1997), and reptiles (Janzen et al., 2002) have retained genetic signatures of survival in northern and southern refugia along the Pacific Coast.

Glacial lakes associated with the ice sheets altered the landscape of western North America in ways that may have affected gene flow. Perhaps the best studied of these lakes is Pleistocene Lake Missoula, a glacial lake that formed in western Montana when the Clark Fork River was dammed by a lobe of the Cordilleran Ice Sheet (Bretz, 1923). Catastrophic flooding occurred when the ice dam broke, and the rushing waters carved

the channeled scablands of eastern Washington (Bretz, 1923, 1925, 1969; Richmond et al., 1965; McKee, 1972; Baldwin, 1981; McPhail and Lindsey, 1986). This process is hypothesized to have occurred approximately 40 (and perhaps as many as 100) times during the Pleistocene (Smith, 2006; Baker, 2009), although most of those flood events were small and would not have been as catastrophic as the most enormous ones (Baker, 2009). The highest magnitude Missoula floods were responsible for the capture of the Palouse River (from the Columbia River) by the lower Snake River (Richmond et al., 1965), and the formation of Palouse Falls, which now stand as a barrier to dispersal for aquatic taxa (Maughan et al., 1980), likely affecting patterns of gene flow for many of the aquatic taxa in the area. Glacial floods were also responsible for completely filling the Willamette Valley in western Oregon (McPhail and Lindsey, 1986; Franklin and Dyrness, 1988), an event that appears to have influenced genetic structure for at least some taxa by forming a barrier between populations of terrestrial organisms (e.g., Miller et al., 2006), and possibly providing more connectivity of aquatic habitats and thus more opportunities for aquatic organisms to disperse.

Glacial lakes and similar flooding events were also operating in northern areas as the ice sheets retreated at the end of the Pleistocene. The Fraser River in British Columbia was connected to the Columbia River (Figure 1.5) through a series of glacial lakes in the Okanagan Valley, but that connection was broken in the late Pleistocene when the Fraser River shifted its course westward and began draining directly into the Pacific Ocean (McPhail and Lindsey, 1986). Okanagan Falls formed in the Okanagan Valley of southern British Columbia, and now stand as a barrier to dispersal for aquatic fauna (McPhail and Lindsey, 1986), although a few species dispersed northward prior to their

formation. Those species continued to disperse northward through glacial lakes when habitats were favorable. Some species gained access to the eastern side of the Rocky Mountains via a glacial lake that formed when the Fraser River became blocked by ice, and spilled into the Parsnip River, a tributary to the Peace River of northern British Columbia and Alberta (McPhail and Lindsey, 1986; Figure 1.5). At least two eastern North American fish species, brassy minnow *Hybognathus hankinsoni* Hubbs and white sucker *Catostomus commersoni* (Lacepede), likely moved westward through the same connection (McPhail and Lindsey, 1986). In British Columbia, the freshwater fish faunas of the Skeena and Nass Rivers in the northwestern part of the province share many species with the Columbia River (McPhail and Lindsey, 1986), suggesting that post-glacial colonization in those areas occurred from the Columbia River system in similar fashion. The Stikine River contains a fish fauna more similar to that of the Bering drainages than that of the Columbia River (McPhail and Lindsey, 1986), so there does not appear to have been any dispersal of freshwater fishes between the Stikine and Nass rivers, and the area between those rivers represents a boundary between Columbia and Bering fish faunas (Figure 1.5).

In addition to the formation of the glacial ice sheets during the Pleistocene (Figure 1.4), the increased precipitation and decreased evaporation associated with lower global temperatures resulted in the formation of pluvial lakes in many of the valleys in the Basin and Range province. Two of the largest pluvial lakes, Lake Bonneville and Lake Lahontan, formed in the the Bonneville and Lahontan basins, respectively (Figure 1.4). Both lakes experienced fluctuating water levels during glacial and interglacial cycles, as evidenced by prominent beach terraces observed above the valley floors of the

Bonneville and Lahontan basins (Morrison, 1965; Stokes, 1988; Reheis and Morrison, 1997; Oviatt, 2002). Fluctuating water levels created aquatic connections between sub-basins, and may have allowed for movement of aquatic taxa between sub-basins. While some aquatic taxa do not appear to have moved between sub-basins despite these aquatic connections (Taylor and Smith, 1981; Taylor, 1985; McKell, 2003), others did (Chen et al., 2007; Chen et al., 2009). Hence, it appears that the influence that pluvial lakes had on genetic diversity of aquatic taxa may have been dependent on the ecological requirements and life histories of individual taxa.

Lake Lahontan experienced fluctuations in water levels throughout the Pleistocene, which established aquatic connections between sub-basins at different times (Morrison, 1965; Reheis and Morrison, 1997; Negrini, 2002; Adams et al., 2008). At least two Lahontan tributaries changed their course of flow during the Pleistocene. The Humboldt River flowed across the Black Rock Desert (in northwestern Nevada) throughout the Miocene and Pliocene, but by the end of the Pleistocene (~13 ka) had changed its course southward to flow into the Carson Sink (Reheis and Morrison, 1997; Figure 1.6). This stream capture event was associated with the last high stand of Lake Lahontan (Reheis and Morrison, 1997). Similarly, the Walker River alternated between flowing into the Carson Sink basin and the Walker Lake basin with various climatic cycles throughout the Pleistocene (Reheis and Morrison, 1997; Figure 1.6). Mid-Pleistocene high stands appear to have had higher water levels than the late Pleistocene high stands, and probably submerged other sub-basins (Reheis, 1999), possibly providing aquatic taxa with connections through which to disperse. However, mapping of shore terraces and spits formed by Lake Lahontan suggests that the lake did not overflow into any of the

surrounding drainages at any time during the Pleistocene (Russell, 1885; Morrison, 1965). Rather, the Lahontan Basin appears to have been isolated from all other drainages for the past two million years (Minckley et al., 1986; Repenning et al., 1995; Reheis and Morrison, 1997; Smith et al., 2002), with the exception of a possible connection to the Death Valley system in the late Pliocene/early Pleistocene (Reheis and Morrison, 1997; Figure 1.3). Hence, aquatic taxa found within the Lahontan Basin have likely been on unique evolutionary trajectories for at least the past two million years.

The Bonneville Basin (Figure 1.7) experienced aquatic connections with other drainage systems during the Pleistocene. One documented connection occurred when the Bear River was captured into the Bonneville Basin. The Bear River flowed into the upper Snake River (via the Portneuf River) in southeast Idaho prior to its capture (Figure 1.7). Approximately 35,000 years ago, tilting of the earth's crust coupled with volcanic activity altered the course of the Bear River, diverting it into Lake Thatcher in southern Idaho, which eventually overflowed and emptied into Lake Bonneville (Bright, 1963; Morrison, 1965; Minckley et al., 1986; Bouchard et al., 1998; Johnson, 2002; Smith et al., 2002). Thereafter, Lake Bonneville experienced an increase in volume until it eventually overflowed its sill at Red Rock Pass in southern Idaho approximately 15,000 years ago and spilled into the Snake River in an event known as the Bonneville Flood (Malde, 1965; Jarrett and Malde, 1987; Johnson, 2002; Figure 1.7). Lake Bonneville emptied into the Snake River until it reached a new stable level (the Provo level, which is marked by the lowest of the beach terraces above the valley floor). The Bonneville Flood is estimated to have lasted between a few weeks and one year (Malde, 1968; O'Connor, 1993). The capture of the Bear River and the Bonneville Flood have both been

hypothesized to have involved the transfer of aquatic taxa (Hubbs and Miller, 1948; Behnke, 2002), and recent studies using molecular data have supported this hypothesis for at least some species of fish (Johnson, 2002; Mock et al., 2006). Differential erosion of the bedrock during the Bonneville Flood is credited with the formation of Shoshone Falls along the Snake River. Shoshone Falls now stands as an impassible barrier for many aquatic taxa, as evidenced by different fish communities above and below the falls. Recent work by Smith and Dowling (2008) also suggests Pleistocene connections between the Bonneville Basin and the upper Colorado River, as well as between the Bonneville Basin and the lower Colorado River via the headwaters of the Virgin River during a Lake Bonneville high stand approximately 650 ka (Oviatt, 2002). Movement of aquatic taxa through that connection is plausible, and would explain the close phylogenetic relationships between haplotypes in Colorado River and Bonneville Basin taxa (Smith and Dowling, 2008). Clearly the climatic oscillations associated with the Pleistocene Epoch afforded many opportunities for dispersal and gene flow via aquatic connections that formed as a result of fluctuating water levels in pluvial and glacial lakes.

Holocene

After the Bonneville Flood, Lake Bonneville stabilized at the Provo level until increased evaporation rates associated with the onset of Holocene desiccation caused water levels to drop again. As water levels dropped, Lake Bonneville became divided into two sub-basins: Lake Gilbert formed in the northern Bonneville Basin, and Lake Gunnison formed in the south (Figure 1.7). Lakes Gilbert and Gunnison shared aquatic connections until approximately 9,700 years ago, when water levels dropped low enough to isolate both lakes (Johnson, 2002). Population fragmentation associated with

decreased connectivity of Lake Bonneville populations appears to have left a genetic signature in some taxa (Johnson, 2002; Mock et al., 2004; Mock et al., 2006). Lake Gunnison eventually dried completely, leaving both the Beaver and Sevier Rivers to flow into Sevier Dry Lake, which now contains water only during very wet years. Lake Gilbert eventually dropped to the level of its modern day remnant, the Great Salt Lake, which is now too saline to support freshwater taxa and serves as a barrier between its northern and southern tributaries.

Lake Lahontan experienced similar fragmentation as its level dropped and sub-basins that shared aquatic connections during the Pleistocene became isolated once again. Today, the main tributaries to Lake Lahontan are no longer connected to one another. The Carson River flows eastward off the east slope of the Sierra Nevada Mountains and into the Carson Sink, a dry lake bed in western Nevada (Figure 1.6). Similarly, the Humboldt River flows westward from eastern Nevada and terminates in the Humboldt Sink, a playa in northwestern Nevada (Figure 1.6). The Carson and Humboldt sinks fill with water only during very wet years; a connection between them remains dry most of the time, although these basins did contain large lakes during Holocene times (Adams, 2003; Yuan et al., 2006). Another Lake Lahontan tributary, the Truckee River, flows from Lake Tahoe (on the California-Nevada border) and terminates in Pyramid Lake near Reno, Nevada. The Walker River in western Nevada now flows into Walker Lake, although, throughout the Holocene it alternated between flowing into Walker Lake and flowing into the Carson Sink, in much the same way as it switched courses during the Pleistocene (Adams, 2003; Yuan et al., 2006). The alternating course of flow of the Walker River may have allowed for dispersal of aquatic organisms between those two

basins. Overall, opportunities for aquatic taxa to move between drainages within the Lahontan Basin have decreased as water levels dropped during the Holocene.

Some researchers have suggested that some aquatic taxa may have dispersed post-glacially to new locations by "hitch-hiking" on avian fauna. The idea of passive dispersal of aquatic invertebrates by attaching to waterfowl is certainly not a new one (see Darwin, 1859), but it has been invoked recently to explain the lack of genetic divergence between populations that occur in aquatic habitats that have been isolated for long periods of time, particularly when they occur along avian migratory routes (Figuerola and Green, 2002; Hershler et al., 2005; Frisch et al., 2007; Liu et al., 2007). However, in the case of freshwater fishes, this hypothesized mode of dispersal has been rejected because their eggs are sensitive to desiccation, especially in dry environments (Smith et al., 2002; Smith and Dowling, 2008). Transplants of certain species (particularly game fish) by fisheries managers has been common throughout western North America in recent years, but such instances are easily identified and explained through the use of molecular markers (Smith and Dowling, 2008), and do not appear to present difficulties for researchers to explain the evolutionary histories of freshwater fish taxa in the majority of cases. Instead, the biogeography of the majority of western North American fish species almost certainly reflects historical distributions and drainage connections rather than recent anthropogenic introductions (Smith and Dowling, 2008).

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Table 1.1: North American cyprinid species that do not occur on the same side of the Continental Divide as the majority of the members of their respective clades. Members of the creek chub – plagopterin and western clades are primarily distributed west of the Rocky Mountains, whereas members of the open posterior myodome clade mostly occur east of the Rocky Mountains.

Species Name	Common Name	Distribution
Creek chub – plagopterin clade		
Couesius plumbeus	lake chub	E + W
Hemitremia flammea	flame chub	E
Margariscus margarita	pearl dace	E
Semotilus atromaculatus	creek chub	E
Semotilus corporalis	fallfish	E
Semotilus lumbee	sandhills chub	E
Semotilus thoreauianus	Dixie chub	E
Open posterior myodome clade		
Algansea aphanea	riffle chub	W
Algansea avia	remote chub	W
Algansea barbata	Lerma chub	W
Algansea lacustris	Pátzcuaro chub	W
Algansea monticola	mountain chub	W
Algansea popoche	popoche chub	W
Algansea tincella	spottail chub	W

Aztecula sallaei	Aztec chub	W
Campostoma ornatum	Mexican stoneroller	E + W
Hybognathus hankinsoni	brassy minnow	E + W
Iotichthys phlegethontis	least chub	W
Mylocheilus caurinus	peamouth	W
Notropis braytoni	Tamaulipus shiner	E + W
Notropis chihuahua	Chihuahua shiner	E + W
Notropis jemazanus	Rio Grande shiner	E + W
Oregonichthys crameri	Oregon chub	W
Oregonichthys kalawatseti	Umpqua chub	W
Pogonichthys ciscoides	Clear Lake splittail	W
Pogonichthys macrolepidotus	splittail	W
Rhinichthys cataractae	longnose dace	E + W
Rhinichthys deaconi	Las Vegas dace	W
Rhinichthys evermanni	Umpqua dace	W
Rhinichthys falcatus	leopard dace	W
Rhinichthys obtusus	western blacknose dace	W
Rhinichthys osculus	speckled dace	W
Rhinichthys umatilla	Umatilla dace	W
Richardsonius balteatus	redside shiner	W
Richardsonius egregius	Lahontan redside shiner	W

Western clade

Gila modesta	Saltillo chub	E
Gila pulchra	Conchos chub	E
Phoxinus cumberlandensis	blackside dace	E
Phoxinus eos	northern redbelly dace	E
Phoxinus erythrogaster	southern redbelly dace	E
Phoxinus neogaeus	finescale dace	E
Phoxinus oreas	mountain redbelly dace	E
Phoxinus saylori	laurel dace	E
Phoxinus tennesseensis	Tennessee dace	E

Figure 1.1: The geographic distributions of *Richardsonius balteatus* and *Richardsonius egregius* (modified after Lee et al., 1980). The range of *R. balteatus* is shaded in grey, with a dashed line showing divisions between two subspecies: *R. b. balteatus*, which occurs in the northern portion of the range, and *R. b. hydrophlox* which occurs in the southeast portion of the range with disjunct populations in southeast Oregon. The range of *R. egregius* is restricted to the Lahontan Basin and is shown in white.

Figure 1.2: Map showing aquatic connections postulated to have existed between modern hydrological basins during the Miocene epoch. Modern rivers are represented by solid black lines, and ancient connections are represented by thick dashed lines. The dashed line next to the question mark represents headwater transfers between the Bonneville Basin and the Green River that are supported by biogeographic evidence, but the exact locations of which are unknown.

Figure 1.3: Map showing aquatic connections postulated to have existed between modern hydrological basins during the Pliocene epoch. Modern Rivers are represented by solid black lines, and ancient connections are represented by thick dashed lines. Arrows represent stream capture events, specifically the capture of the Snake River by the Columbia River system, and capture of the headwaters of the Willamette River by the Umpqua River system. Question marks represent connections that are supported by biogeographic evidence, but the exact locations are unknown. The Continental Divide is approximated by the red dotted line.

Figure 1.4: Map of North America during the Pleistocene epoch showing the locations of postulated Pleistocene refugia. Refugia are labeled as follows: Beringia (Be), Chehalis River Valley (Ch), lower Columbia River (LC), upper Columbia River (UC), Haida Gwaii (HG), and Klamath-Siskiyou (KS). Pluvial Lakes Bonneville (Bo) and Lahontan (La) are shown in blue.

Figure 1.5: Map showing routes of post-glacial colonization for some aquatic taxa after ice sheets retreated. Dashed lines represent aquatic connections that no longer exist between drainages. The thick black line represents a transition zone of aquatic fauna. Areas below the line share an affinity with the Columbia River system, whereas areas above the line are closely associated with the aquatic fauna of Beringia.

Figure 1.6: Pluvial Lake Lahontan. The grey dashed line represents the boundaries of the Lahontan Basin. Blue lines represent the modern day Humboldt and Walker Rivers. Blue dashed lines represent former direction of flow of the Humboldt River into what is now the Black Rock Desert (BR), and flow of the Walker River into Carson Sink (CS). The Humboldt River now flows into the Humboldt Sink (HS).

Figure 1.7: Map of the Bonneville Basin. The dark gray shaded area represents Lake Bonneville at its highest levels. The light grey area shows the location of Lake Gilbert and Lake Gunnison, which formed as water levels dropped during the Holocene. The dashed line represents the course of the Bear River as it flowed into the Snake River prior

to its capture into the Bonneville Basin. The black arrow shows the location and direction of the Bonneville Flood, which occurred at the end of the Pleistocene.

Figure 1.1

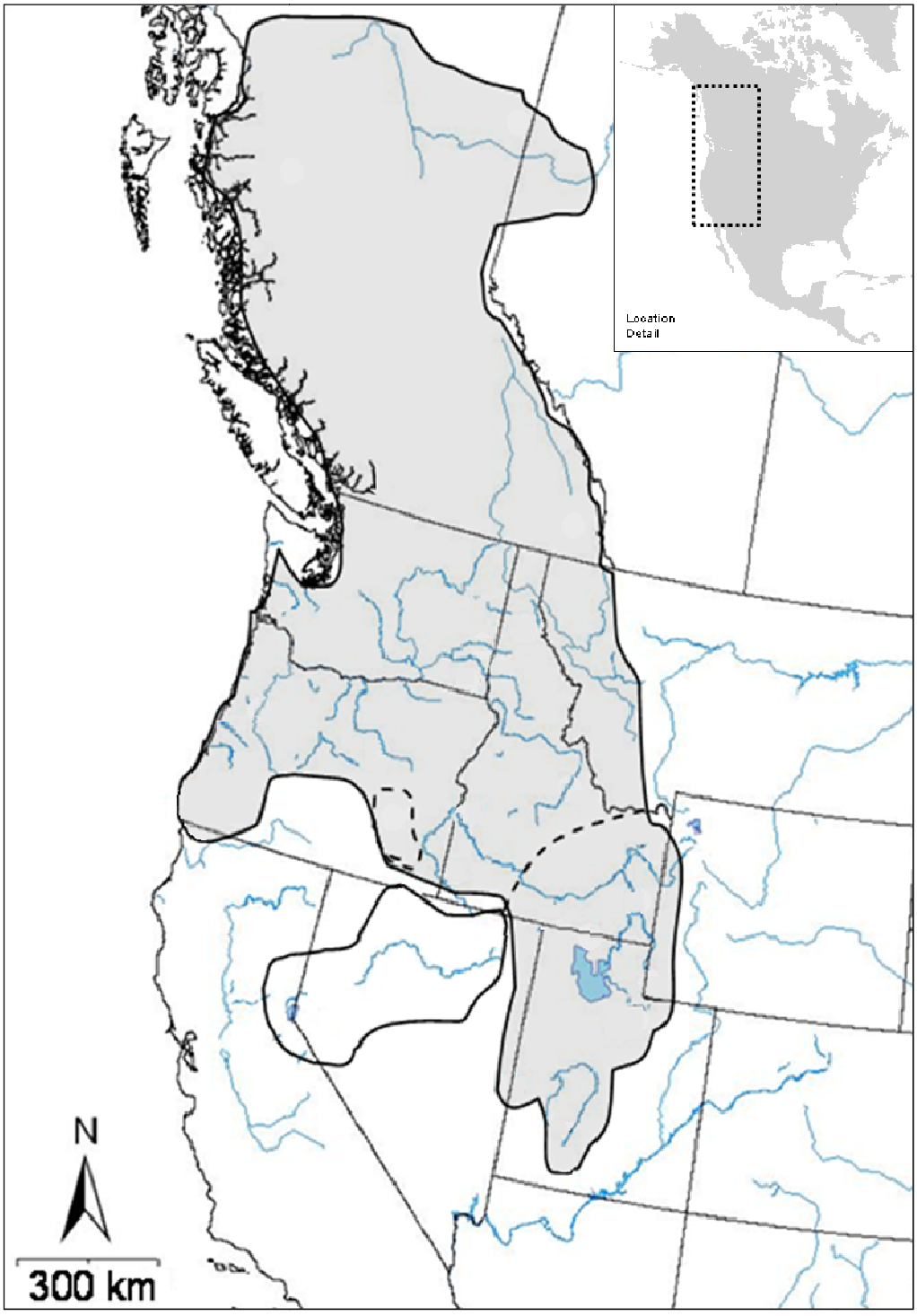


Figure 1.2

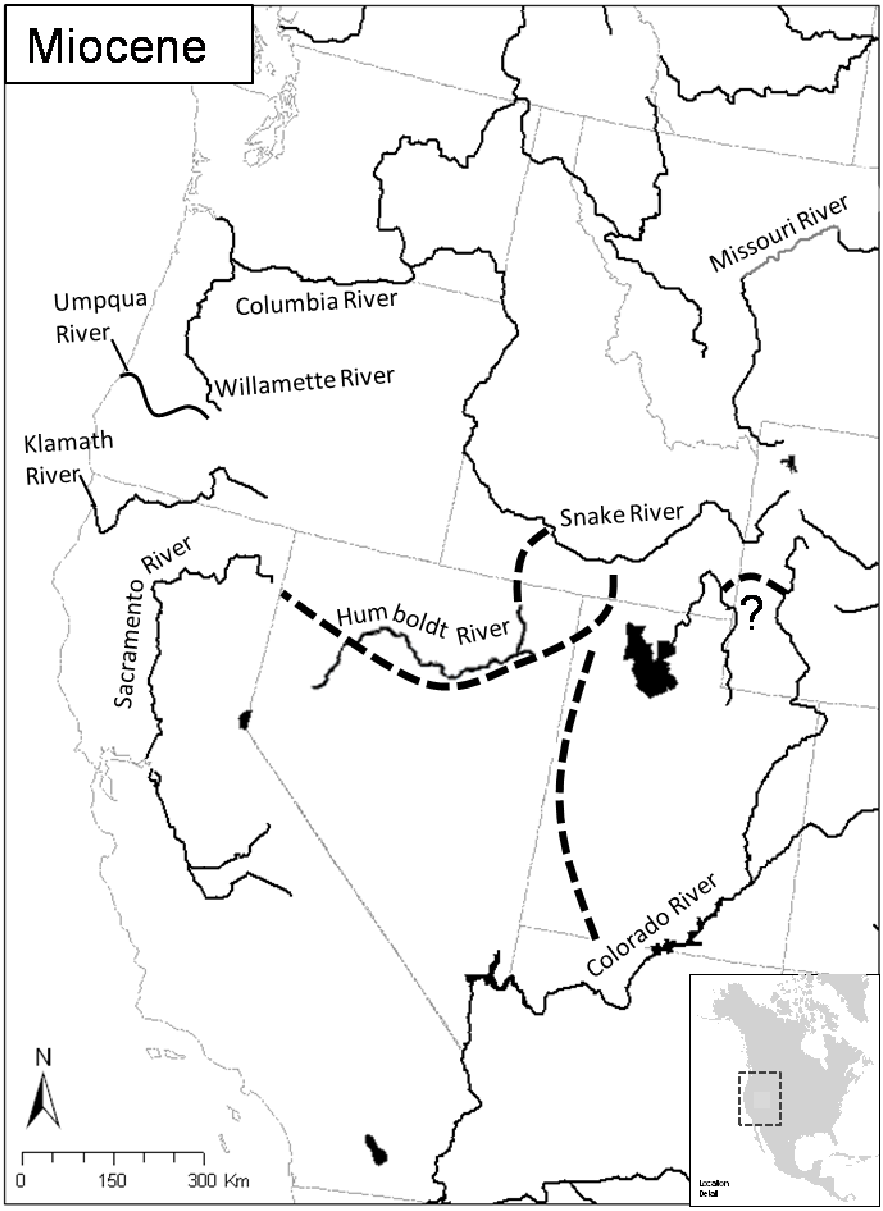

Miocene
Missouri River
Umpqua River
Columbia River
Willamette River
Klamath River
Snake River
Sacramento River
Humboldt River
?
Colorado River
N
0
150
300 Km

Figure 1.3

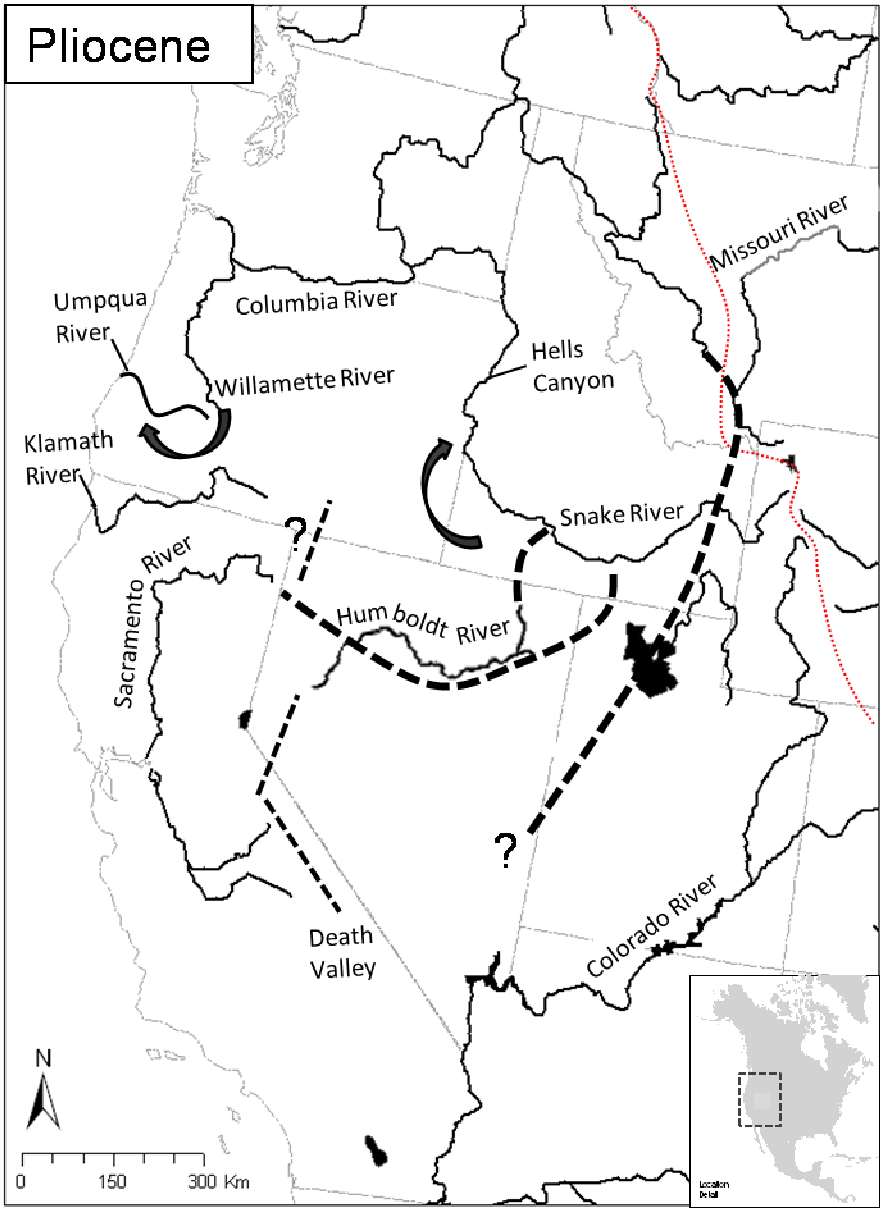

Pliocene
Missouri River
Umpqua River
Columbia River
Hells Canyon
Willamette River
Klamath River
Snake River
Sacramento River
Humboldt River
?
?
Death Valley
Colorado River
N
0
150
300 Km

Figure 1.4

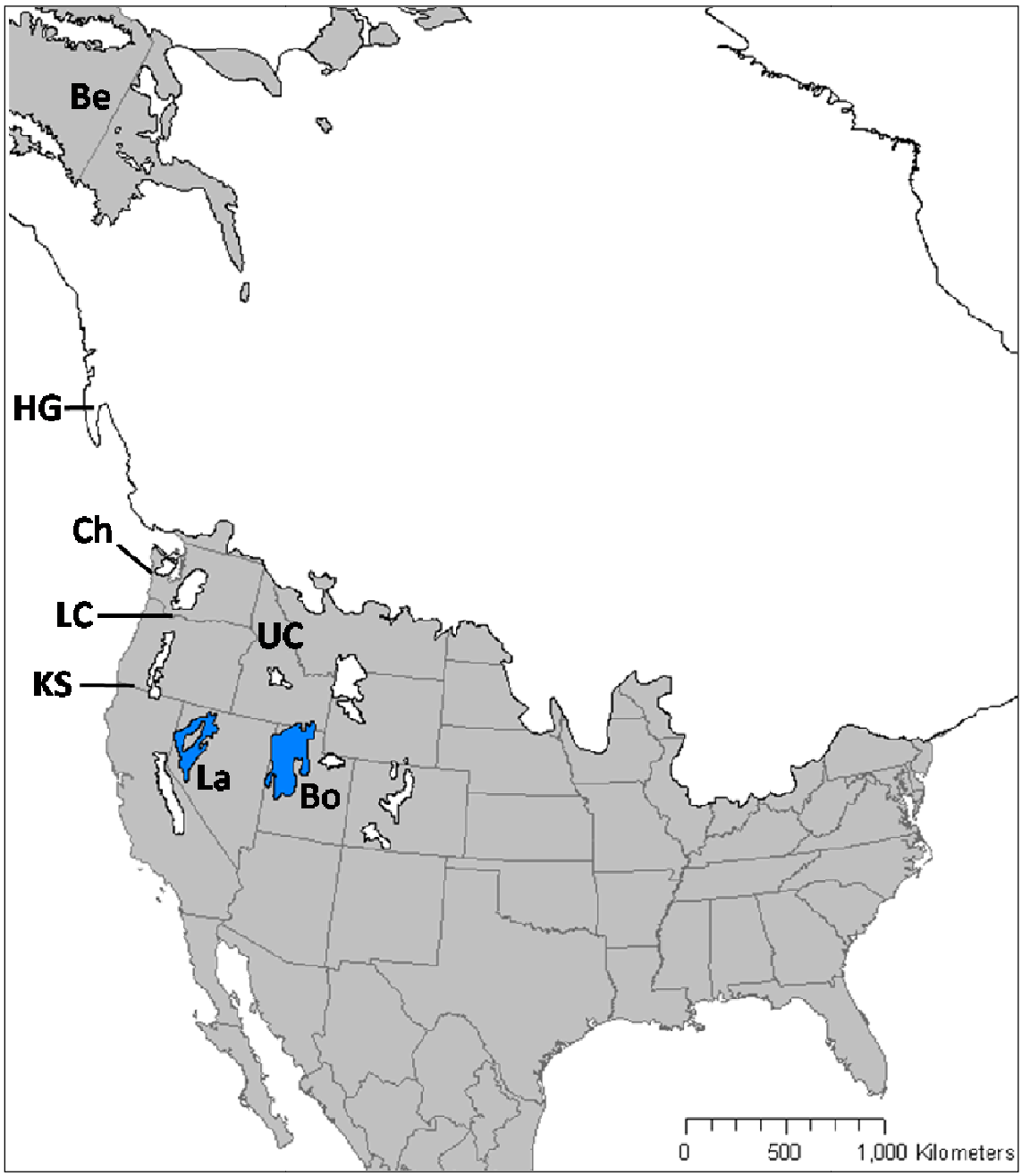


Figure 1.5

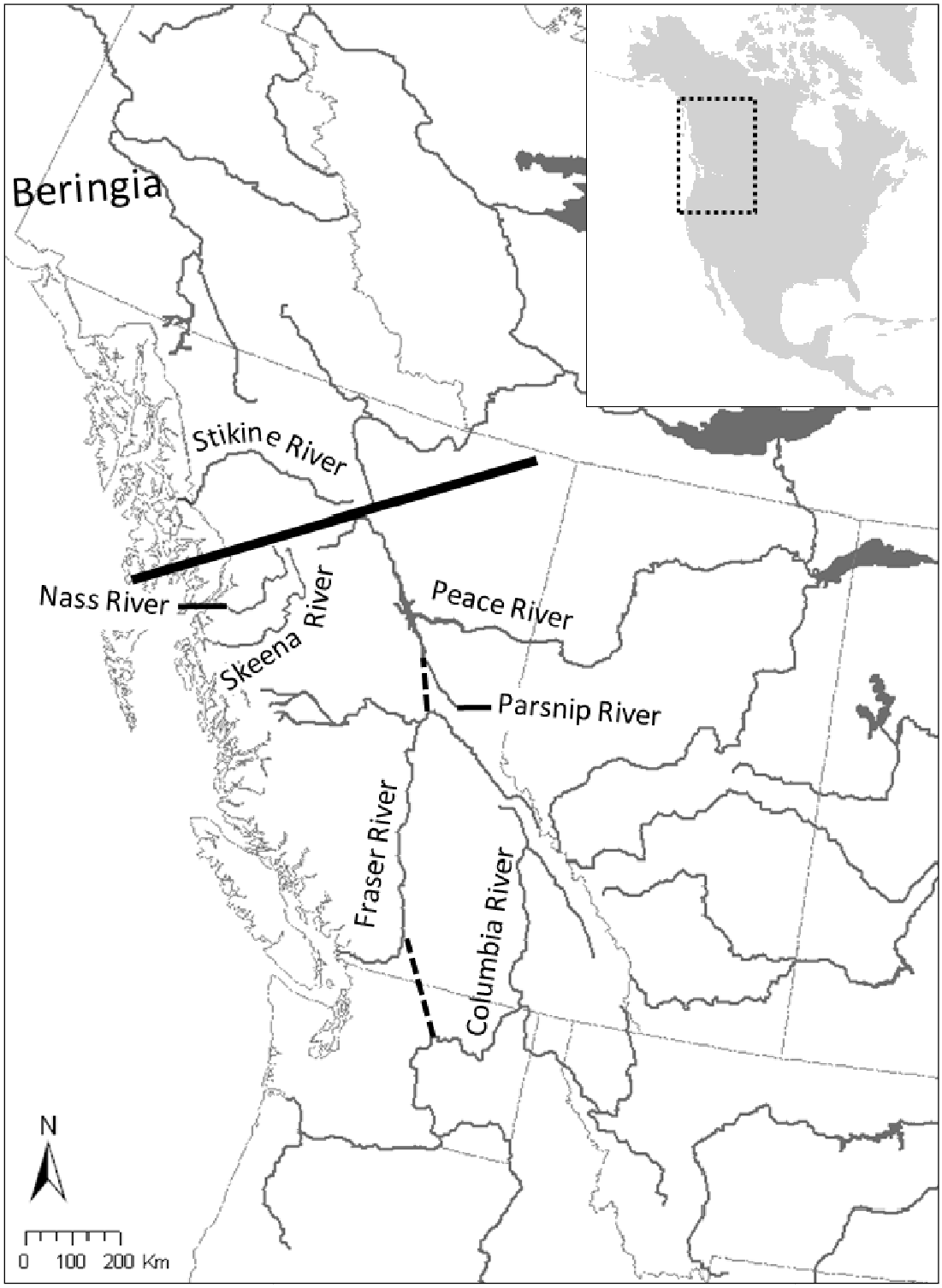

Beringia
Stikine River
Nass River
Skeena River
Peace River
Parsnip River
Fraser River
Columbia River
N
0 100 200 Km

Figure 1.6

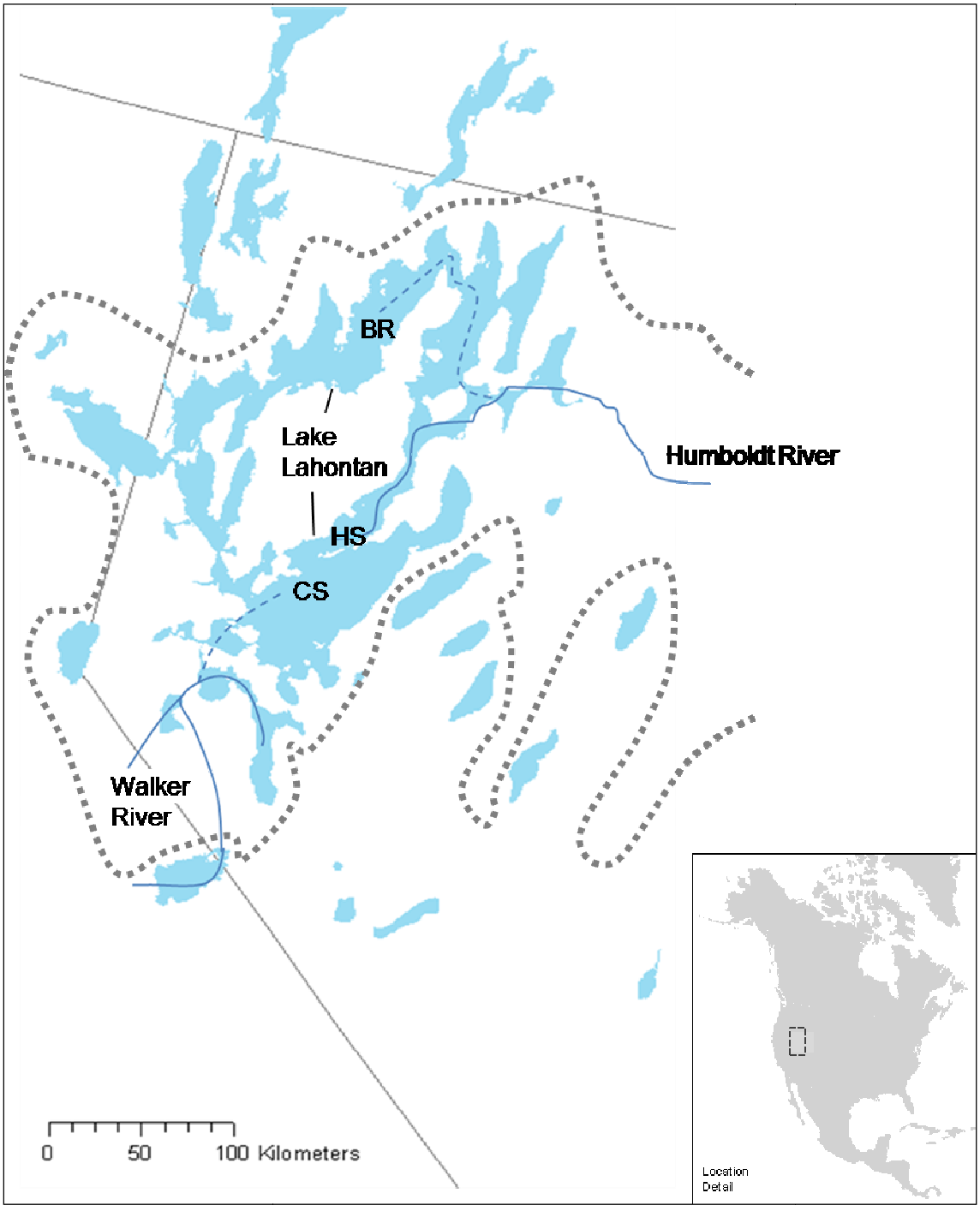
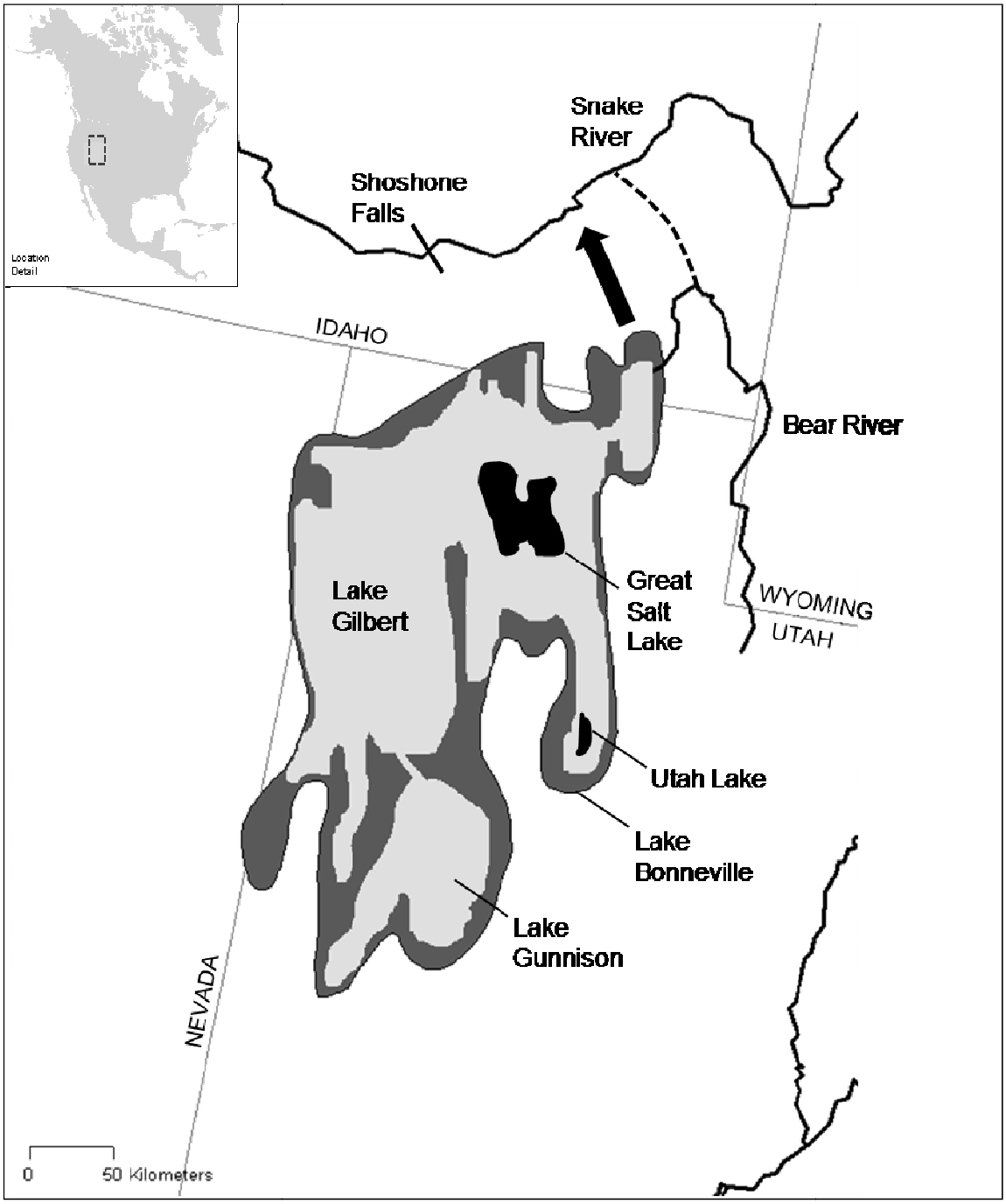

BR
Lake
Lahontan
HS
CS
Humboldt River
Walker
River
0
50
100 Kilometers
Location
Detail

Figure 1.7


Location
Detail
Snake
River
Shoshone
Falls
IDAHO
Bear River
Lake
Gilbert
Great
Salt
Lake
WYOMING
UTAH
Utah Lake
Lake
Bonneville
Lake
Gunnison
NEVADA
0
50 Kilometers

CHAPTER 2

PHYLOGENETIC RELATIONSHIPS OF THE WESTERN NORTH AMERICAN CYPRINID GENUS *RICHARDSONIUS*, WITH AN OVERVIEW OF PHYLOGEOGRAPHIC STRUCTURE

Introduction

The cumulative geological and climatic changes that molded the Cenozoic North American landscape have profoundly influenced patterns of diversification across numerous taxa (e.g., Minckley et al., 1986; Riddle, 1995; Klicka and Zink, 1997; Soltis et al., 1997; Brunsfeld et al., 2001; Hershler and Sada, 2002; Hewitt, 2004; Castoe et al., 2007; Liu and Hershler, 2007; Kohn and Fremd, 2008). If, as seems likely, the evolution of many North American taxa has been influenced both by recent changes in global climate as well as by temporally deeper geological changes to the landscape (Riddle, 1996), those taxa should exhibit shallow genetic structure superimposed on deep divergences. Indeed, such hierarchical structure has been demonstrated for a variety of taxa (e.g., Demboski and Cook, 2001; Wilke and Duncan, 2004; Alexander and Riddle, 2005; Carstens et al., 2005a; Steele et al., 2005).

Perhaps due to their reduced vagility in comparison, aquatic taxa tend to maintain a more intact signature of responses to geological processes and climate change than do terrestrial organisms (Bernatchez and Wilson, 1998), as has been demonstrated among various freshwater fishes around the globe (e.g., Bermingham and Avise, 1986; McGuigan et al., 2000; Unmack, 2001; Smith and Bermingham, 2005; Swartz et al., 2007; Zemlak et al., 2008). The evolution of freshwater fishes in western North America has been characterized by long term isolation of populations, punctuated by sporadic

dispersal among hydrological basins during major events such as floods or stream captures, some of which coincide with major climatic events (Smith, 1981; Minckley et al., 1986; Johnson, 2002; Smith et al., 2002; Mock et al., 2006).

Richardsonius is a genus of western North American freshwater fish from the family Cyprinidae that contains two species: redside shiner *Richardsonius balteatus* (Richardson) and Lahontan redside shiner *Richardonius egregius* (Girard). However, the state of Oregon recognizes two additional species, coastal redside shiner *R. siuslawi* and hotspring redside shiner *R. thermophilus*, claiming that both species were "lumped with *R. balteatus* without evidence" (ODFW, 2005). In this study, I use the conventional two species classification of *R. balteatus* and *R. egregius* (as recognized by the American Fisheries Society, the American Society of Ichthyologists and Herpetologists, and the Integrated Taxonomic Information System). *Richardsonius* is of biogeographic interest because of its wide distribution throughout several western North American drainages (Figure 2.1). Numerous ancient connections have existed between these drainage basins at different times throughout the Paleogene, suggesting a number of opportunities for isolation and dispersal as drainage systems evolved (Taylor, 1985; Minckley et al., 1986; Smith et al., 2002; Spencer et al., 2008). Moreover, *R. balteatus* exhibits morphological (Hubbs and Miller, 1948; Lindsey, 1953; Smith, 1966; McPhail and Lindsey, 1986; Minckley et al., 1986; LaRivers, 1994; Smith et al., 2002) and ecological differences among populations (Houston and Belk, 2006) that may be due, in part, to a history of geographic isolation. Some of these morphological differences (primarily the number of anal fin rays), coupled with geographic distributions, are the basis for dividing *R. balteatus* into two subspecies, *R. b. balteatus* and *R. b. hydrophlox*.

Monophyly of *Richardsonius* has never been rigorously tested or questioned. Given that recent studies have uncovered cryptic genetic diversity within other North American cyprinid genera (Johnson et al., 2004; Schönhuth et al., 2008; Houston et al., *in press*), it is prudent to evaluate the monophyly of *Richardsonius* prior to conducting detailed phylogeographic studies. Several studies have provided what appear to be robust evolutionary hypotheses regarding the phylogenetic relationships of many North American cyprinid genera (e.g., Mayden, 1989; Mayden, 1991; Cavender and Coburn, 1992; Coburn and Cavender, 1992; Dowling and Naylor, 1997; Simons et al., 2003; Johnson et al., 2004; Blum et al., 2008). *Richardsonius* belongs to the *Mylocheilus* sub-clade of Mayden's Open Posterior Myodome (OPM) clade (Mayden, 1989; Simons et al., 2003), which contains four genera: *Clinostomus, Mylocheilus, Pogonichthys,* and *Richardsonius* (Simons et al., 2003). Within the *Mylocheilus* sub-clade, *Richardsonius* and *Clinostomus* were most recently postulated to be sister genera (Simons et al., 2003), although, because one genus was not included in recent studies (see below), the extant sister-genus to *Richardsonius* remains unclear.

Richardsonius is somewhat unique among western North American cyprinids because its hypothesized sister taxon, *Clinostomus*, is a genus that occurs east of the Rocky Mountains, suggesting that dispersal across the Continental Divide has occurred. Like *Richardsonius*, *Clinostomus* contains only two species: redside dace *Clinostomus elongatus* (Kirtland), which occurs in the Great Lakes region, and rosyside dace *Clinostomus funduloides* Girard, which occurs in the southeastern United States (Lee et al., 1980; Figure 2.1). The genera *Clinostomus* and *Richardsonius* have long been considered to be closely related, and have even been grouped in the same genus at times

(for review, see Simons and Mayden, 1998; 1999). The sister relationship of these two genera appears to be supported both by morphology (Coburn and Cavender, 1992) and by mitochondrial DNA (mtDNA) sequence data (Simons and Mayden, 1999; Simons et al., 2003). However, recent mtDNA evidence suggests that another genus, *Iotichthys*, may also be closely related to *Richardsonius* (Smith et al., 2002). The least chub *Iotichthys phlegethontis* (Cope) is a monotypic species that occurs in the northern Bonneville Basin (Figure 2.1; Lee et al., 1980; Mock and Miller, 2005). To my knowledge, there have been few phylogenetic studies to date that include *Clinostomus*, *Iotichthys* and *Richardsonius*. The first was based on morphological characters and showed sister relationships between *Clinostomus* and *Richardsonius*, and between *Iotichthys* and Utah chub *Gila atraria* (Girard), another western North American cyprinid (Coburn and Cavender, 1992). Conversely, recent mtDNA work showed a sister relationship between *Iotichthys* and *Richardsonius*, although *Clinostomus* was not included in those analyses (Smith et al., 2002). A recent phylogeny published by Estabrook et al. (2007) supported a sister relationship between *Iotichthys* and *Richardsonius*, and showed *Clinostomus* to be sister to a *Richardsonius – Iotichthys* clade. However, those authors were investigating questions on higher level phylogenetic relationships and molecular clock rates, so only one individual was sequenced for each of the three genera and they did not specifically address this issue.

Here, I employ an array of phylogenetic analyses to address the following: First, I use a multiple gene tree approach to address phylogenetic relationships of the *Mylocheilus* sub-clade of Mayden's OPM clade, with particular attention to testing alternative hypotheses regarding *Clinostomus* vs. *Iotichthys* as the extant sister genus to

Richardsonius. Second, given the uncertain phylogenetic placement of *Iotichthys*, and its close geographic proximity to *R. balteatus* and *R. egregius* (Figure 2.1), it is possible that *Iotichthys* could render *Richardsonius* paraphyletic. Therefore, I assess the monophyly of *Richardsonius* by conducting phylogenetic analyses that incorporate specimens representing both nominal species and *I. phlegethontis*. Third, I evaluate the reciprocally monophyletic status of *R. balteatus* and *R. egregius* using multiple samples from throughout the geographic distributions of both species, as well as the two subspecies of *R. balteatus*. Finally, I employ molecular dating techniques using fossil calibrations to establish a time frame for the evolution of this group of fishes and qualitatively assess the subsequent phylogeographic implications.

Materials and Methods

Family-level investigation: sampling and analysis

To ensure that I did not exclude any taxa that could potentially be sister to *Richardsonius* from my analyses, I obtained cytochrome *b* (cyt *b*) gene sequences from GenBank for all available (as of May 2009) North American cyprinids. To avoid potential problems that could result from including sequences with too much missing data, I used only sequences that contained between 1,100-1,143 base pairs. The resulting data set contained 135 species from 44 genera (Table 2.1). These phylogenetic analyses included only 44 of the 60 (~73%) currently recognized North American cyprinid genera and 155 of the 307 (~50%) currently recognized species (Nelson et al., 2004), plus the proposed monotypic species *Codoma ornata* (Schönhuth et al., 2008). I feel confident that there are no other cyprinid genera likely to be sister to *Richardsonius* because an

additional five genera are accounted for in other analyses and none of these appear to be close relatives to *Richardsonius* (e.g., Simons and Mayden, 1998; Simons et al., 2003). In other words, 49 of the 60 (~82%) currently recognized genera have been incorporated into recent molecular phylogenetic analyses and none other than *Clinostomus* or *Iotichthys* appear as a potential sister genus to *Richardsonius*. The remaining eleven genera are either presumed to be extinct (thus unavailable for phylogenetic analyses), occur in Mexico or have been introduced to North America recently and thus are unlikely to be close relatives. Likewise, many of the species missing from these analyses belong to the speciose shiner clade (with many belonging to the genus *Notropis*), and thus are not likely to be more closely related to *Richardsonius* than other members of the shiner clade.

I aligned cyt *b* sequences automatically using Sequencher v. 4.6 (Gene Codes Corp.) and corrected the alignment by eye, using the amino acid sequence for reference. I analyzed the data using maximum parsimony (MP) and maximum likelihood (ML) optimality criteria, as well as Bayesian inference. For MP analysis, I used PAUP* v. 4.0b10 (Swofford, 2002) to perform a heuristic search with 10 random stepwise addition replicates and tree bisection-reconnection (TBR) branch swapping. Gaps were treated as missing data because cyt *b* is a protein-coding gene, and the only missing data occurred where the complete gene was not available from GenBank. I estimated nodal support by performing 100 bootstrap pseudo-replicates (Felsenstein, 1985). Cyprinids as divergent as those from Europe, Asia and North America do not exhibit saturation at any codon position within the cyt *b* gene (Doadrio and Carmona, 2004; Sasaki et al., 2007), so I did not down-weight the third codon position for this analysis. For ML analysis, I selected

the model of sequence evolution using Modeltest (Posada and Crandall, 1998). The model selected under the Akaike Information Criterion (AIC) was GTR + I + G. I used TreeFinder (Jobb, 2005) to reconstruct a phylogeny, and performed 100 bootstrap replicates to estimate nodal support (Felsenstein, 1985). I performed Bayesian analyses using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001), and partitioned the data by codon position. I used MrModeltest v. 2.2 (Nylander, 2004) to find the appropriate model of sequence evolution for each partition. The models selected were HKY + I + G for the first base position, and GTR + I + G for the second and third base positions. I employed a Markov Chain Monte Carlo approach in Bayesian analysis, with one cold chain and three heated chains. I ran the analysis for 4,000,000 generations, sampling every 100 generations. In order to get appropriate levels of mixing between chains I had to lower the temperature from the default setting of 0.20 to 0.03. I discarded the first 1,000,000 generations (25%) as burn-in, and used a majority rule consensus of the remaining topologies for posterior probabilities.

Genus-level investigation: Sampling

Some samples were available for my use from the ichthyological collection at the Monte L. Bean Life Science Museum (MLBM) at Brigham Young University, and some were provided by other researchers, but I collected the majority of the specimens I used in this study. I sampled natural populations of *R. balteatus* and *R. egregius* throughout their native ranges using a beach seine, minnow traps, or a backpack electroshocker. Sampling localities for *Richardsonius* populations are shown in Figure 2.2, with detailed descriptions listed in Table 2.2. Because I had samples from only one population per species for *Clinostomus* and *Iotichthys* (except *C. elongatus* for which I had two

populations) those populations are not marked on the map, but they are listed in Table 2.2. I euthanized the fish by administering a lethal dose of tricaine methanesulfonate (MS-222). I placed whole specimens in 95% ethanol to preserve tissues, and deposited these specimens as vouchers in the MLBM collection. All laboratory protocols and field sampling methods were approved by UNLV IACUC Protocol No. R701-0703-179.

Genus-level investigation: DNA sequencing and analyses

In the laboratory, I removed muscle tissue from specimens and extracted whole genomic DNA from these tissues using the Qiagen DNeasy Tissue extraction protocol. Successful extractions were verified by visualizing the extraction product via ultraviolet radiation following gel electrophoresis on a 0.8% agarose gel. I used the polymerase chain reaction (PCR) to amplify the markers I used in our analyses (see below). I purified PCR products of successful amplifications using the Qiagen PCR Purification kit following the manufacturer's directions.

I amplified the mitochondrial control region (CR) and the protein coding cyt *b* gene because they are two of the most rapidly evolving markers in the mitochondrial genome of fishes (Broughton and Reneau, 2006), and have been useful in determining phylogenetic relationships and phylogeographic patterns that stem from Neogene events in many western North American taxa (e.g., Nielson et al., 2001; Dowling et al., 2002; Johnson, 2002; Carstens et al., 2004; Johnson et al., 2004; Carstens et al., 2005b; Mock and Miller, 2005; Steele et al., 2005; Mock et al., 2006). I amplified CR using the primers L-Pro and MRT-2 (Meyer et al., 1994; Ptacek and Breden, 1998), and cyt *b* using the primers HA-a and LA-a (Dowling and Naylor, 1997). The thermal profile I used to amplify both mtDNA markers consisted of an initial denature of 95° C for 4 minutes,

followed by 35 cycles of 95° C for 30 seconds, 50° C for 30 seconds, and 72° C for 90 seconds, followed by a final extension of 72° C for 7 minutes and a rapid cool down to 4° C.

When making phylogenetic inferences it is desirable to use multiple unlinked markers (Hillis, 1996), and CR and cyt *b* are maternally inherited as a single unit (Avise, 2004). Hence, I also sequenced a nuclear marker, the first intron of the S7 ribosomal protein gene (S7). The S7 intron has recently been shown to have sufficient variation to be phylogenetically informative for other cyprinid genera (e.g., Johnson et al., 2004; He et al., 2008; Moyer et al., 2009). I amplified S7 using the primers S7RPEX1F and S7RPEX2R (Chow and Hazama, 1998) for a subset of twenty-five individuals representing all the major clades observed in the mitochondrial dataset. The thermal profile I used to amplify S7 began with an initial denature of 95° C for 1 minute, followed by 30 cycles of 95° C for 30 seconds, 58° C for 30 seconds, and 72° C for 2 minutes, followed by a final extension of 72° C for 10 minutes and a rapid cool down to 4° C.

I performed cycle sequencing reactions using Big Dye chemistry (Applied Biosystems, Inc.), sequencing in both directions, and using the same primers I used for amplification of gene segments in PCR. In certain cases, it was necessary to use internal primers in additional sequencing reactions to complete the sequence. Internal primers for cyt *b* were modified from primers designed by Dowling and Naylor (1997) as follows: HDrs (5’ – GGG TTA TTT GAC CCT GTT TCG T – 3’; modified from HD-a), LDrs (5’ – CCA TTT GTC ATC GCC GGT GC – 3’; modified from LD-a), and LErs (5’ – CCC ACC ACA TAT TCA ACC – 3’; modified from LE-s). I also designed primer Sq7Hrs

(5’ – ATG CTA AAT AAT AGG GCG AGG AC – 3’; modified from Sq7H, Houston et al., *in press*) for use as an internal sequencing primer for cyt *b*. I used primers 12Rrs (5’ – CAT CTG GTT CCT ATT TCA GG – 3’; modified from 12R, Johnson, 2002) and newly developed CR7H (5’ – TAG GGG GTA GGG GGG TTT GTC – 3’) as internal primers for sequencing some CR samples. Additionally, I developed an internal sequencing primer, S7INT3F (5’- TAG CCG CCT AGC CGG TGA ATT – 3’), for use as needed to complete the S7 sequence for some individuals. All sequencing was carried out on an ABI 3130 automated sequencer. All sequences have been deposited in GenBank (Accession Numbers GU182504-GU182876).

I aligned all DNA sequences using the automatic assembly function in Sequencher v. 4.6 (Gene Codes Corp.) and made corrections manually. I included GenBank sequences of the closest available taxon to *Richardsonius* for use as a reference to trim sequences to the correct length: *R. balteatus* for aligning cyt *b*, *G. atraria* for aligning CR, and northern leatherside chub *Lepidomeda copei* (Jordan and Gilbert) for aligning S7 (GenBank accession numbers AY096011, AF481762, and AY825461 respectively). I used amino acid sequence as a reference for aligning and editing the protein coding cyt *b* sequences. As might be expected for non-coding sequences, there were individuals that had insertion/deletion sequences (indels) for CR, S7, or both. There were few gaps in the CR sequences among ingroup taxa, so I aligned CR sequences using Sequencher and made corrections manually, making efforts to minimize the overall number of gaps whenever possible. Some of the individuals sequenced for S7 were heterozygous for indels. I inspected the chromatograms of S7 heterozygotes and assigned the appropriate IUPAC nucleotide ambiguity code for every base position that had a double peak. I then

used the program InDelligent (Dmitriev and Rakitov, 2008) to locate the position of the indels and separate the two alleles. I aligned the separated alleles in Sequencher, and manually edited the alignment so that gaps were in the locations specified by InDelligent. I converted the aligned sequences to python format and then ran the program Phase 2.1 (Stephens et al., 2001; Stephens and Scheet, 2005) to assign posterior probabilities to the base calls, leaving bases with posterior probabilities below 0.95 as ambiguous characters. After running the sequences through Phase, I converted the output file from python format to Nexus format for phylogenetic analyses.

Because CR and cyt *b* are mtDNA markers and thus inherited as a unit, I concatenated them in these analyses. I generated phylogenetic trees for the concatenated mtDNA data set using MP and ML optimality criterion. To minimize the number of terminal taxa in our phylogenetic analyses, I removed redundant haplotypes from the concatenated data set using MacClade v. 4.08 (Maddison and Maddison, 2005) and used the reduced data set in these phylogenetic analyses. I used the same programs and settings as outlined for the family-level analyses (above) with the following exceptions: I treated gaps as a fifth character state in MP analysis to account for indel sequences in CR, the model selected by Modeltest was GTR + I + G for the ML analysis, and I performed 1,000 and 100 bootstrap replicates for MP and ML analyses respectively. I partitioned the mtDNA data by gene and codon position for Bayesian analysis. I used the following models of sequence evolution for each Bayes partition, as suggested by MrModeltest: SYM + I for the first codon position of cyt *b*, F81 for the second codon position of cyt *b*, GTR + G for the third codon position of cyt *b*, and GTR + I + G for CR. I set the temperature to 0.05 for Bayesian analysis. I used the same settings and programs for the

nuclear S7 data set, with the following exceptions: I performed 10,000 bootstrap replicates in MP analysis. I did not down-weight codon positions because S7 sequences do not exhibit saturation in cyprinids (He et al., 2008). I used the model HKY + G for ML analysis, and performed 1,000 bootstrap replicates. Because S7 is a non-coding nuclear intron, I analyzed the data under a single model of nucleotide evolution rather than partitioning by codon position in Bayesian analysis. The model F81 + G was selected by MrModeltest, and I set the temperature to 0.07 for Bayesian analysis. I rooted all topologies using peamouth *Mylocheilus caurinus* (Richardson) as the outgroup taxon because it is hypothesized to be a close relative to *Richardsonius* and *Clinostomus*.

Genus-level investigation: Molecular dating

To obtain divergence time estimates, I used a Bayesian approach implemented using the uncorrelated lognormal relaxed clock model in the program BEAST v.1.4.8 (Drummond et al., 2006; Drummond and Rambaut, 2007). I included all individuals for which I had sequenced cyt *b*, CR and S7 in my phylogenetic analyses. I added the sequence data as two separate alignments. I concatenated the two linked mtDNA markers for the first alignment, and included S7 sequences as a second, unlinked alignment. I used jModeltest (Posada, 2008) as implemented in PhyML (Guindon and Gascuel, 2003) to select a model of sequence evolution for both the mitochondrial and nuclear data sets, as well as to estimate priors for various model parameters (e.g., gamma, GTR substitutions, proportion of invariant sites, etc.). I selected the GTR + I + G substitution model for the mtDNA alignment, and the HKY + G substitution model for the S7 alignment based on the jModeltest results, and used the Yule process speciation model to establish the prior on the tree.

I calibrated two nodes on the tree using fossil data. The first fossil I used was the earliest unambiguous *Mylocheilus* fossil, which was taken from the Chalk Hills Formation, Idaho, and dates to 7.0 Ma (Dowling et al., 2002; Smith et al., 2002). I used the *Mylocheilus* fossil to calibrate the basal node on the phylogeny because that node represents the most recent common ancestor between *Mylocheilus* and the rest of the ingroup. The second fossil I used was that of *Richardsonius durranti* (now extinct), which was taken from the Glenns Ferry Formation, Idaho, and is the earliest known *Richardsonius* fossil (Smith, 1975). The Glenns Ferry Formation was deposited approximately 3.5 Ma (Neville et al., 1979; Kimmel, 1982), so I conservatively used that date as a minimum age for the node representing the most recent common ancestor of *Iotichthys* and *Richardsonius*. I ran the MCMC chain for 40,000,000 generations in BEAST, sampling every 1,000 generations, and I discarded the first 4,000,000 steps (10%) as burn-in. To increase effective sample size (ESS) values, I repeated the analysis a second time and pooled the data from the two runs.

I calculated average percent sequence divergence between species using MEGA 4.1 (Tamura et al., 2007) to obtain a mutation rate for cyt *b* among the species belonging to the *Mylocheilus* sub-clade of North American cyprinids. I calculated mutation rate as percent sequence divergence per million years by dividing the average percent sequence divergence between species by the mean divergence time estimates between those same species as generated by the BEAST analyses.

Results

Family-level investigation

Phylogenetic analyses of the North American cyprinid cyt *b* sequences obtained from GenBank yielded trees congruent with the phylogram shown in Figure 2.3 regardless of approach, although some of the nodes supported in one type of analysis are not well supported in others (i.e., some nodes with high ML bootstrap values and high Bayesian posterior probabilities had low, or no bootstrap support in MP analysis). Hence, I marked only nodes which received high support values in all three types of analyses as well supported (Figure 2.3). Nevertheless, phylogenetic relationships for the *Mylocheilus* sub-clade and its members received high support values in MP, ML and Bayesian analyses (Figure 2.4). Hence, there do not appear to be any additional members of the *Mylocheilus* sub-clade other than those outlined above (see introduction). The phylogram (Figure 2.4) shows a sister relationship between *Iotichthys* and *Richardsonius*, and a sister relationship between *Clinostomus* and the *Iotichthys–Richardsonius* clade.

Genus-level investigation: DNA sequencing

DNA sequencing yielded 1,140 bp of cyt *b*, 958-965 bp of CR for 169 individuals, and 852-869 bp of S7 for 25 individuals. Both CR and S7 exhibited length polymorphisms due to indel sequences. Gaps in the CR alignment ranged from 1 to 4 bp in length, whereas gaps in the S7 alignment ranged from 1 to 15 bp in length (the longest occurred in *M. caurinus* only). The final alignments (including gaps) were 973 and 905 bp for CR and S7, respectively. Of the 1,140 bp of cyt *b*, 864 characters were constant, and 212 were variable characters that were parsimony informative. Control region had 807 constant and 137 parsimony informative characters. The S7 sequences had 759

constant characters, and 62 parsimony informative characters. However, when just the two *Richardsonius* species were included in the S7 alignment, the sequence alignment contained 891 total characters for 29 taxa, and only 16 variable characters that were parsimony informative, suggesting that this marker evolves too slowly to effectively evaluate reciprocal monophyly of *R. balteatus* and *R. egregius*.

Genus-level investigation: Mitochondrial DNA phylogeny

All phylogenetic analyses performed on the concatenated mtDNA data resulted in phylogenetic trees that were consistent with the results of the family level analyses, with well supported nodes showing *Iotichthys* as the sister genus to *Richardsonius*, and *Clinostomus* as sister to the *Iotichthys – Richardsonius* clade (Figure 2.5). *Richardsonius* appears to be a monophyletic genus, and *R. balteatus* and *R. egregius* are reciprocally monophyletic species. Shallow nodes indicate that there are three major clades within *R. balteatus* (see Figure 2.5): Clade B is comprised of individuals from the Bonneville Basin and upper Snake River; clade Co is a coastal clade, containing individuals from the Pacific Coast and lower Columbia River drainage populations; and clade Ci is an inland clade that consists of individuals from the upper Columbia River and British Columbia populations. Clades B and Ci are well supported (Figure 2.5), whereas Clade Co is not (it was supported by ML bootstrap values, but not MP bootstrap values or Bayesian posterior probabilities). Collapsing that node would result in a series of monophyletic lineages among coastal populations, but all coastal haplotypes are much more similar to each other than they are to haplotypes from clades B and Ci. Well supported nodes (Figure 2.5) show separate western and eastern Lahontan Basin clades within *R. egregius*. The eastern and western Lahontan clades are not entirely reciprocally monophyletic

because one western haplotype appears in an eastern Lahontan Basin population , and one eastern haplotype appears in a western population (Figures 2.2 and 2.4). Similarly, the inland Columbia clade and the Bonneville clade within *R. balteatus* are not reciprocally monophyletic because the most widespread inland Columbia haplotype appears in one of the northern Bonneville populations (Figures 2.2 and 2.4).

Genus-level investigation: Nuclear DNA phylogeny

The S7 dataset confirmed that *Iotichthys* is the sister taxon to *Richardsonius*, and *Clinostomus* is sister to the *Richardsonius – Iotichthys* clade (Figure 2.6). The S7 phylogeny also shows *Richardsonius* to be monophyletic. There is little resolution within the genus *Richardsonius*, and *R. balteatus* and *R. egregius* are not shown to be reciprocally monophyletic species, presumably due to a lack of phylogenetically informative characters (see above). Rather, the phylogeny shows a polytomy among most of the individuals of *Richardsonius*, with only a few well supported nodes depicting relationships among similar haplotypes. There does appear to be moderate support for at least some of the clades recovered in the mtDNA phylogeny. For example, four of the five Bonneville haplotypes cluster together (MN8042, TE7214a, TE7216 and TP7284), but the remaining haplotype (TE7214b) came from a heterozygote and branches out of a basal polytomy (Figure 2.6). Similarly, *R. egregius* haplotypes cluster into one of two clades, although one of those two clades contains a *R. balteatus* haplotype as well, albeit with low nodal support (Figure 2.6).

Genus-level investigation: Molecular dating

Phylogenies and divergence time estimates reveal a pattern consistent with relatively shallow phylogenetic structure within each of the two *Richardsonius* species that is

superimposed upon much deeper divergences among genera. All divergence time estimates are shown surrounded by 95% confidence intervals in Figure 2.7. Mean genetic diversification among the three *R. balteatus* clades is estimated to have occurred in the early Pleistocene (mtDNA: $1.6 \pm 2.4\times10^{-2}$ Ma [all divergence time estimates are listed in the text as mean ± standard error]; nuDNA: $1.8 \pm 7.5\times10^{-3}$ Ma), as is diversification between the eastern and western clades of *R. egregius* (mtDNA: $1.4 \pm 2.3\times10^{-2}$ Ma; nuDNA: $1.5 \pm 6.7\times10^{-3}$ Ma). Time to most common recent ancestor (tmrca) of the two *Richardsonius* species is estimated to be in the late Pliocene (mtDNA: $2.8 \pm 2.0\times10^{-2}$ Ma; nuDNA: $2.1 \pm 8.6\times10^{-3}$ Ma). The estimated split between *Iotichthys* and *Richardsonius* is estimated to have occurred in the mid-Pliocene (mtDNA: $3.7 \pm 1.5\times10^{-2}$ Ma; nuDNA: $3.5 \pm 6.6\times10^{-3}$ Ma). Divergence between *Clinostomus* and the *Iotichthys – Richardsonius* clade is estimated to have occurred in the late-Miocene to early-Pliocene (mtDNA: $5.8 \pm 2.3\times10^{-2}$ Ma; nuDNA: $4.9 \pm 9.2\times10^{-3}$ Ma). Average percent sequence divergences among species along with rates of genetic divergence for cyt *b* are given in Table 2.3. Sequence divergence ranged from 1.4 to 2.2 percent sequence divergence per million years, rates which are consistent with published mutation rates for cyt *b* in other cyprinids (e.g., Dowling et al., 2002; Smith et al., 2002; Berendzen et al., 2008).

Discussion

Phylogenetic systematics

These results advance knowledge of the phylogenetic relationships within a subset of North American cyprinids, specifically the *Mylocheilus* sub-clade of Mayden's OPM

clade (Mayden, 1989), by supporting the hypothesis that *Iotichthys* rather than *Clinostomus* is the sister genus to *Richardsonius*, and thus *Iotichthys* is not sister to *Gila* as hypothesized previously (see Coburn and Cavender, 1992). A sister relationship between *Iotichthys* and *Richardsonius* is consistent with the close genetic distances between the genera reported by Smith et al. (2002). *Clinostomus* is sister to the *Iotichthys – Richardsonius* clade. Therefore, a sister relationship between *Clinostomus* and *Richardsonius* (Simons and Mayden, 1998; 1999; Simons et al., 2003) is not supported. These phylogenetic relationships are consistent with the phylogeny published by Estabrook et al. (2007)

The phylogenies reconstructed by the analysis of both the mtDNA and the nuclear data sets (Figures 4 and 5) suggest that the current classification of *Richardsonius* is correct. *Richardsonius* appears to be a monophyletic genus based on these results. Likewise, the mtDNA phylogeny (Figure 2.5) shows that *R. balteatus* and *R. egregius* are reciprocally monophyletic species. However, the phylogeny produced by analyses of S7 (Figure 2.6) does not show reciprocal monophyly of the two *Richardsonius* species. Rather, the genus remains an unresolved polytomy in this phylogeny, likely because of the tendency for nuclear DNA sequences to evolve (and sort) at a slower rate than mtDNA sequences, as evidenced by the paucity of phylogenetically informative sites in the S7 sequence data. Even with the lack of resolution within *Richardsonius*, the S7 phylogeny is consistent with the mtDNA phylogeny.

Based on the mtDNA results, the subspecies designation of *R. b. balteatus* and *R. b. hydrophlox* may need revision. The Bonneville clade matches the subspecific designation of *R. b. hydrophlox*, except for the disjunct distribution in southeastern

Oregon (Figure 2.2). While I sampled only one population from the disjunct portion of the distribution of *R. b. hydrophlox*, all the individuals I sequenced from that population carried inland Columbia haplotypes rather than Bonneville Basin/Upper Snake River haplotypes (Figure 2.2). The occurrence of strictly inland Columbia haplotypes in the disjunct populations of *R. b. hydrophlox* is not consistent with subspecific designations, although, both *R. b. balteatus* and *R. b. hydrophlox* have been reported to occur in the area (Minckley et al., 1986). If the fish in the Harney-Malheur Basin are not in fact consistent with a genetically-defined *R. b. hydrophlox*, then their similarities in morphology to Bonneville Basin/Upper Snake fish (as reported by McPhail and Lindsey, 1986) may be due to convergent evolution in response to selective pressures in similar environments rather than common ancestry. Alternatively, a hypothesized hydrological connection between the John Day River (tributary to the Columbia River) and the Silvies River in southeast Oregon may have allowed for secondary contact to occur between sub-species (Bisson and Bond, 1971; McPhail and Lindsey, 1986). More intensive sampling in the area is necessary to resolve this issue.

Biogeographic implications

The biogeographic implications of the divergence time estimates yielded by these analyses are many. The Rocky Mountains began forming in the Late Cretaceous and reached their maximum height by the middle of the Eocene (Saleeby, 2003; English and Johnston, 2004; Spencer et al., 2008), and now stand as a formidable barrier to dispersal for aquatic taxa. It is postulated that cyprinids did not migrate to North America from Asia until much later, in the mid-Oligocene (Briggs, 1979; Cavender, 1991; Simons and Mayden, 1998; Briggs, 2005; Nelson, 2006). Therefore, the eastern distribution of

Clinostomus contrasted with the western distribution of its *Richardsonius – Iotichthys* sister clade, as well as the western distribution of *Mylocheilus* and *Pogonichthys*, suggests that *Clinostomus* crossed the Continental Divide to obtain its current distribution in eastern North America. Our divergence time estimates are consistent with such a scenario. The 95% confidence intervals surrounding the estimates for divergence between *Clinostomus* and *Iotichthys – Richardsonius* show that these genera split in the late Miocene to mid-Pliocene (Figure 2.7). There are at least two documented cases of aquatic connections across the present day Continental Divide during that time period: when what are now tributaries to the Missouri River were connected to the Saskatchewan River, or when they were connected to the Snake River during the late Miocene and Pliocene (Smith, 1981; Smith et al., 2000; Hershler and Gustafson, 2001; Hershler et al., 2008).

Divergence between *Iotichthys* and *Richardsonius* is estimated to have occurred during the Pliocene (Figure 2.7). The Great Basin, where these genera occur, began forming in the Eocene as a result of block faulting and extension of the earth's crust, and continued to evolve throughout the Miocene as a result of tectonic activity in western North America (Minckley et al., 1986; Stokes, 1988; Kohn and Fremd, 2008). The Bonneville Basin, where *I. phlegethontis* and *R. b. hydrophlox* occur, formed in the eastern Great Basin, whereas the Lahontan Basin, where *R. egregius* occurs, formed in the western Great Basin. The boundaries of these basins were largely in place by late Miocene when tectonic activity subsided, but biogeographic evidence suggests that they shared aquatic connections during the Pliocene (Miller, 1958; Taylor, 1985; Minckley et al., 1986). It is possible that divergence between these genera occurred when aquatic

connections between the two basins were severed, but the exact timing and location of these postulated hydrological connections are still unknown. Alternatively, the occurrence of *R. durranti* fossils in Glenns Ferry deposits (Smith, 1975) indicates a Pliocene distribution of *Richardsonius* in the Snake River drainage. Biogeographic evidence supports a large river connection between the Snake and Colorado rivers during the Miocene and Pliocene via the Bonneville Basin (e.g., Taylor, 1985; Johnson et al., 2004; Oakey et al., 2004; Smith and Dowling, 2008; Spencer et al., 2008; Houston et al., *in press*), although geological evidence for such a connection is lacking (Powell, 2005; Pederson, 2008). Divergence between *Iotichthys* and *Richardsonius* may have occurred when those connections ceased, although this scenario does not explain why neither genus occurs naturally in the Colorado River system.

Divergence between *R. balteatus* and *R. egregius* occurred in the late Pliocene to early Pleistocene (Figure 6). The timing of this split is consistent with the capture of the Snake River by the Columbia River system, which occurred 2.5-3.2 Ma when a tributary to the prehistoric Salmon River eroded a sill of the basin containing Pliocene Lake Idaho, causing massive spill-over (Wheeler and Cook, 1954; Repenning et al., 1995; Link et al., 2002; Beranek et al., 2006). Capture of the Snake River by the Columbia system may have placed *R. balteatus* and *R. egregius* on separate evolutionary trajectories depending on the path of the Snake River prior to the capture event. A Miocene connection between the Snake River and the Sacramento River is supported by geomorphic and biogeographic evidence (Wheeler and Cook, 1954; Minckley et al., 1986; Smith et al., 2002). Two pathways have been proposed for that connection. One postulated connection is supported by fossil and current distributions of mollusks (Taylor and Smith,

1981; Taylor, 1985) and fishes (Kimmel, 1975; Smith, 1975; Smith et al., 1982; Smith et al., 2000; Smith and Cossell, 2002) and indicates a westward flow of the Snake River across southern Oregon to the Sacramento or Klamath rivers. The other involved flow of the Snake River southwestward into the Humboldt River (in northern Nevada) which then entered the Sacramento system. This route is supported by mammal fossils (Repenning et al., 1995), molecular data from gastropods (Hershler and Liu, 2004), and sedimentary zirconium (Link et al., 2005; Beranek et al., 2006). The time constraints on the hypothesized Snake – Humboldt – Sacramento connection are between 9.8 Ma when the Humboldt River first began to form (Wallace et al., 2008), and 2.5 – 3.2 Ma when the Snake River was captured into the Columbia River system (Link et al., 2002; Beranek et al., 2006). The capture and draining of Lake Idaho would have effectively disrupted the Snake – Humboldt connection. Regardless of which pathway the Snake River followed prior to its capture, the Lahontan Basin, where *R. egregius* occurs, has remained isolated from its surrounding drainages since approximately 2 Ma (Minckley et al., 1986; Repenning et al., 1995; Reheis and Morrison, 1997; Smith et al., 2002). The complete isolation of the Lahontan Basin is well within the confidence intervals surrounding the estimate of divergence time between *R. balteatus* and *R. egregius*.

Each species of *Richardsonius* exhibits significant phylogeographic structure that is worthy of further investigation. The occurrence of shallow clades of *R. balteatus* that are estimated to be early Pleistocene in origin (Figures 4 and 6) indicates that there may have been at least three glacial age distributions for the species. It also appears that divergence between two clades within *R. egregius* preceded events of the late Pleistocene (Figure 2.7). The Bonneville/Upper Snake clade containing *R. b. hydrophlox* also exhibits

phylogenetic structure (Figure 2.5). These results suggest that gene flow may not have been prominent throughout pluvial lakes during the Pleistocene. Some researchers have stated that pluvial lakes were short enough in duration (in the sense of evolutionary time) that they did not play a prominent role in the evolution of aquatic taxa (Taylor and Smith, 1981; Taylor and Bright, 1987; McKell, 2003), which explain the existence of such phylogeographic structure. A biological reason for this could be that while *R. balteatus* and *R. egregius* can be aggressive colonizers and both may occur in lakes, where they are typically restricted to warmer shoreline habitats (La Rivers, 1994; Lindsey and Northcote, 1963; Smith et al., 2000). Dispersal through deep waters may not be likely due to threat of predation by large piscivorous fish. It is known that cutthroat trout (which inhabited pluvial lakes Bonneville and Lahontan) shift to a piscivorous diet upon reaching larger body sizes in big water bodies (May et al., 1978). Hence, cutthroat trout may have posed a predatory barrier to minnows in those pluvial lakes.

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Table 2.1: List of species and accession numbers for the cyt *b* sequences obtained from GenBank for phylogenetic analyses.

Species	Accession #	# Base Pairs Used in Phylogenetic Analyses
Acrocheilus alutaceus	AF452076	1,140
Agosia chrysogaster	AF452081	1,126
Algansea avia	EU082509	1,141
Algansea lacustris	EU082506	1,141
Algansea tincella	EU082472	1,141
Aztecula sallaei	EU082472	1,141
Campostoma anomalum	AF452079	1,127
Campostoma oligolepis	DQ486836	1,143
Campostoma ornatum	DQ486789	1,140
Campostoma pauciradii	DQ486791	1,140
Clinostomus elongatus	KK7295[a]	1,140
Clinostomus funduloides	BR7256[a]	1,140
Codoma ornata	EU082516	1,140
Couesius plumbeus	AY281053	1,140
Cyprinella bocagrande	DQ324100	1,141
Cyprinella formosa	DQ324099	1,141
Cyprinella garmani	DQ324102	1,141
Cyprinella lutrensis	AB070206	1,141
Cyprinella proserpina	DQ324101	1,141

Cyprinella rutila	EU082524	1,141
Cyprinella spiloptera	U66605	1,140
Dionda catostomops	EU082481	1,139
Dionda diaboli	EU082494	1,141
Dionda dichroma	EU082484	1,141
Dionda episcopa	EU082490	1,141
Dionda ipni	EU082480	1,141
Dionda mandibularis	EU082487	1,141
Dionda melanops	EU082497	1,141
Dionda nigrotaeniata	EU082503	1,141
Dionda serena	DQ324080	1,141
Eremichthys acros	AF370117	1,140
Erimystax cahni	AY486010	1,116
Erimystax dissimilis	AY486011	1,116
Erimystax harryi	AY486032	1,116
Erimystax insignis	AY486042	1,116
Erimystax x-punctatus	AY486054	1,115
Gila atraria	EU747197	1,140
Gila boraxobius	AF370042	1,140
Gila cypha	AF452074	1,140
Gila orcuttii	AF370118	1,140
Gila pandora	EU747196	1,140
Gila robusta	NC008105	1,141

Hemitremia flammea	AY281054	1,140
Hesperoleucus symmetricus	AF370116	1,140
Hybognathus amarus	EU811098	1,140
Hybognathus argyritis	EU811094	1,140
Hybognathus hankinsoni	AF452080	1,140
Hybognathus hayi	EU811092	1,140
Hybognathus nuchalis	EU811096	1,140
Hybognathus placitus	EU811086	1,140
Hybognathus regius	EU811088	1,140
Hybopsis amblops	AF117152	1,140
Hybopsis winchelli	AF117165	1,140
Iotichthys phlegethontis	AY641427	1,101
Lepidomeda albivalis	AF452089	1,140
Lepidomeda aliciae	AY825486	1,140
Lepidomeda copei	AY825444	1,140
Lepidomeda mollispinus	AF452092	1,140
Lepidomeda vittata	AF452088	1,134
Luxilus albeolus	U66598	1,140
Luxilus cardinalis	U66601	1,140
Luxilus cerasinus	U66599	1,140
Luxilus chrysocephalus	U66596	1,140
Luxilus coccogenis	U66603	1,140
Luxilus cornutus	U66597	1,140

Luxilus photogenis	AF352281	1,140
Luxilus pilsbryi	U66602	1,135
Luxilus scepticus	AF352283	1,140
Luxilus telescopus	AF352290	1,140
Luxilus zonatus	U66600	1,140
Luxilus zonistius	U66604	1,140
Lythrurus ardens	AY096007	1,140
Lythrurus atrapiculus	U17271	1,141
Lythrurus bellus	U17275	1,141
Lythrurus fumeus	U17269	1,141
Lythrurus lirus	U17273	1,141
Lythrurus roseipinnis	X66456	1,137
Lythrurus snelsoni	U17272	1,141
Lythrurus umbratilis	U17274	1,141
Margariscus margarita	AF452072	1,140
Meda fulgida	AF452094	1,130
Moapa coriacea	AF452075	1,135
Mylocheilus caurinus	AF117169	1,140
Mylopharodon conocephalus	EU747200	1,131
Nocomis biguttatus	AY486057	1,116
Nocomis leptocephalus	EU082468	1,141
Nocomis micropogon	AF452077	1,138
Notropis amabilis	AF352269	1,140

Notropis ammophilus	AF117160	1,120
Notropis amoenus	AF352270	1,140
Notropis anogenus	AY140698	1,140
Notropis ariommus	AY281057	1,140
Notropis asperifrons	AF261219	1,131
Notropis atherinoides	AY281062	1,140
Notropis blennius	AF117171	1,140
Notropis boops	AF352261	1,140
Notropis boucardi	AF469159	1,141
Notropis buchanani	AY281058	1,140
Notropis calientis	AF469143	1,141
Notropis candidus	AF352275	1,140
Notropis chrosomus	AF352262	1,140
Notropis dorsalis	AF117175	1,140
Notropis edwardranevi	AF352263	1,140
Notropis girardi	AF352276	1,140
Notropis heterodon	AY140697	1,141
Notropis heterolepis	AY140696	1,141
Notropis jemezanus	AF352277	1,140
Notropis longirostris	AF352264	1,140
Notropis micropteryx	EU084791	1,133
Notropis moralesi	AF469157	1,141
Notropis nubilus	AF352265	1,140

Notropis oxyrhynchus	AF352278	1,140
Notropis percobromus	EU084780	1,140
Notropis perpallidus	AF352279	1,140
Notropis potteri	AF352266	1,140
Notropis rafinesquei	AF117187	1,140
Notropis rubellus	AF117195	1,140
Notropis sabinae	AF117199	1,140
Notropis shumardi	AF352284	1,140
Notropis simus	EU811099	1,140
Notropis stilbius	AF352286	1,140
Notropis stramineus	DQ536429	1,141
Notropis suttkusi	AF352288	1,140
Notropis texanus	AF352267	1,140
Opsopoeodus emilae	U17270	1,141
Orthodon microlepidotus	AF452073	1,140
Phenacobius mirabilis	NC008112	1,141
Phenacobius uranops	AY486056	1,116
Phoxinus erythrogaster	AY281055	1,140
Pimephales notatus	U66606	1,140
Pimephales vigilax	AF117203	1,140
Plagopterus argentissimus	AF452090	1,140
Platygobio gracilis	EU811100	1,140
Pogonichthys macrolepidotus	AY096009	1,140

Pteronotropis euryzonus	AF261223	1,141
Pteronotropis hubbsi	AF261224	1,131
Pteronotropis hypselopterus	AF261227	1,131
Pteronotropis signipinnis	AF261230	1,136
Pteronotropis welaka	AF261232	1,141
Ptychocheilus lucius	EU747222	1,114
Ptychocheilus oregonensis	EU747203	1,123
Ptychocheilus umpquae	EU747204	1,123
Relictus solitarius	AF370115	1,140
Rhinichthys atratulus	AF452078	1,140
Rhinichthys cataractae	DQ990251	1,140
Rhinichthys evermanni	EU780890	1,140
Rhinichthys fulcatus	DQ990284	1,140
Rhinichthys obtusus	DQ990250	1,140
Rhinichthys osculus	DQ990316	1,140
Richardsonius balteatus	AY096011	1,140
Richardsonius egregius	TK7264[a]	1,140
Semotilus atromaculatus	AF452082	1,133
Siphateles alvordensis	AF370041	1,140
Siphateles bicolor	AF370106	1,140
Yuriria alta	AF469163	1,141

[a] Denotes sequences that were not available on GenBank but came from this study instead.

Table 2.2: Sampling localities for each of the specimens included in phylogenetic analyses for this study. Accession numbers are listed for the Las Vegas Tissue (LVT) collection at the University of Nevada Las Vegas where tissues are stored. Accession numbers are listed for the Monte L. Bean Museum (MLBM) at Brigham Young University where voucher specimens are deposited. The numbers of individuals sequenced for cytochrome *b* (cyt *b*), control region (CR), and the first nuclear intron of the S7 ribosomal protein gene (S7) are also listed.

Locality	**Latitude/Longitude**	**LVT Accession #**	**MLBM Accession #**	**Individuals Sequenced**		
				cyt *b*	**CR**	**S7**
Clinostomus elongatus						
WISCONSIN						
Sleighton Creek (Kickapoo River; KK), Monroe County	43.839 N, 90.534 W	7294 – 7298	59122 – 59126	5	5	2
West Branch Raccoon Creek (RC), Rock County	42.564 N, 89.214 W	8289-90, 8292	59179-80, 59182	3	3	0

Clinostomus funduloides						
TENNESSEE						
Big Richland Creek (BR), Humphreys County	36.160 N, 87.760 W	7254 – 7258	58239 – 58243	5	5	1
Iotichthys phlegethontis						
UTAH						
Lucin Pond (LU), Box Elder County	41.348 N, 113.906 W	8237 – 8241	63825 – 63829	5	5	1
Mylocheilus caurinus						
OREGON						
Columbia River	exact location unknown	8285	59412	1	1	1
Richardsonius egregius						
NEVADA						
McDermitt Creek (MC), Humboldt County	41.970 N, 117.836 W	7881 – 7885	63759 – 63763	5	5	1

North Fork Little Humboldt River (LH), Humboldt County	41.692 N, 117.247 W	9846 – 9850	112052 – 112056	5	5	0
T Creek (T), Elko County	41.525 N, 115.247 W	8277 – 8281	99317 – 99321	5	5	0
Truckee River (TK), Washoe County	39.588 N, 119.448 W	7264-5, 7267-8	63054-5, 63057-8	4	4	1
West Walker River (WK), Lyon County	38.740 N, 119.400 W	7275 – 7278	62099 – 62102	4	4	2
Richardsonius balteatus						
BRITISH COLUMBIA						
Antonelli Creek (AN), Peace Region	56.334 N, 120.154 W	9721 – 9725	63951 – 63955	5	5	0
Doris Lake (DO), Skeena Region	54.945 N, 126.552 W	9731 – 9735	63965 – 63969	5	5	0
Kettle River (KT), Okanagan Region	49.013 N, 118.200 W	9001 – 9005	084184 – 084188	5	5	1

Similkameen River (SK), Okanagan Region	49.175 N, 119.768 W	8991 – 8994	84171 – 84174	4	4	0
IDAHO						
Big Bear Creek (Clearwater River; CW), Latah County	46.600 N, 116.660 W	8247 – 8251	138772 – 138776	5	5	1
Blackfoot River (BK), Bingham County	43.230 N, 112.030 W	7851 – 7855	58911 – 58915	5	5	0
Cold Creek (Goose Creek; GS), Cassia County	42.093 N, 113.933 W	7314 – 7318	61222 – 61226	5	5	0
Coeur d'Alene River (CD), Shoshone County	47.553 N, 116.257 W	6301 – 6305	63627 – 63631	5	5	0
Hurry Back Creek (HB), Owyhee County	42.581 N, 116.676 W	7861 – 7865	63743 – 63747	5	5	1
Salmon River (SL), Custer County	44.639 N, 114.122 W	7871 – 7875	63780 – 63784	5	5	1

Teton River (TE), Teton County	43.750 N, 112.200 W	7215 – 7218	63689 – 63692	4	4	2
OREGON						
Callapooia River (CA), Linn County	44.461 N, 123.076 W	6291 – 6295	63642 – 63646	5	5	1
Callapooya Creek (Umpqua River; UM), Douglas County	43.413 N, 123.207 W	8267 – 8271	68425 – 68429	5	5	0
Donner und Blitzen River (DB), Harney County	42.801 N, 118.967 W	9252 – 9255	114036 – 114039	4	4	2
Elk Creek (EK), Josephine County	42.033 N, 123.750 W	7334 – 7338	59297 – 59301	5	5	2
Hunter Creek (HN), Curry County	42.352 N, 124.353 W	7234 – 7238	63653 – 63657	5	5	1
South Fork John Day River (SJ), Grant County	44.424 N, 119.540 W	7224 – 7228	63673 – 63677	5	5	1

Siuslaw River (SI), Lane County	44.000 N, 123.689 W	8452 – 8456	63929 – 63933	5	5	1
UTAH						
Beaver Creek (Weber River; WB), Summit County	40.626 N, 111.163 W	8257-8, 8260-1	69873-4, 69876-7	4	4	0
Blue Creek (BL), Box Elder County	41.952 N, 112.723 W	7300 – 7303	68244 – 68247	4	4	0
Lake Creek (LK), Millard County	38.767 N, 114.048 W	7244 – 7248	68439 – 68443	5	5	0
Little Reservoir (LT), Beaver County	38.250 N, 112.480 W	7324 – 7328	63718 – 63722	5	5	0
Main Creek (MN), Wasatch County	40.394 N, 111.442 W	8042 – 8046	63798 – 63802	5	5	1
Tropic Reservoir (TP), Garfield County	37.580 N, 112.250 W	7284 – 7288	63705 – 63709	5	5	1

WASHINGTON						
North Fork Palouse River (PL), Whitman County	46.920 N, 117.339 W	8551 – 8555	63622 – 63626	5	5	0
Yakima River (YK), Yakama County	46.417 N, 120.333 W	7354-6, 7358	63583-5, 63587	4	4	0
WYOMING						
LaChappelle Creek (LC), Uinta County	41.127 N, 110.787 W	8078-9, 8081	63810-1, 63813	3	3	0

Table 2.3: Pairwise comparisons of average percent sequence divergence among species of the *Mylocheilus* sub-clade of North American cyprinids (above diagonal). Percent sequence divergence per million years for cyt *b* between the same species (below diagonal).

	C. elongatus	*C. funduloides*	*I. phlegethontis*	*M. caurinus*	*P. macrolepidotus*	*R. balteatus*	*R. egregius*
C. elongatus	–	4.4	10.4	12.0	14.1	9.2	9.7
C. funduloides	1.7	–	9.9	12.4	13.7	8.5	9.0
I. phlegethontis	1.8	1.7	–	13.7	15.1	8.2	9.0
M. caurinus	1.8	1.8	2.0	–	9.3	12.1	12.1
P. macrolepidotus	1.8	2.0	2.2	2.0	–	13.5	13.9
R. balteatus	1.6	1.5	2.2	1. 8	2.0	–	3.9
R. egregius	1.7	1.6	2.4	1.8	2.0	1.4	–

Figure 2.1: Map depicting the natural distributions of the seven species belonging to the *Mylocheilus* sub-clade of North American cyprinids. Two species, *M. caurinus* and *R. balteatus*, overlap through much of their ranges, so that of *M. caurinus* is outlined with a dashed line to show the difference.

Figure 2.2: A detailed distribution map of the native ranges of *R. balteatus* and *R. egregius*. The range for *R. balteatus* is shown in grey, with a dashed line approximating the boundaries between *R. b. hydrophlox* and *R. b. balteatus*. *Richardsonius balteatus hydrophlox* is in the southeast portion of the range with a disjunct distribution in southeast Oregon (surrounded by the dashed line), whereas *R. b. balteatus* occurs in the northwest portion of the range. The native range for *R. egregius* is not shaded. Circles mark sampling localities used in this study, and are color-coded according to clade (see Figure 2.5). Circles with multiple colors represent populations that contain haplotypes from more than one clade.

Figure 2.3: Phylogram depicting relationships of North American cyprinids based on cyt *b* sequences available from GenBank. Asterisks mark nodes that are well supported in maximum parsimony, maximum likelihood and Bayesian analyses (Bootstraps > 70; Posterior Probabilities > 0.95).

Figure 2.4: Phylogenetic relationships of the *Mylocheilus* sub-clade of Mayden's Open Posterior Myodome Clade of North American cyprinids (Mayden, 1989; Simons et al., 2003) based on analyses of the cyt *b* data set obtained from GenBank. Numbers above branches represent MP and ML bootstrap values, respectively, whereas numbers below branches represent posterior probability values. The grey box on the phylogeny on the

left illustrates the position of the *Mylocheilus* sub-clade relative to the other North American cyprinids included in the family-level analyses (see Figure 2.3 for the family-level phylogeny in its entirety).

Figure 2.5: Maximum likelihood tree showing the relationships between *Clinostomus*, *Iotichthys,* and *Richardsonius* based on mtDNA sequence data. Numbers above nodes represent MP and ML bootstrap values, respectively, whereas numbers below nodes represent posterior probabilities. *Richardsonius balteatus* is subdivided into three major clades. Individuals in those clades occur in the Bonneville Basin/upper Snake River drainage (B), along the Pacific Coast (Co), and further inland in the upper Columbia River drainage (Ci). *Richardsonius egregius* is subdivided into Eastern (E) and Western (W) clades. Colored bars for each of the clades are so that clades can be cross referenced with sampling locality (see Figure 2.2).

Figure 2.6: Phylogenetic tree reconstructed from DNA sequence data of the first nuclear intron of the S7 ribosomal gene (S7). Numbers above nodes represent MP and ML bootstrap values, respectively, whereas numbers below nodes represent posterior probabilities. Terminal taxa are labeled with the species name, followed by parentheses containing the population abbreviation and LVT number for each individual as given in Table 2.2. The letters ‘a’ and ‘b’ designate the sequences of heterozygous individuals.

Figure 2.7: Phylogeny showing estimates of divergence times between species in the genera *Clinostomus, Iotichthys,* and *Richardsonius*. Numbers above nodes represent divergence time estimates (in millions of years) based on mtDNA sequence data, and are surrounded by grey bars representing 95% confidence intervals surrounding the mean.

Numbers below nodes represent divergence time estimates based on nuDNA sequence data, and are surrounded by white bars representing 95% confidence intervals. Black circles represent calibration points from fossil data, with C_1 and C_2 referring to 7.0 Ma and 3.5 Ma fossils of *Mylocheilus* and *Richardsonius*, respectively.

Figure 2.1

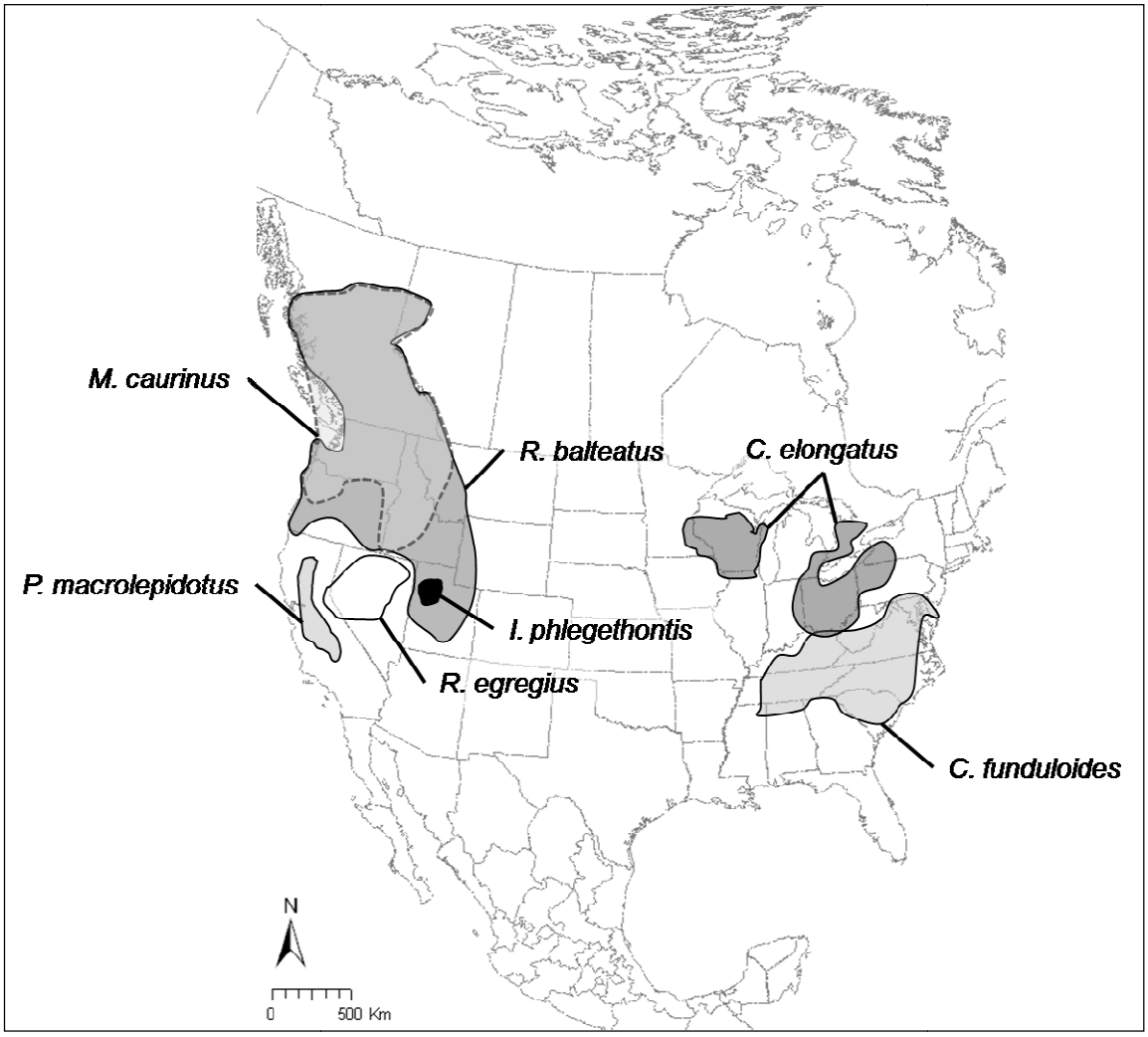

M. caurinus
R. balteatus
C. elongatus
P. macrolepidotus
I. phlegethontis
R. egregius
C. funduloides
N
0
500 Km

Figure 2.2

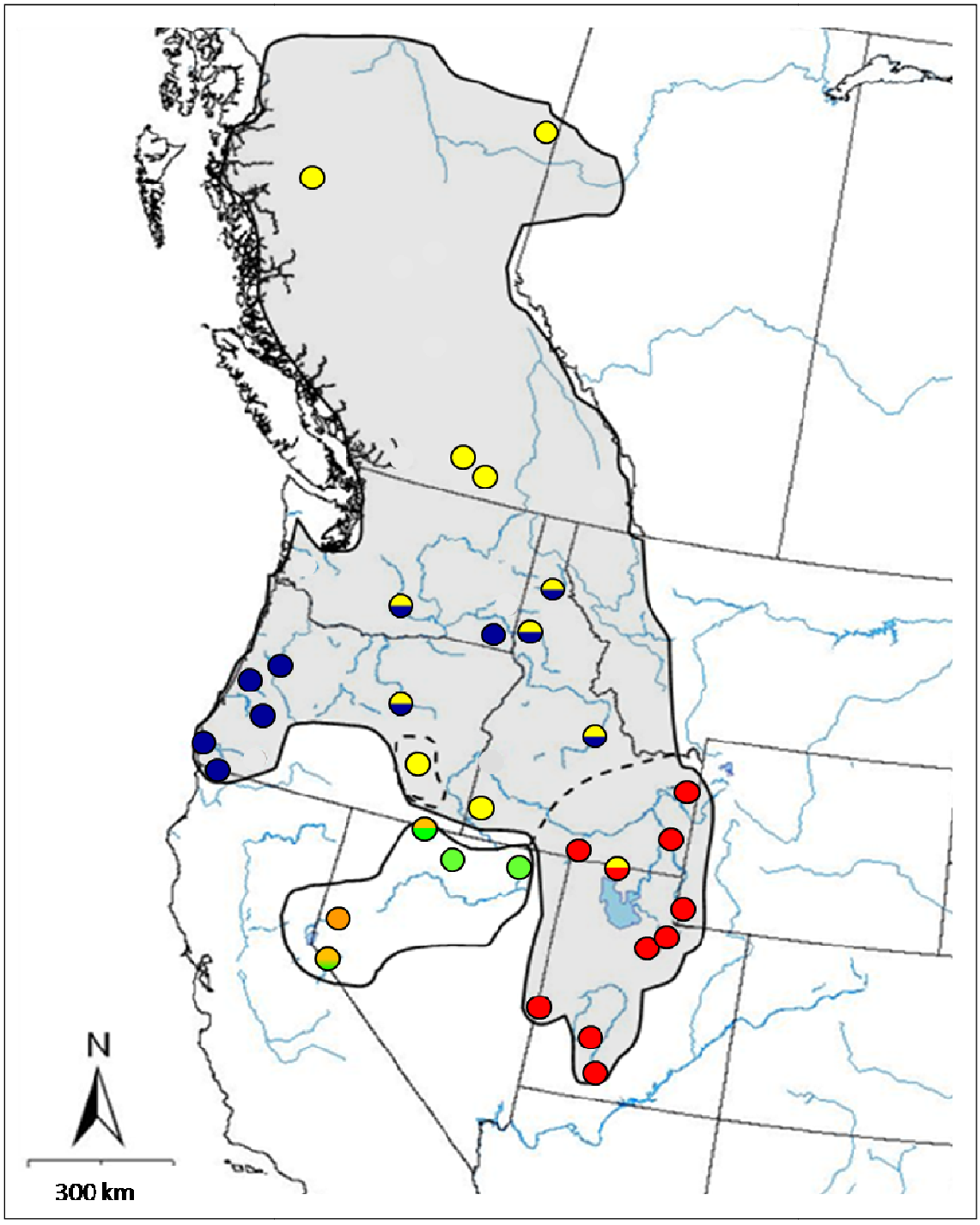
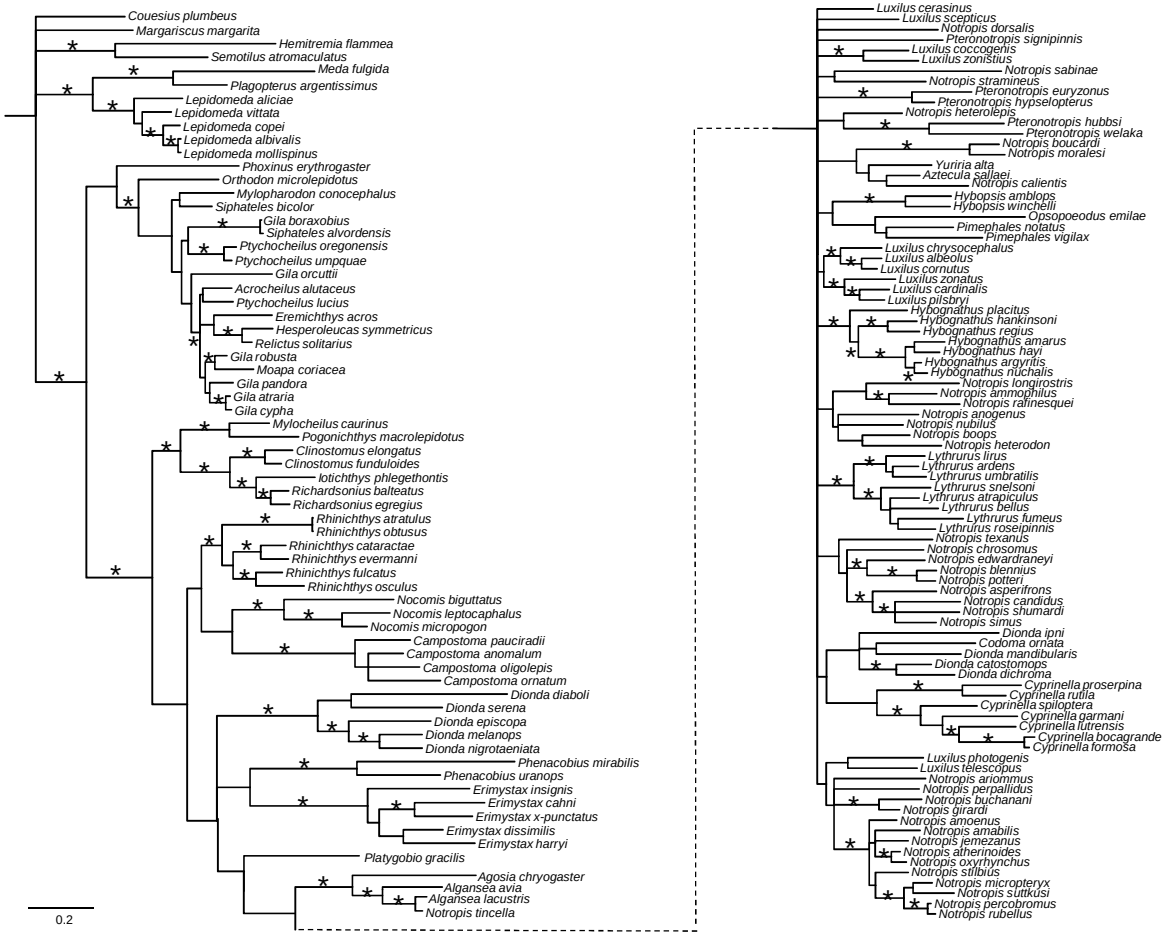


Figure 2.3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Margariscus margarita
Hemitremia flammea
Semotilus atromaculatus
Meda fulgida
Plagopterus argentissimus
Lepidomeda aliciae
Lepidomeda vittata
Lepidomeda copei
Lepidomeda albivalis
Lepidomeda mollispinus
Phoxinus erythrogaster
Orthodon microlepidotus
Mylopharodon conocephalus
Siphateles bicolor
Gila boraxobius
Siphateles alvordensis
Ptychocheilus oregonensis
Ptychocheilus umpquae
Gila orcuttii
Acrocheilus alutaceus
Ptychocheilus lucius
Eremichthys acros
Hesperoleucas symmetricus
Relictus solitarius
Gila robusta
Moapa coriacea
Gila pandora
Gila atraria
Gila cypha
Mylocheilus caurinus
Pogonichthys macrolepidotus
Clinostomus elongatus
Clinostomus funduloides
Iotichthys phlegethontis
Richardsonius balteatus
Richardsonius egregius
Rhinichthys atratulus
Rhinichthys obtusus
Rhinichthys cataractae
Rhinichthys evermanni
Rhinichthys fulcatus
Rhinichthys osculus
Nocomis biguttatus
Nocomis leptocaphalus
Nocomis micropogon
Campostoma pauciradii
Campostoma anomalum
Campostoma oligolepis
Campostoma ornatum
Dionda diaboli
Dionda serena
Dionda episcopa
Dionda melanops
Dionda nigrotaeniata
Phenacobius mirabilis
Phenacobius uranops
Erimystax insignis
Erimystax cahni
Erimystax x-punctatus
Erimystax dissimilis
Erimystax harryi
Platygobio gracilis
Agosia chryogaster
Algansea avia
Algansea lacustris
Notropis tincella
0.2
Luxilus cerasinus
Luxilus scepticus
Notropis dorsalis
Pteronotropis signipinnis
Luxilus coccogenis
Luxilus zonistius
Notropis sabinae
Notropis stramineus
Pteronotropis euryzonus
Pteronotropis hypselopterus
Notropis heterolepis
Pteronotropis hubbsi
Pteronotropis welaka
Notropis boucardi
Notropis moralesi
Yuriria alta
Aztecula sallaei
Notropis calientis
Hybopsis amblops
Hybopsis winchelli
Opsopoeodus emilae
Pimephales notatus
Pimephales vigilax
Luxilus chrysocephalus
Luxilus albeolus
Luxilus cornutus
Luxilus zonatus
Luxilus cardinalis
Luxilus pilsbryi
Hybognathus placitus
Hybognathus hankinsoni
Hybognathus regius
Hybognathus amarus
Hybognathus hayi
Hybognathus argyritis
Hybognathus nuchalis
Notropis longirostris
Notropis ammophilus
Notropis rafinesquei
Notropis anogenus
Notropis nubilus
Notropis boops
Notropis heterodon
Lythrurus lirus
Lythrurus ardens
Lythrurus umbratilis
Lythrurus snelsoni
Lythrurus atrapiculus
Lythrurus bellus
Lythrurus fumeus
Lythrurus roseipinnis
Notropis texanus
Notropis chrosomus
Notropis edwardraneyi
Notropis blennius
Notropis potteri
Notropis asperifrons
Notropis candidus
Notropis shumardi
Notropis simus
Dionda ipni
Codoma ornata
Dionda mandibularis
Dionda catostomops
Dionda dichroma
Cyprinella proserpina
Cyprinella rutila
Cyprinella spiloptera
Cyprinella garmani
Cyprinella lutrensis
Cyprinella bocagrande
Cyprinella formosa
Luxilus photogenis
Luxilus telescopus
Notropis ariommus
Notropis perpallidus
Notropis buchanani
Notropis girardi
Notropis amoenus
Notropis amabilis
Notropis jemezanus
Notropis atherinoides
Notropis oxyrhynchus
Notropis stilbius
Notropis micropteryx
Notropis suttkusi
Notropis percobromus
Notropis rubellus

Figure 2.4

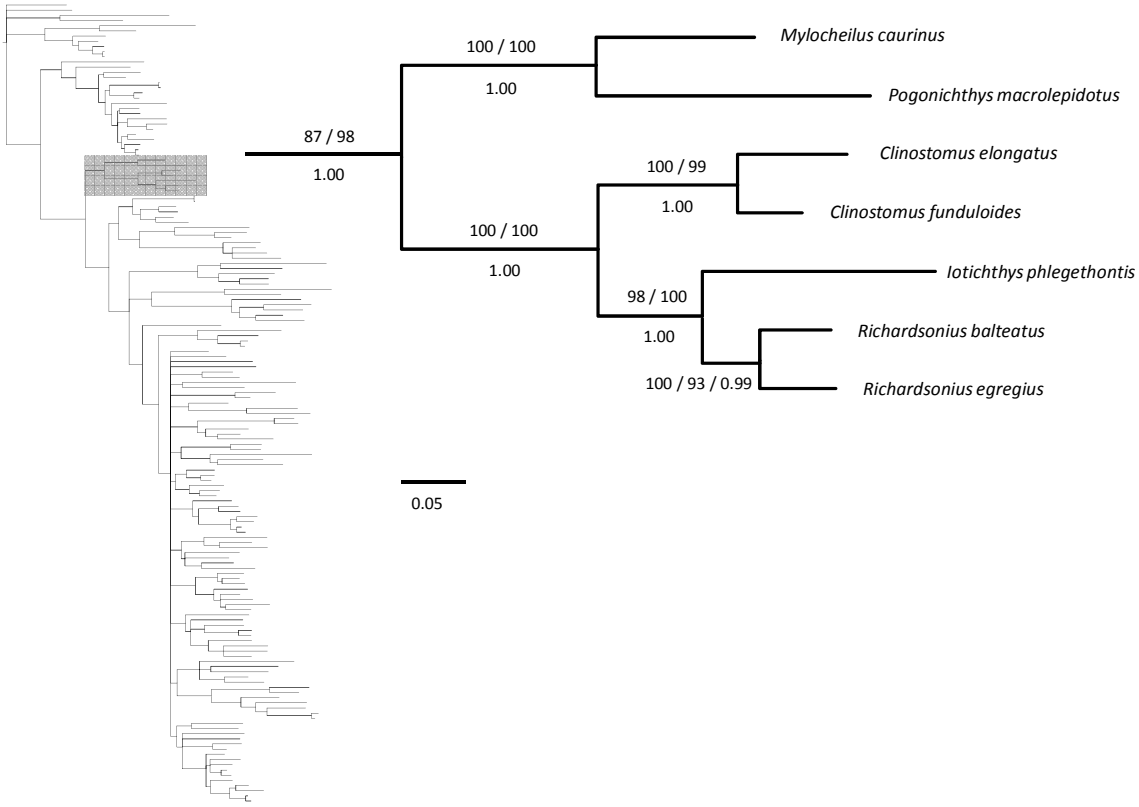

100 / 100
1.00
Mylocheilus caurinus
Pogonichthys macrolepidotus
87 / 98
1.00
100 / 99
1.00
Clinostomus elongatus
Clinostomus funduloides
100 / 100
1.00
Iotichthys phlegethontis
98 / 100
1.00
Richardsonius balteatus
100 / 93 / 0.99
Richardsonius egregius
0.05

Figure 2.5

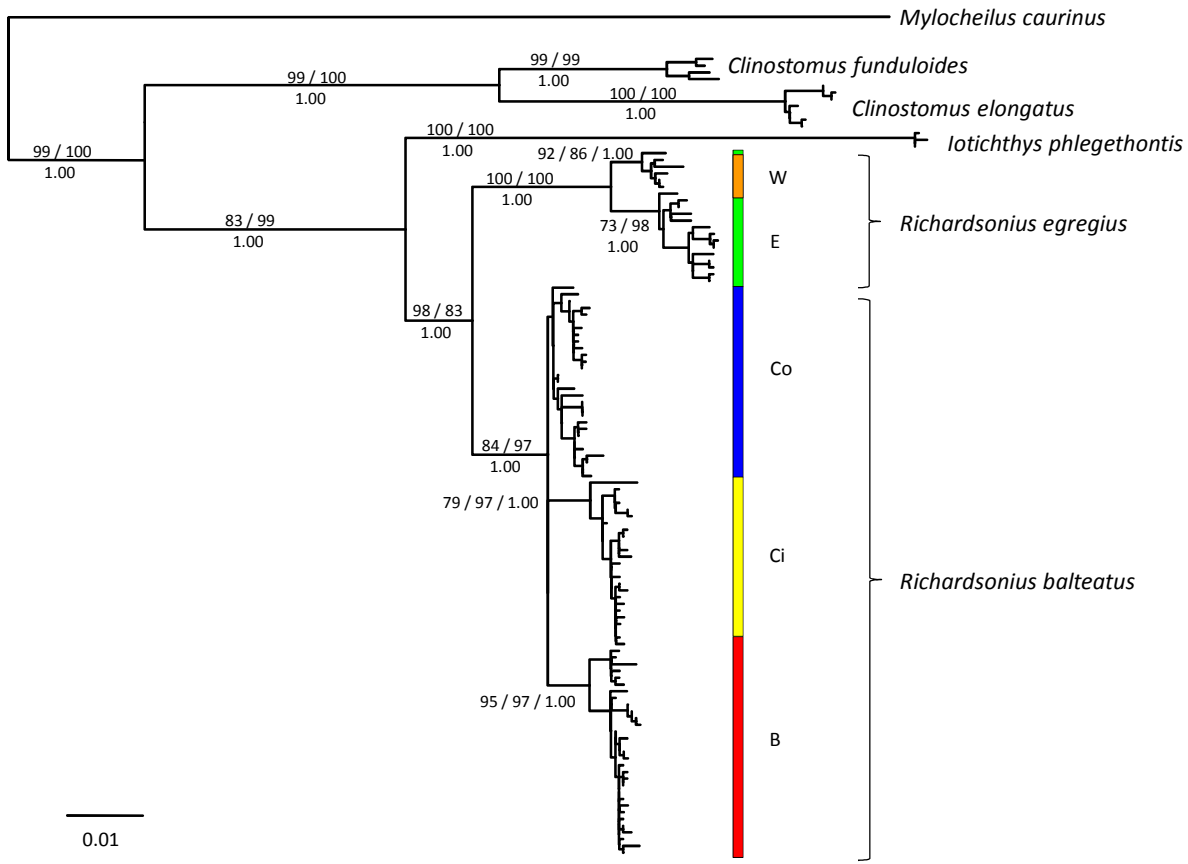

Mylocheilus caurinus
99 / 99
1.00
Clinostomus funduloides
99 / 100
1.00
100 / 100
1.00
Clinostomus elongatus
100 / 100
1.00
Iotichthys phlegethontis
99 / 100
1.00
92 / 86 / 1.00
100 / 100
1.00
W
73 / 98
1.00
E
Richardsonius egregius
83 / 99
1.00
98 / 83
1.00
Co
84 / 97
1.00
79 / 97 / 1.00
Ci
Richardsonius balteatus
95 / 97 / 1.00
B
0.01

Figure 2.6

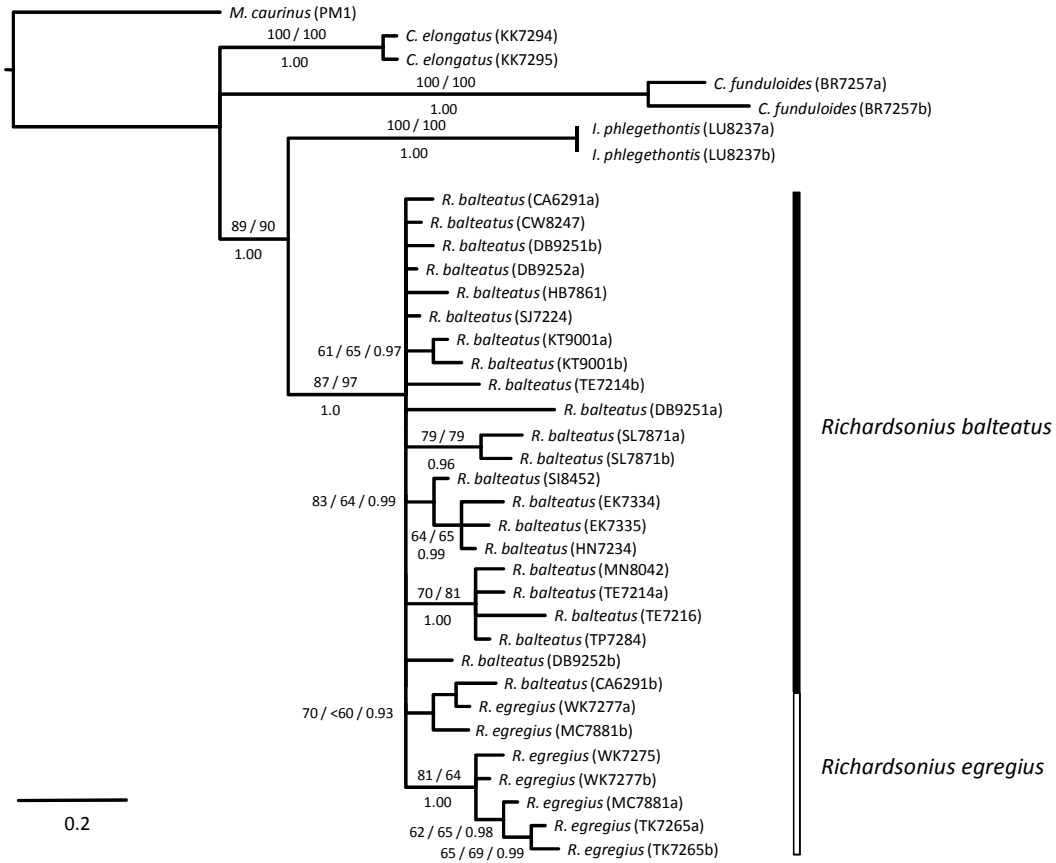
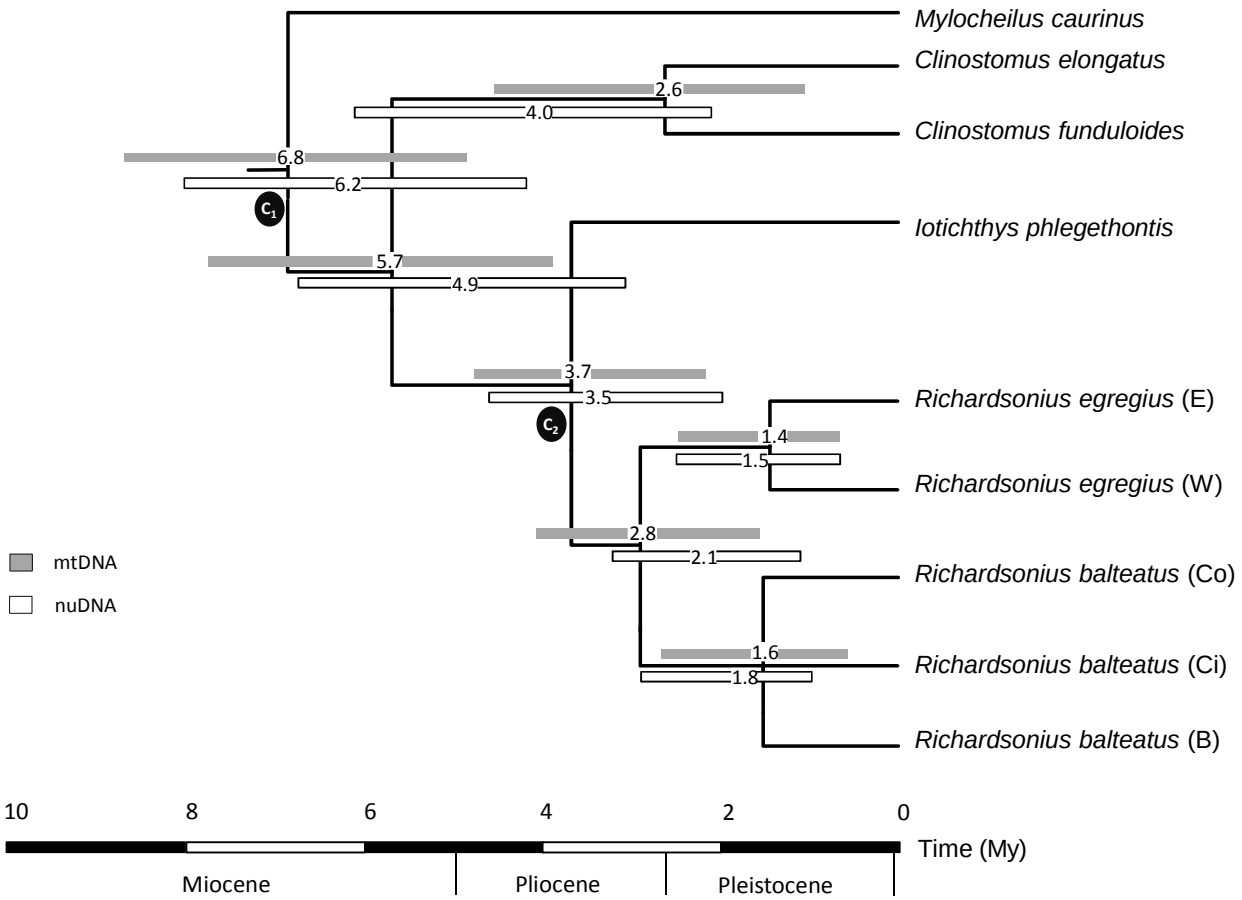

M. caurinus (PM1)
100 / 100
1.00
C. elongatus (KK7294)
C. elongatus (KK7295)
100 / 100
1.00
C. funduloides (BR7257a)
C. funduloides (BR7257b)
100 / 100
1.00
I. phlegethontis (LU8237a)
I. phlegethontis (LU8237b)
89 / 90
1.00
R. balteatus (CA6291a)
R. balteatus (CW8247)
R. balteatus (DB9251b)
R. balteatus (DB9252a)
R. balteatus (HB7861)
R. balteatus (SJ7224)
61 / 65 / 0.97
R. balteatus (KT9001a)
R. balteatus (KT9001b)
87 / 97
1.0
R. balteatus (TE7214b)
R. balteatus (DB9251a)
79 / 79
0.96
R. balteatus (SL7871a)
R. balteatus (SL7871b)
83 / 64 / 0.99
R. balteatus (SI8452)
R. balteatus (EK7334)
64 / 65
0.99
R. balteatus (EK7335)
R. balteatus (HN7234)
70 / 81
1.00
R. balteatus (MN8042)
R. balteatus (TE7214a)
R. balteatus (TE7216)
R. balteatus (TP7284)
R. balteatus (DB9252b)
70 / <60 / 0.93
R. balteatus (CA6291b)
R. egregius (WK7277a)
R. egregius (MC7881b)
81 / 64
1.00
R. egregius (WK7275)
R. egregius (WK7277b)
R. egregius (MC7881a)
62 / 65 / 0.98
R. egregius (TK7265a)
65 / 69 / 0.99
R. egregius (TK7265b)
0.2
Richardsonius balteatus
Richardsonius egregius

Figure 2.7


Mylocheilus caurinus
Clinostomus elongatus
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4.0
Clinostomus funduloides
6.8
6.2
C1
Iotichthys phlegethontis
5.7
4.9
3.7
3.5
C2
Richardsonius egregius (E)
1.4
1.5
Richardsonius egregius (W)
2.8
2.1
Richardsonius balteatus (Co)
1.6
1.8
Richardsonius balteatus (Ci)
Richardsonius balteatus (B)
mtDNA
nuDNA
10
8
6
4
2
0
Time (My)
Miocene
Pliocene
Pleistocene

CHAPTER 3

INVESTIGATING THE ROLE OF PLUVIAL LAKE LAHONTAN ON INTRA-SPECIFIC GENETIC VARIATION OF THE LAHONTAN REDSIDE SHINER *RICHARDSONIUS EGREGIUS*

Introduction

Molecular biogeography and phylogeography have been used in recent years to successfully address questions about the processes responsible for shaping genetic diversity of many taxa (Riddle et al., 2008; Avise, 2009; Mantooth and Riddle, *in press*). The use of molecular techniques has allowed researchers to evaluate patterns of genetic diversity resulting from vicariance and dispersal, and from population demographic changes (such as range expansions or contractions), and will continue to do so as molecular data continue to become easier and less expensive to generate.

Geologic and climatic processes have contributed to the alteration of the western North American landscape throughout the Cenozoic. Processes such as plate tectonics, volcanism, block faulting, and glaciations have shaped drainage patterns in western North America, and thus have influenced genetic structure of numerous aquatic taxa (e.g., Hershler and Sada, 2002; Smith et al., 2002; Hershler et al., 2007; Spencer et al., 2008). Episodes of vicariance, caused by disruption of aquatic connections, have led to genetic divergence among species and genera (e.g., Smith et al., 2002; Liu et al., 2003; Houston et al., *in press*), but dispersal has also affected genetic structuring within and among drainages (e.g., Kauwe et al., 2004; Liu and Hershler, 2007; Boizard et al., 2009). Given the complex history of the western North American landscape and its influence on the genetic diversification of many organisms, it is likely that additional aquatic taxa will

exhibit phylogeographic structure that is consistent with changes to the landscape that have alterred drainage patterns.

The Lahontan Basin is an endorheic basin (i.e., a closed drainage with no outlet to the ocean) located in the western United States. It is part of the western Great Basin (Figure 3.1), which formed as a result of uplift and extension of the earth's crust beginning in the Eocene, continuing sporadically through the Miocene, and slowing thereafter but continuing to undergo extension through present time (Stokes, 1988; Sonder and Jones, 1999; Horton et al., 2004). During its formation, geologic processes (such as block faulting) helped to divide the Lahontan Basin into numerous sub-basins. Geologic and biogeographic evidence suggests that the Lahontan Basin has shared aquatic connections with surrounding drainages (e.g., Bonneville Basin, Columbia River, Snake River, etc.) at various times throughout the Neogene (Miller, 1958; Taylor and Smith, 1981; Taylor, 1985; Minckley et al., 1986; Repenning et al., 1995; Link et al., 2002; Hershler and Liu, 2004; Link et al., 2005; Beranek et al., 2006), but the Lahontan Basin appears to have remained isolated from surrounding drainages since the end Pliocene/early Pleistocene (Russell, 1885; Morrison, 1965; Minckley et al., 1986; Repenning et al., 1995; Reheis and Morrison, 1997; Smith et al., 2002).

During the Pleistocene, pluvial lakes formed in valleys within the Lahontan Basin; the largest of these lakes was Lake Lahontan (Figure 3.1). Fluctuating water levels in Lake Lahontan established aquatic connections between various sub-basins at different times, but all lakes were contained within the Lahontan Basin (Morrison, 1965; Reheis and Morrison, 1997; Negrini, 2002; Reheis et al., 2002). Connections between sub-basins during lake high stands may have provided dispersal corridors for aquatic taxa

within the Lahontan Basin during the Pleistocene (Reheis, 1999), and appears to have done so for at least some western Great Basin aquatic taxa (Chen et al., 2007; 2009). However, water levels dropped with the onset of Holocene desiccation, leaving most of the former tributaries to Lake Lahontan isolated from one another (Negrini, 2002; Adams et al., 2008).

Aquatic taxa are often considered to have low or restricted vagility because they are dependent on aquatic connections for dispersal (e.g., Bernatchez and Wilson, 1998; Boizard et al., 2009; but see Figuerola and Green, 2002; Hershler et al., 2005; Frisch et al., 2007; Liu et al., 2007 for recent examples of dispersal of aquatic mollusks via attachment to waterfowl). Within these geographic constraints, many aquatic species within the Lahontan Basin would be predicted to have been on unique evolutionary trajectories (at least) since the basin became isolated from surrounding areas. Moreover, division of the Lahontan Basin into sub-basins may have provided a mechanism for genetic divergence within the basin as a result of vicariance. Many narrowly distributed aquatic species and sub-species within the Lahontan Basin are consistent with such a scenario. For example, many species of freshwater snails are restricted to single spring systems within the Lahontan Basin (Taylor, 1985; Liu et al., 2003; Liu and Hershler, 2005; Hershler et al., 2007), whereas some freshwater fishes have unique lineages that are restricted to small drainages and springs within the basin (e.g., speckled dace *Rhinichthys osculus* (Girard) - 11 lineages; tui chub *Siphateles bicolor* (Girard) - 6 lineages). The evolution of these various lineages is believed to have been driven largely by late Paleogene geologic activity within the Lahontan Basin (Taylor, 1985; Oakey et al., 2004; Liu and Hershler, 2005).

Some widespread fish species within the Lahontan Basin are not subdivided into narrowly distributed subspecies within the basin. These widespread species may have arrived in the Lahontan Basin prior to its complete isolation from surrounding drainages, and subsequently dispersed throughout the basin as aquatic connections made such movement possible. Alternatively, these species may exhibit yet uncovered cryptic genetic diversity. Many of these widespread fish species occur not only in the Lahontan Basin, but in its surrounding drainages as well (Sigler and Sigler, 1987). Examples include cutthroat trout *Oncorhynchus clarkii* Richardson (although the subspecies Lahontan cutthroat trout *O. c. henshawi*, is solely distributed throughout the Lahontan Basin; Behnke, 1992), mountain whitefish *Prosopium williamsoni* (Girard), and Paiute sculpin *Cottus beldingii* Eigenmann and Eigenmann. The only two widespread endemic fish species in the Lahontan Basin are Lahontan redside shiner *Richardsonius egregius* (Girard) and Tahoe sucker *Catostomus tahoensis* (Gill and Jordan) (Smith et al., 2002). In this study I investigate phylogeographic structure within *R. egregius*.

Richardsonius egregius occurs in aquatic habitats ranging from small streams to large rivers and lakes, and is found in most watersheds within the Lahontan Basin (Lee et al., 1980; Figure 3.2). The species is no longer found in some headwater regions where it once occurred (LaRivers, 1994). Since *R. egregius* is widespread throughout the basin, a testable hypothesis is that the species will exhibit a relatively shallow phylogenetic structure that is consistent with a panmictic Lake Lahontan population in the Late Pleistocene (when pluvial lakes provided aquatic connections between various sub-basins) that later experienced simultaneous isolation of populations when habitats became fragmented as a result of Holocene desiccation (Figure 3.3a). Alternatively, populations

of *R. egregius* may have experienced sequential isolation (as opposed to simultaneous isolation) as habitats became more fragmented, in which case the phylogenetic structure within the species would still be shallow, but loss of alleles through genetic drift could provide phylogenetic structure among populations if those populations experienced genetic bottlenecks (Figure 3.3b). Yet another testable hypothesis is that *R. egregius* could exhibit deeper phylogenetic structuring (Figure 3.3b) that is more consistent with ancient geological changes to the landscape if pluvial lakes were of minimal importance in the dispersal of aquatic taxa as suggested by some researchers (e.g., Taylor and Smith, 1981; Taylor and Bright, 1987). The expected difference between the last two scenarios (Figure 3.3b) is the time since genetic divergence occurred (i.e., if pre-Pleistocene events had the most influence on the genetic structure of *R. egregius* populations, divergence times would be much earlier than if late-Pleistocene events had the most influence). Here, I use a molecular genetic approach to differentiate between these alternative hypotheses. Specifically, I use mitochondrial DNA (mtDNA) to investigate phylogeographic patterns within *R. egregius* at various levels. First, I generated a molecular phylogeny and used fossil data and a molecular clock approach to estimate divergence times among clades. Second, I evaluated genetic diversity within and among populations by estimating population genetic parameters. Finally, I used a coalescent approach to estimate population demographic parameters and to determine the likelihood of contemporary gene flow between populations.

Materials and Methods

I sampled specimens of *R. egregius* from five naturally occurring populations throughout the Lahontan Basin in northern Nevada using either a beach seine or a backpack electroshocker. Sampling localities were McDermitt Creek (MC), North Fork Little Humboldt River (LH), T Creek, (T), Truckee River (TK), and West Walker River (WK), and are shown in Figure 3.2 with additional details given in Table 3.1. I euthanized individuals by administering an overdose of tricaine methanesulfonate (MS-222), and placed them into 95.0% ethanol to preserve tissues. I deposited the specimens into the fish collection at the Monte L. Bean Life Science Museum (MLBM) at Brigham Young University to be housed as voucher specimens (Table 3.1). Tissue samples are stored in the Las Vegas Tissue (LVT) collection at the University of Nevada, Las Vegas (Table 3.1).

I extracted whole genomic DNA from muscle tissues using the Qiagen DNeasy tissue kit. I sequenced the cytochrome *b* (cyt *b*) mtDNA gene because it is one of the most rapidly evolving mtDNA markers in freshwater fishes (Broughton and Reneau, 2006) and thus should be informative for detecting phylogenetic structure resulting from late Paleogene or Quaternary events. I amplified cyt *b* via the polymerase chain reaction (PCR) using the primers HA-a and LA-a (Dowling and Naylor, 1997). The PCR cocktails had a total reaction volume of 12.5 µl, and consisted of DNA template (100 ng), nuclease free water (2.25 µl), RNA primers (10 pmoles each), and Promega GoTaq green master mix (6.25 µl). I used the following thermal profile for PCR: an initial denaturation at 95.0° C for 4 minutes was followed by 35 cycles of denaturation at 95.0° C for 30 seconds, annealing at 50.0° C for 30 seconds, and extension at 72.0° C for 90

seconds, followed by a final extension at 72.0° C for 7 minutes and a rapid cool down to 4.0° C. I determined success of DNA extractions and PCR amplifications by visualizing the products under ultraviolet radiation following electrophoresis through a 0.8% agarose gel. I purified PCR products using the Qiagen QIAquick PCR purification kit.

I performed cycle sequencing reactions on the purified PCR products using the ABI Big Dye Terminator protocol (Applied Biosystems, Foster City, CA). Reaction cocktails were 20 µl in volume, and consisted of purified PCR product (100 ng), nuclease free water (12.2 µl), 2.5X Tris buffer (3.2 µl), 25 mM $MgCl_2$ (0.8 µl), RNA primer (6 pmoles), and dye terminator reaction mix (0.5 µl). I used the same primers for sequencing as I did for PCR amplification, and sequenced both the heavy and light strands for each individual. I used the following thermal profile for cycle sequencing: 25 cycles of denaturation at 95.0° C for 20 seconds, annealing at 50.0° C for 15 seconds, and extension at 60.0° C for 60 seconds, followed by a rapid cool down to 4.0° C. I removed excess dye terminator reaction mix from the cycle sequencing reaction products using Centrisep spin columns. I performed all sequencing on an ABI 3130 automated sequencer.

I sequenced cyt *b* for 51 individuals, including 45 *R. egregius* and six individuals representing outgroup taxa. I aligned DNA sequences using the automatic assembly function in Sequencher v. 4.8 (Gene Codes Corporation, 2007). As a reference for alignment, I used a cyt *b* sequence from the sister species to *R. egregius*, the redside shiner *Richardsonius balteatus* (Richardson) (GenBank accession number AY096011). Because cyt *b* is a protein-coding gene I also used amino acid sequence as a reference in

aligning the DNA sequences. I visually inspected chromatograms and made corrections to the sequences manually. There were no gaps in the final alignment.

I generated phylogenies using maximum parsimony (MP) and maximum likelihood (ML) optimality criteria, and Bayesian inference methods. I used PAUP* (Swofford, 2002) to generate MP trees, employing a heuristic search with ten random addition replicates and TBR branch swapping. I generated estimates of nodal support by performing 1,000 bootstrap pseudo-replicates (Felsenstein, 1985). I used Modeltest (Posada and Crandall, 1998) to select the appropriate model of sequence evolution, and generated ML phylogenies using TreeFinder (Jobb, 2005), performing 1,000 bootstrap replicates to estimate nodal support. For Bayesian analysis, I partitioned the data by codon position and used MrModeltest v. 2.2 (Nylander, 2004) to select the model of sequence evolution for each partition. I employed a Markov Chain Monte Carlo (MCMC) approach with one cold chain and three heated chains. To get suitable amounts of switching between chains, I lowered the temperature setting from the default setting of T=0.20 to T=0.07. I ran Bayesian analysis for 4,000,000 generations, sampling every 100 generations. I discarded the first 1,000,000 generations (25%) as burn-in, and used the majority rule consensus of the remaining topologies for posterior probabilities. I used *R. balteatus*, least chub *Iotichthys phlegethontis* (Cope), redside dace *Clinostomus elongatus* (Kirtland), rosyside dace *Clinostomus funduloides* Girard, and peamouth *Mylocheilus caurinus* (Richardson) as outgroup taxa because they are hypothesized to be the closest living relatives of *R. egregius* (Simons et al., 2003; Estabrook et al., 2007). I rooted phylogenies to *M. caurinus* in all analyses because of the outgroup taxa used in this study *M. caurinus* is the most distant relative to *R. egregius*.

I estimated divergence times between clades on the phylogeny using a Bayesian approach, as implemented using the uncorrelated lognormal relaxed clock model in the program BEAST (Drummond et al., 2006; Drummond and Rambaut, 2007). I used jModeltest (Posada, 2008), as implemented in PhyML (Guindon and Gascuel, 2003), to select the appropriate model of sequence evolution, and based on those results used the GTR + I + G substitution model in BEAST. I set priors for model parameters (e.g., GTR substitutions, gamma, proportion of invariant sites) using the jModeltest output, and used the Yule process model of speciation set a prior on the tree. I calibrated the tree using fossil dates for *Mylocheilus* and *Richardsonius*. I established a minimum age for the basal node of the tree (i.e., the node representing the most recent common ancestor of *Mylocheilus* and *Richardsonius*) using a 7 Ma *Mylocheilus* fossil recovered from the Chalk Hills Formation, Idaho (Dowling et al., 2002; Smith et al., 2002). The earliest known *Richardsonius* fossil, *Richardsonius durranti* (now extinct), was recovered from the Pliocene Glenns Ferry Formation in southwest Idaho (Smith, 1975). The Glenns Ferry Formation was deposited approximately 3.5 Ma (Neville et al., 1979; Kimmel, 1982), so I conservatively used this estimate as the minimum age for the node representing the common ancestor of *Richardsonius* and its sister genus, *Iotichthys*. I ran the MCMC chain for 40,000,000 steps in BEAST, sampling every 1000 steps. The first 4,000,000 steps (10%) were discarded as burn-in. To increase effective sample sizes (ESS) to adequate values (i.e., ESS values above 200), I performed the BEAST analysis a second time and combined the output from both runs.

To evaluate genetic diversity within and among populations, I obtained population parameter estimates of haplotype diversity (Hd) and nucleotide diversity (π) for each

population using the program DNAsp (Rozas et al., 2003). I used Arlequin v. 3.11 (Excoffier et al., 2005) to calculate average percent sequence divergence among populations, Wright's F_{ST} statistics (Wright, 1921; 1978), and Fu's Fs (Fu, 1997) and Tajima's D (Tajima, 1989) neutrality statistics for each population. Significant values of Fu's Fs and Tajima's D are indicative of selection acting on a locus, or, in the case of mtDNA (which exhibits neutral or near neutral rates of sequence evolution), of demographic histories of a population. Significantly negative values are often used to infer rapid population expansion, whereas significantly positive values are used to identify populations that have experienced recent bottlenecks. I also performed analysis of molecular variance (AMOVA), as implemented in Arlequin v. 3.11, to explore genetic variation at hierarchical geographic levels, including grouping samples by region (i.e., eastern vs. western Lahontan Basin, as dictated by clades on the phylogeny), and by populations within regions (i.e., sampling localities).

I performed coalescent analyses based on the isolation with migration model, as implemented in the software program IM (Hey and Nielsen, 2004), to determine whether recent migration between eastern and western Lahontan Basin populations has occurred. I performed preliminary analyses using default values for priors (see Hey and Nielson, 2004; Hey, 2007a; Hey, 2007b), and adjusted each value in subsequent runs based on those preliminary results. I used the following parameter values as priors for the final IM analyses: upper bounds on priors for theta of eastern (θ_1) and western (θ_2) populations were set at 80, upper bounds on priors for migration for eastern (m_1) and western (m_2) Lahontan populations were set at 5, and the upper bound on the prior for divergence time (*t*) was set at 8. I used 20 chains and ran the analysis for 30,000,000 generations,

discarding the first 500,000 generations as burn-in. To ensure convergence on the same values, I performed three separate runs using a different random number seed for each one. Each of the three runs produced near identical parameter values, so I used the means of those values to calculate effective population sizes (N_1 and N_2 for eastern and western populations, respectively), and migration rates between populations (m_1 and m_2). I performed calculations using a mutation rate of 1.4% sequence divergence per million years for cyt *b* (see Chapter 2), and a generation time of 2.5 years (Lee et al., 1980).

Results

DNA sequencing yielded 1,140 base pairs of the cyt *b* gene, of which 932 were constant and 135 were parsimony informative characters. There were 29 haplotypes among all populations of *R. egregius*. All DNA sequences will be deposited in the GenBank online data base upon publication of this work. The model selected by Modeltest for ML analysis (and by jModeltest for Bayesian analyses in BEAST) was GTR + I + G under the Akaike Information Criterion (AIC). For partitioned Bayesian inference I selected the K80 model for the first codon position, F81 model for the second codon position, and GTR + G for the third codon position based on the AIC output of MrModeltest.

The results of MP, ML and Bayesian analyses yielded the same phylogeny, albeit with varying levels of nodal support for some clades (Figure 3.4). *Richardsonius egregius* appears to be a monophyletic species and is divided into two major clades: one clade primarily consists of individuals from the eastern Lahontan Basin populations (i.e., North Fork Little Humboldt River, McDermitt Creek, and T Creek; individuals from

these populations are labelled on the phylogeny as LH, MC, and T, respectively), and the other is comprised of individuals from western Lahontan Basin populations (i.e., Truckee River and Walker River; individuals from these populations are labelled on the phylogeny as TK and WK, respectively). Two individuals carried haplotypes that were not consistent with their geographic locations. One individual from a western locality (WK 7275) carried an eastern haplotype that branched from the eastern clade's basal node. Similarly, an individual from an eastern population (MC 7883) carried a haplotype that branched from the western clade's basal node. There was little phylogenetic structure within the eastern and western Lahontan clades.

Divergence time estimates (Figure 3.5) show that the eastern and western Lahontan clades of *R. egregius* split from each other approximately 2.6 Ma. Mean divergence time estimates show that genetic diversification of the eastern and western Lahontan clades occurred approximately 2.0 and 1.8 Ma respectively. Confidence intervals around these mean divergence time estimates are wide (see Figure 3.5) because we used a single mtDNA marker in this study.

Haplotype diversity was high and nucleotide diversity was low in each of the populations included in this study (Table 3.2). Fu's Fs and Tajima's D values were not significant for any of the populations, although Fu's Fs was significant for all populations combined (Fs = -5.743, P = 0.002). Corrected average pairwise differences and F_{ST} values are presented in Table 3.3. F_{ST} values were significant in 8 of the 10 population comparisons; the only pairwise comparisons that were not significant were between the Truckee River and North Fork Little Humboldt River populations, and between the T Creek and McDermitt Creek populations. These three populations occur in the eastern

Lahontan Basin (Figure 3.2; Table 3.1). Corrected average pairwise differences among populations were generally higher between eastern and western Lahontan populations than between populations within the same geographic area. AMOVA results show that the highest proportion of genetic variation (61.85%) is explained by geographic structuring between groups (i.e., the eastern and western Lahontan Basin), with the second highest proportion explained by genetic structuring within populations (33.91%), and a small amount of the overall variation (4.24%) explained by populations within groups (i.e., sampling localities) (Table 3.4).

IM analyses estimated θ_1 to be 54.8, θ_2 to be 42.5, θ_A to be 30.9, both m_1 and m_2 to be 2.5×10^{-8}, and *t* to be 3.25. Estimated posterior distributions for these model parameters are shown in Figure 3.6. Demographic parameter estimates and 95% confidence intervals calculated using these estimates are listed in Table 3.5. Effective population sizes of eastern and western Lahontan Basin populations are estimated to be 687,000 and 533,000, respectively. Those estimates are larger than effective population size of the ancestral population, which was estimated to be 387,000, although confidence intervals surrounding all these estimates are large (see Table 3.5) because the estimates are based on a single marker. The probabilities of migration between eastern and western Lahontan Basin populations are low. The marginal curve for *t* did not return to zero (see Figure 3.6), which is common when sample sizes are small or the data are not able to identify the model (Hey, 2005; Hey, 2007a), thus the results for *t* were not reliable for these data. Therefore, I was not able to use IM to estimate divergence time between eastern and western Lahontan Basin populations with any degree of confidence.

Discussion

The topologies produced by these phylogenetic analyses indicate that *R. egregius* is a monophyletic species (see Figure 3.4). Monophyly of *R. egregius* is consistent with the fact that the species is endemic to the Lahontan Basin, and thus has been isolated for at least two million years (Minckley et al., 1986; Repenning et al., 1995; Reheis and Morrison, 1997; Smith et al., 2002). *Richardsonius egregius* does exhibit deep divergence between eastern and western Lahontan Basin populations, as evidenced by the two main clades on the phylogeny (Figure 3.4) and the divergence time estimates between them (Figure 3.5). Significant population pairwise F_{ST} values indicate high levels of population structure between eastern and western Lahontan Basin populations (Table 3.3), and AMOVA results show that the east/west split explains the majority of the genetic variation observed within the species (Table 3.4).

Divergence time estimates should be interpreted with caution, particularly when based on a single locus, because confidence intervals surrounding estimates tend to be large under such conditions (as they are in this study). Moreover, the use of fossil data can lead to erroneous results if nodes are not calibrated correctly (see Ho et al., 2008). Nevertheless, the results of BEAST analyses still provide important insight into the timing of diversification within *R. egregius*. The split between the eastern and western Lahontan clades of *R. egregius* occurred approximately 2.6 Ma, with a 95% confidence interval ranging from the late Miocene to mid Pleistocene (Figure 3.5). Even with such a wide confidence interval surrounding that divergence time estimate, the two clades clearly diverged prior to the last glacial maximum (18 ka), as indicated by the lower limit to the confidence interval being mid-Pleistocene in age (see Figure 3.5). The same is true

for diversification within the eastern and western Lahontan clades, which is estimated to have occurred approximately 2.0 and 1.8 Ma, respectively. I used a conservative approach when calibrating nodes using fossil data by assigning fossils to the earliest nodes they could represent on the phylogeny. Placing those fossils on more recent nodes would result in even older divergence time estimates than those presented here. Hence, these divergence time estimates reveal that the deepest divergences within *R. egregius* occurred before the late Pleistocene high stands of Lake Lahontan, and collectively support the assertion that the effect that pluvial lakes had on the dispersal of some aquatic taxa was minimal (Taylor and Smith, 1981; Taylor and Bright, 1987).

The observed pattern of deep divergence between eastern and western populations within the Lahontan Basin is not unique to *R. egregius*. A similar east/west split has been demonstrated among populations of Lahontan cutthroat trout (Nielsen and Sage, 2002) and speckled dace (McKell, 2003). Other researchers have studied Lahontan cutthroat trout and uncovered a third group to the north in the Quinn River system (to which McDermitt Creek is a tributary) in addition to an east/west split among Lahontan Basin populations (see Peacock and Kirchoff, 2004 and references therein). Significant population pairwise F_{ST} values between McDermitt Creek and North Fork Little Humboldt River (Table 3.3) suggest that *R. egregius* might exhibit a similar pattern of isolation of the McDermitt Creek population that could be better detected if samples were analyzed using more rapidly evolving markers such as microsatellites. Additional comparative phylogeographic studies are warranted based on the seemingly congruent east/west pattern of divergence among multiple Lahontan Basin fish taxa. Unfortunately, without good estimates of divergence times between eastern and western populations of

speckled dace and Lahontan cutthroat trout it is not possible to determine whether the shared pattern reflects phylogeographic congruence among these species, or if the pattern represents pseudo-congruence. Pseudo-congruence occurs when multiple taxa exhibit similar phylogeographic patterns spatially, but the patterns differ temporally and thus do not result from the same event (Mantooth and Riddle, *in press*).

The appearance of a western haplotype in an eastern locality, and an eastern haplotype in a western population (see Figure 3.4) could result from contemporary gene flow, or from incomplete lineage sorting. That fact that both anomalous haplotypes branch from the basal nodes of their respective clades suggests that the pattern formed via incomplete lineage sorting. The results of IM analyses (Figure 3.6; Table 3.5) support such a conclusion, because they indicate that the probabilities of migration from eastern to western Lahontan Basin populations (m_1), and from western to eastern Lahontan Basin populations (m_2) are low ($m_1 = 2.00 \times 10^{-8}$; $m_2=2.00 \times 10^{-8}$). Hence, it does not appear that contemporary migration between eastern and western Lahontan populations of *R. egregius* occurs, even though eastern and western populations may be connected in very wet years. Within the last few decades both the Carson and Humboldt Sinks have filled with water, essentially creating an aquatic connection between the Humboldt River (to which the North Fork Little Humboldt River and T Creek are tributaries) and the Carson and Walker Rivers, which flow into the Carson Sink in the western Lahontan Basin (Adams et al., 2008). Such connections typically form marshy areas that are short-lived, and therefore opportunities for migration between the eastern and western populations would be minimal, particularly if ecological requirements prevented the species from dispersing through such aquatic connections. Ecological requirements have recently been

demonstrated to be an influential factor in the dispersal of freshwater fishes across drainage divides in other parts of the world (e.g., Thacker et al., 2007; Burridge et al., 2008).

Non-significant values of Tajima's D and Fu's F_S statistics suggest that the five populations of *R. egregius* included in this study have not experienced recent population bottlenecks, which is somewhat surprising given the relatively recent reduction in size and connectivity of water bodies within the Lahontan Basin during the Holocene, along with significant F_{ST} values (Table 3.3) that are suggestive of considerable isolation of populations. Non-significant values of those statistics also indicate that the populations have not undergone recent expansion, even though certain aspects of the data could be interpreted as suggesting that expansion has occurred. Each population exhibits high haplotype diversity and low nucleotide diversity (Table 3.2), which is typical of populations that have undergone recent, rapid expansion from an ancestral population with a small effective population size (Avise, 2000). Mean estimates of effective population sizes produced by IM analyses hint at population expansion in the eastern and western Lahontan Basin because mean estimates of effective population size for contemporary populations are larger than that of the ancestral population (Table 3.5). However, the confidence intervals surrounding those estimates of effective population size are very large, so there is not a significant difference between contemporary and ancestral populations. Hence, I conclude from these data that these five populations of *R. egregius* have been relatively stable over time. The apparent stability of these populations could be explained by the ecology of the *R. egregius*. The ability of the species to survive in many different types of aquatic habitats (i.e., lakes, rivers, streams)

along with their small body size and short generation time may have allowed them to adjust to changing environmental conditions and persist in these areas without a significant reduction in population sizes.

The overall lack of deep phylogenetic structure within the eastern and western Lahontan clades (Figure 3.4) could be due to recent aquatic connections that may have allowed gene flow among eastern Lahontan Basin populations, as well as between western Lahontan Basin populations (but not between eastern and western populations). The North Fork Little Humboldt River and T Creek in the eastern Lahontan Basin are still connected via the Humboldt River in northern Nevada (Figure 3.2), and the North Fork Little Humboldt River and McDermitt Creek were still connected at the end of the Pleistocene. It is possible that cyt *b* evolves too slowly to be informative for detecting phylogeographic patterns within such a recent time frame. Even if gene flow does not occur between the Little Humboldt and McDermitt populations now that they are no longer connected, there may not have been enough time for an accumulation of mutations that would allow for the detection of a phylogeographic pattern. Use of more rapidly evolving markers in future studies may help detect additional genetic variation that was not detected in this study. Populations of Lahontan cutthroat trout exhibit significant genetic variation within drainages in the Lahontan Basin when analyzed using microsatellite data (e.g., Mary's River – Neville et al., 2006). Also, some drainages with Lahontan cutthroat trout contain population densities that are tied to topography (e.g., McDermitt Creek – Boxall et al., 2008), which means that topography affects migration patterns and gene flow within drainages. While my sampling was not detailed enough to investigate migration patterns of *R. egregius* within drainages for comparison, it stands to

reason that the same factors that contribute to population structure of Lahontan cutthroat trout within drainages might contribute to population structure of *R. egregius* within drainages, and to the overall genetic diversity of the species. Additional geographic sampling coupled with the utilization of more rapidly evolving markers (such as microsatellites) would be useful in determining whether there are populations of *R. egregius* that I did not sample that exhibit reduced genetic variation because of population bottlenecks, particularly given that studies using microsatellites have uncovered significant genetic variation among populations of other Great Basin cyprinids (e.g. Chen et al., 2009).

Additional studies using additional sampling and multiple unlinked loci would be useful in looking at patterns of migration and gene flow within and between drainages, similar to work that has been done on other Lahontan Basin species (e.g., Lahontan cutthroat trout; see Neville et al., 2006). Moreover, the use of multiple unlinked loci would help reduce confidence intervals around divergence time estimates (Arbogast et al., 2002; Kumar, 2005), which in turn would help to better understand the timing of evolution of phylogroups within this species. Patterns of congruence of coalescence among unlinked loci both spatially and temporally lend more support for causation of divergences by documented geological or climatic events (Kuo and Avise, 2005; Riddle et al., 2008). Therefore, studies using additional markers would be useful in better identification of the processes that shaped genetic diversification within *R. egregius*.

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Table 3.1: The five natural populations of *Richardsonius egregius* from the Lahontan Basin that were sampled for genetic analyses, along with abbreviations used to label terminal taxa on tree figures in parentheses, location of each population within the Lahontan Basin, Las Vegas Tissue (LVT) numbers (also used to label terminal taxa on tree figures), and Monte L. Bean Museum (MLBM) numbers.

Sampling Locality	Clade	Latitude	Longitude	Elevation	LVT #	MLBM #
McDermitt Creek (MC) Humboldt County, NV	Eastern	41.970 N	117.836 W	1,810 m	7881 – 7890	63759 – 63768
North Fork Little Humboldt River (LH) Humboldt County, NV	Eastern	41.692 N	117.247 W	1,730 m	9841 – 9850	112047 – 112056
T Creek (T) Elko County, NV	Eastern	41.525 N	115.247 W	1,768 m	8277 – 8283	99317 – 99323
Truckee River (TK) Washoe County, NV	Western	39.588 N	119.448 W	1,273 m	7264-7265, 7267-7273	63054-63055, 63057-63063
West Walker River (WK) Lyon County, NV	Western	38.740 N	119.400 W	1,485 m	7275-7278, 7282-7283, 10151-10153	62099-62102, 62106-62110

Table 3.2: Population genetic parameters of five naturally occurring populations of *Richardsonius egregius* located throughout the Lahontan Basin, as estimated by the program DNAsp. Parameter estimates are as follows: sample size (n), haplotype diversity (Hd), nucleotide diversity (π), Tajima's D statistic (D), F_s is Fu's F statistic (F_s). Significant values of D and Fs are shown in bolded font. Standard deviations of parameter estimates are given in parentheses.

Population	n	# of haplotypes	Hd	π	D	F_s
North Fork Little Humboldt River	10	8	0.933 (± 0.077)	0.00612 (±0.00014)	-0.2829	-1.134
McDermitt Creek	10	5	0.844 (±0.080)	0.00852 (±0.00238)	-0.4046	3.547
T Creek	7	6	0.952 (±0.096)	0.00672 (±0.00077)	0.1345	-0.354
Truckee River	9	5	0.722 (±0.159)	0.00221 (±0.00065)	-1.4922	-0.354
West Walker River	9	6	0.889 (±0.091)	0.00580 (±0.00192)	-1.2481	0.616
Total	45	29	0.972 (±0.011)	0.01208 (±0.00043)	0.0849	**-5.743**

Table 3.3: Corrected average percent sequence divergences among five populations of *R. egregius* located throughout the Lahontan Basin (above diagonal), and population pairwise F_{ST} values for the same five populations (below diagonal). Populations are abbreviated as in Table 3.1. Statistically significant values ($p < 0.05$) are listed in bolded font.

	North Fork Little Humboldt River	McDermitt Creek	T Creek	Truckee River	West Walker River
North Fork Little Humboldt River	-	**0.15**	0.02	**1.45**	**1.19**
McDermitt Creek	**0.17**	-	0.04	**1.10**	**0.87**
T Creek	0.02	0.05	-	**1.25**	**1.01**
Truckee River	**0.78**	**0.67**	**0.74**	-	**0.06**
West Walker River	**0.67**	**0.55**	**0.62**	**0.13**	-

Table 3.4: Results of AMOVA. Populations were categorized as either eastern or western Lahontan Basin according to phylogenetic placement. Grouping the samples by clade explained the majority of the molecular variation observed within these samples of *R. egregius*.

Source of Variation	d.f.	Sum of Squares	Variance Components	% of Molecular Variation Explained
Among groups (i.e., eastern vs. western Lahontan Basin)	1	139.233	6.11454 Va	61.85
Among populations within groups (i.e., sampling localities)	3	21.273	0.41879 Vb	4.24
Within populations	40	134.622	3.35290 Vc	33.91
Total	44	294.622	9.88623	

Table 3.5: Estimates of demographic parameters calculated using model parameter estimates from the output of IM analyses: Effective population size in the eastern Lahontan Basin (N_1), effective population size in the western Lahontan Basin (N_2), effective population size of the ancestral population (N_A), time (in years) since the divergence of eastern and western populations (t), probability of migration into the eastern Lahontan Basin from the western Lahontan Basin per year (m_1), probability of migration into the western Lahontan Basin from the eastern Lahontan Basin per year (m_2), the effective rate at which alleles come into the eastern Lahontan Basin from the western Lahontan Basin per generation ($2N_1m_1$), and effective rate at which alleles come into the western Lahontan Basin from the eastern Lahontan Basin per generation ($2N_2m_2$).

Demographic Parameter	Estimate	95% Confidence Interval
N_1	687,000	419,000 – 1,480,000
N_2	533,000	298,000 – 1,510,000
N_A	387,000	176,000 – 8,832,000
m_1	2.00×10^{-8}	$2.00 \times 10^{-8} – 1.88 \times 10^{-6}$
m_2	2.00×10^{-8}	$2.00 \times 10^{-8} – 2.45 \times 10^{-6}$
$2N_1m_1$	0.0685	0.0418 – 13.93
$2N_2m_2$	0.0531	0.0297 – 18.51

Figure 3.1: Map of western North America depicting the outline of the Great Basin, with the dashed line approximating the boundary of the Lahontan Basin in the western Great Basin. The pluvial lakes that filled many of the valleys within the Lahontan Basin during the Pleistocene are shown in grey on the map on the right.

Figure 3.2: Distribution map showing the approximate range of *R. egregius*. Sampling localities for the five populations included in this study are marked with open circles. Populations are as follows: McDermitt Creek (MC), North Fork Little Humboldt River (LH), T Creek (T), Truckee River (TK), and West Walker River (WK).

Figure 3.3: Alternative hypotheses regarding phylogenetic structure of *R. egregius*. Phylogeny A) depicts a shallow structure consistent with a panmictic Lake Lahontan population which became fragmented after the Pleistocene. Phylogeny B) represents the expected phylogenetic structure that might occur if events pre-dating the late Pleistocene were the most influential in guiding the evolution of the species. Population abbreviations are the same as those given in Table 3.1, and branching patterns coincide with the order in which populations might have become isolated from one another based on geographic proximity.

Figure 3.4: Phylogenetic tree produced by Bayesian analysis. Bootstrap values from MP and ML analyses are listed above branches, whereas posterior probabilities from Bayesian analysis are listed below branches. Eastern and western Lahontan clades of *R. egregius* are depicted by the black and white bars respectively. Asterisks indicate

individuals that carried haplotypes that were not consistent with their geographic location (i.e., a western haplotype found in an individual from an eastern Lahontan Basin population, and an eastern haplotype carried by an individual from a western population).

Figure 3.5: Confidence intervals surrounding divergence time estimates for A) the genus *Richardsonius* (3.3 Ma), B) *Richardsonius egregius* (2.6 Ma), C) the Eastern Lahontan Clade of *R. egregius* (2.0 Ma), and D) the Western Clade of *R. egregius* (1.8 Ma). Note that even with broad confidence intervals, diversification within this group of cyprinids did not occur in the late Pleistocene. Terminal taxa are labeled with population abbreviations followed by the LVT accession number assigned to that individual. Population abbreviations are as follows: McDermitt Creek (MC), North Fork Little Humboldt River (LH), T Creek (T), Truckee River (TK), and Walker River (WK). Black circles mark fossil calibrations. The circle labeled as C_1 represents a 7.0 million year old *Mylocheilus* fossil (Dowling et al., 2002; Smith et al., 2002), and the circle labeled as C_2 represents a 3.5 million year old *R. durranti* fossil (Smith, 1975).

Figure 3.6: Population model parameter estimates from IM analyses. Plots are labeled as follows: Population mutation rate for the eastern Lahontan Basin (θ_1), population mutation rate for the western Lahontan Basin (θ_2), population mutation rate for the ancestral population from which the eastern and western Lahontan Basin populations originated (θ_A), time since the eastern and western clades diverged (t), the migration rate, per mutation, into the eastern Lahontan Basin from the west (m_1), and migration rate, per mutation, into the western Lahontan Basin from the east (m_2).

Figure 3.1

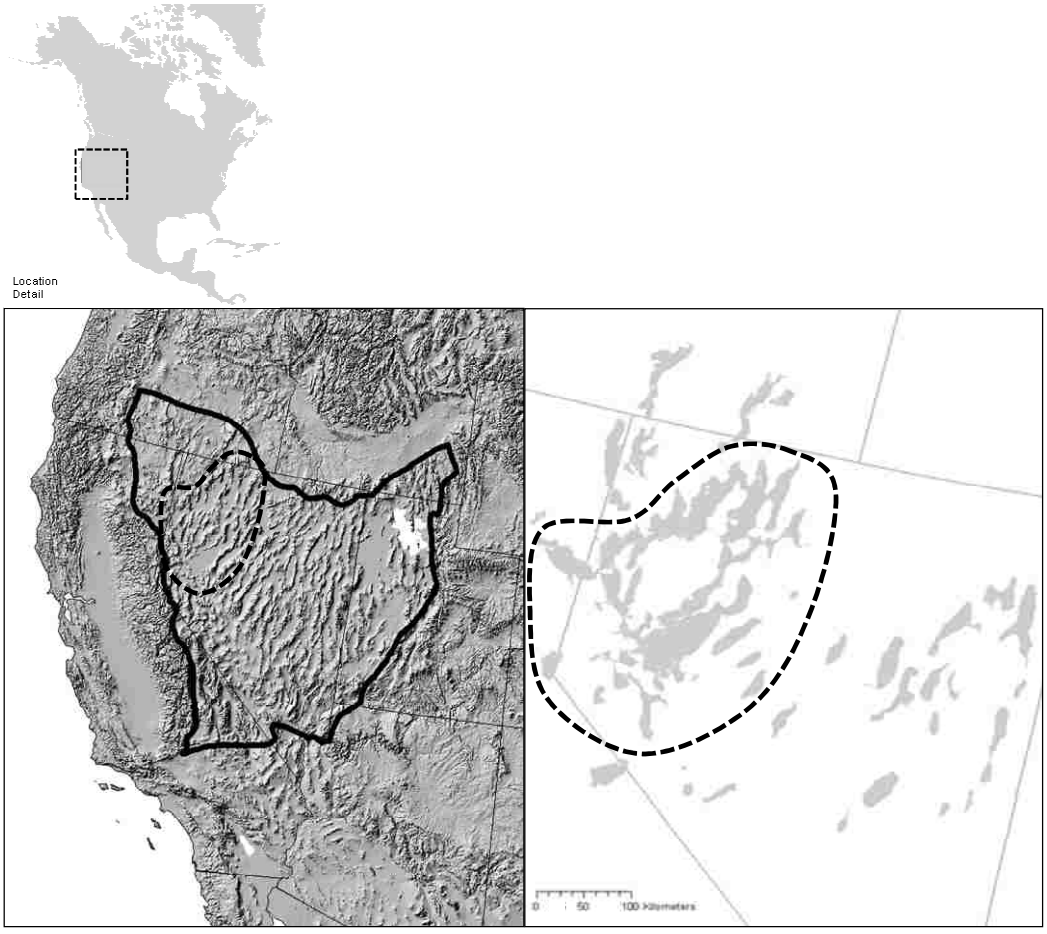


Figure 3.2

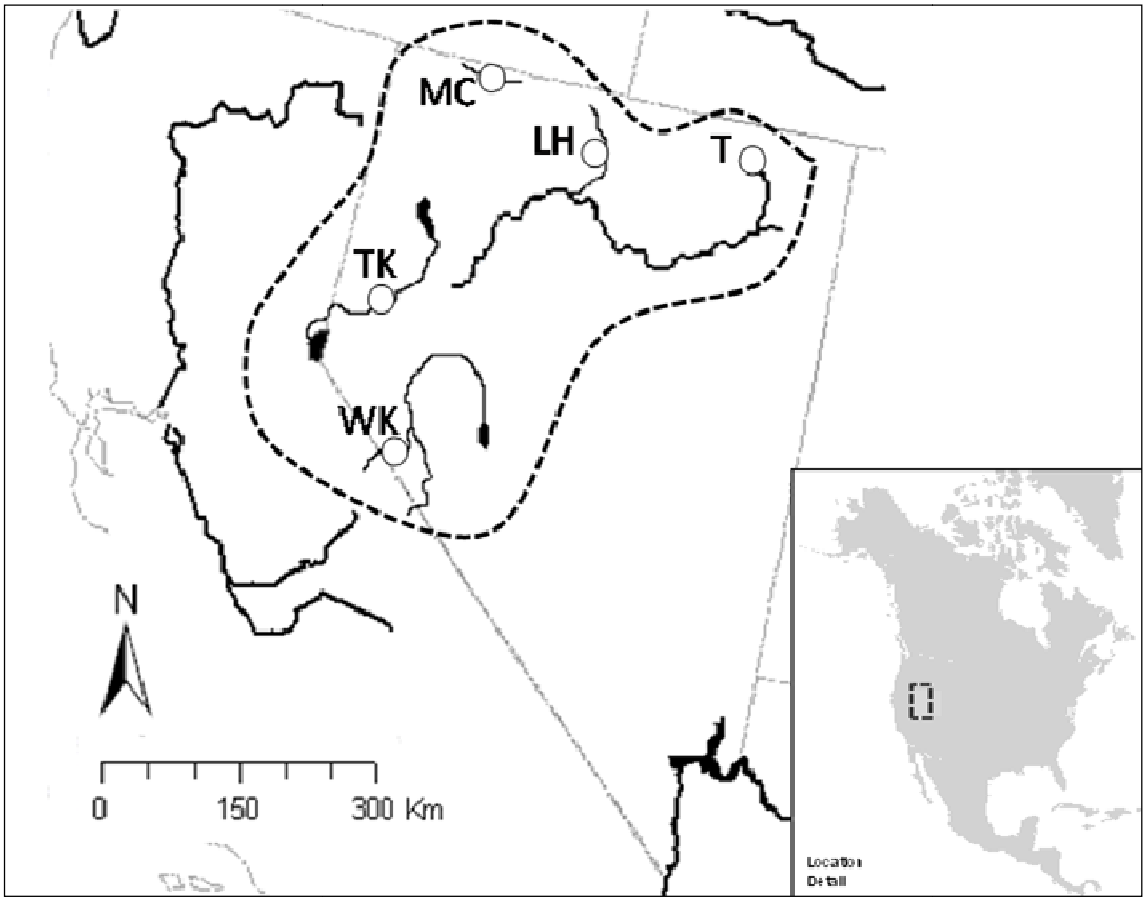


Figure 3.3

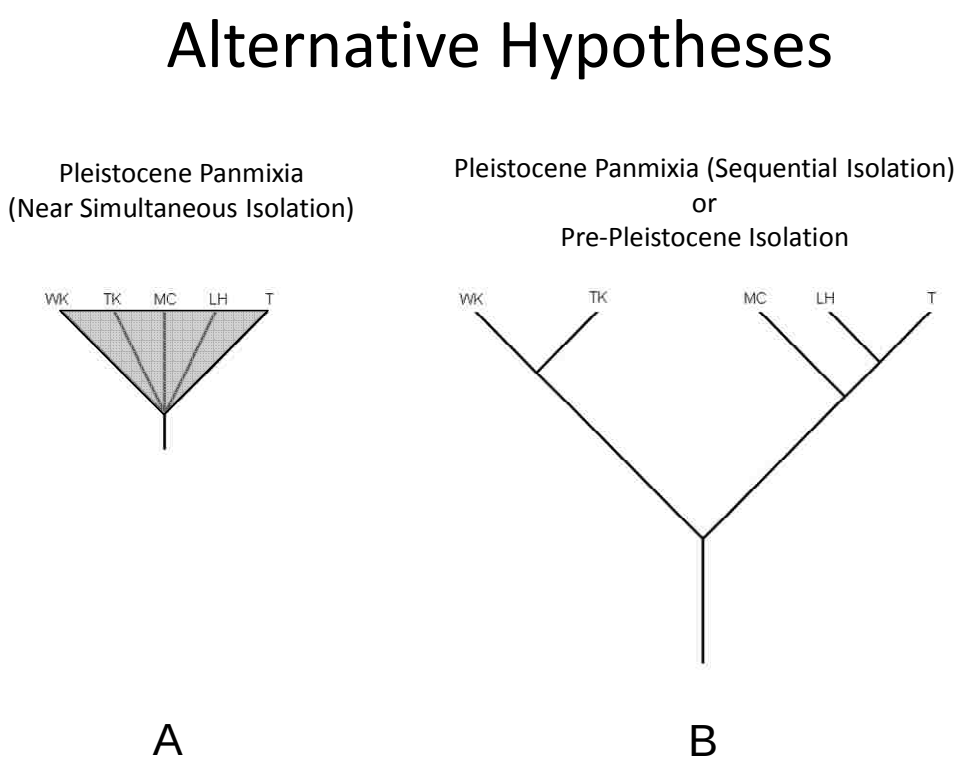

Alternative Hypotheses
Pleistocene Panmixia
(Near Simultaneous Isolation)
WK
TK
MC
LH
T
A
Pleistocene Panmixia (Sequential Isolation)
or
Pre-Pleistocene Isolation
WK
TK
MC
LH
T
B

Figure 3.4

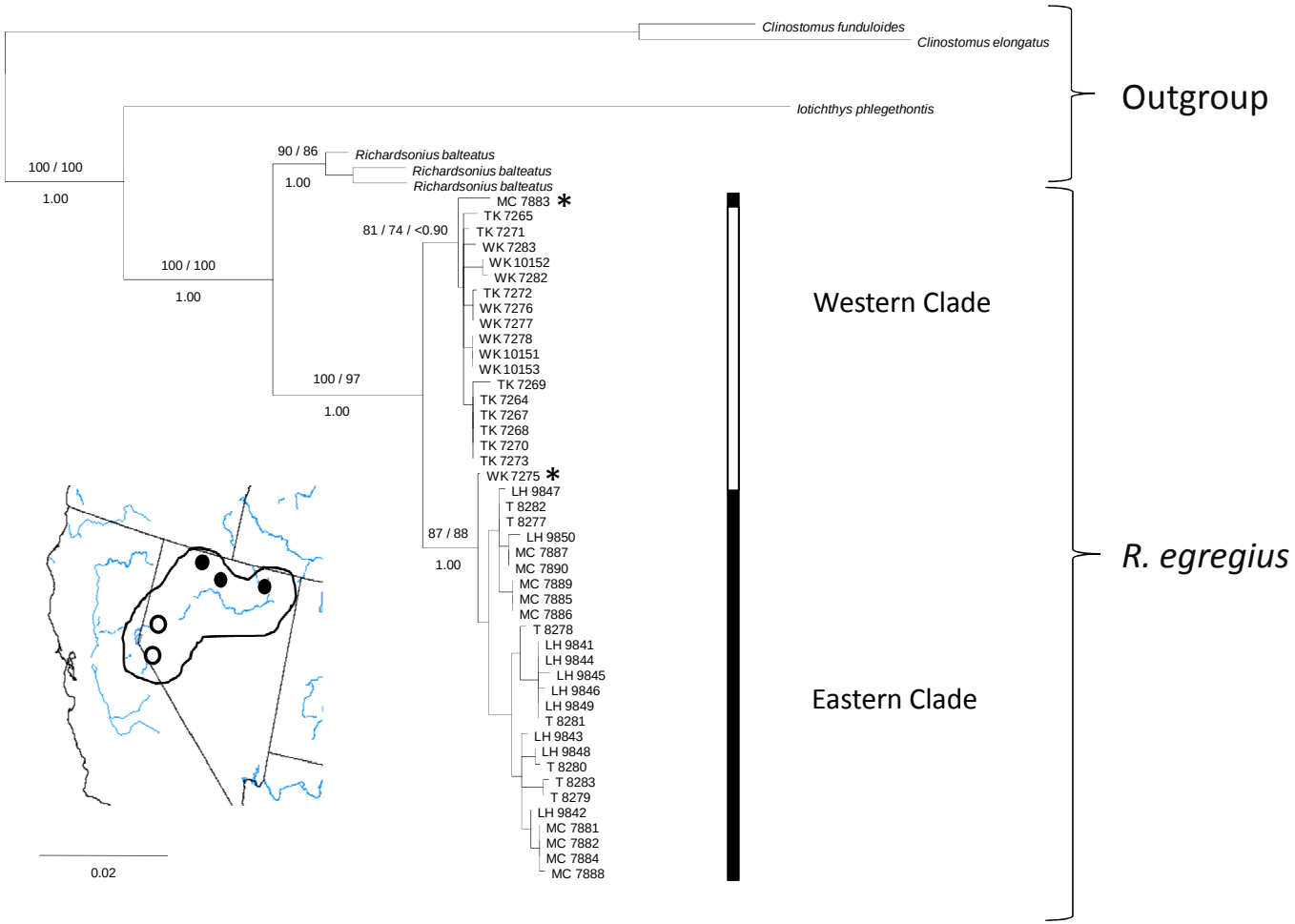
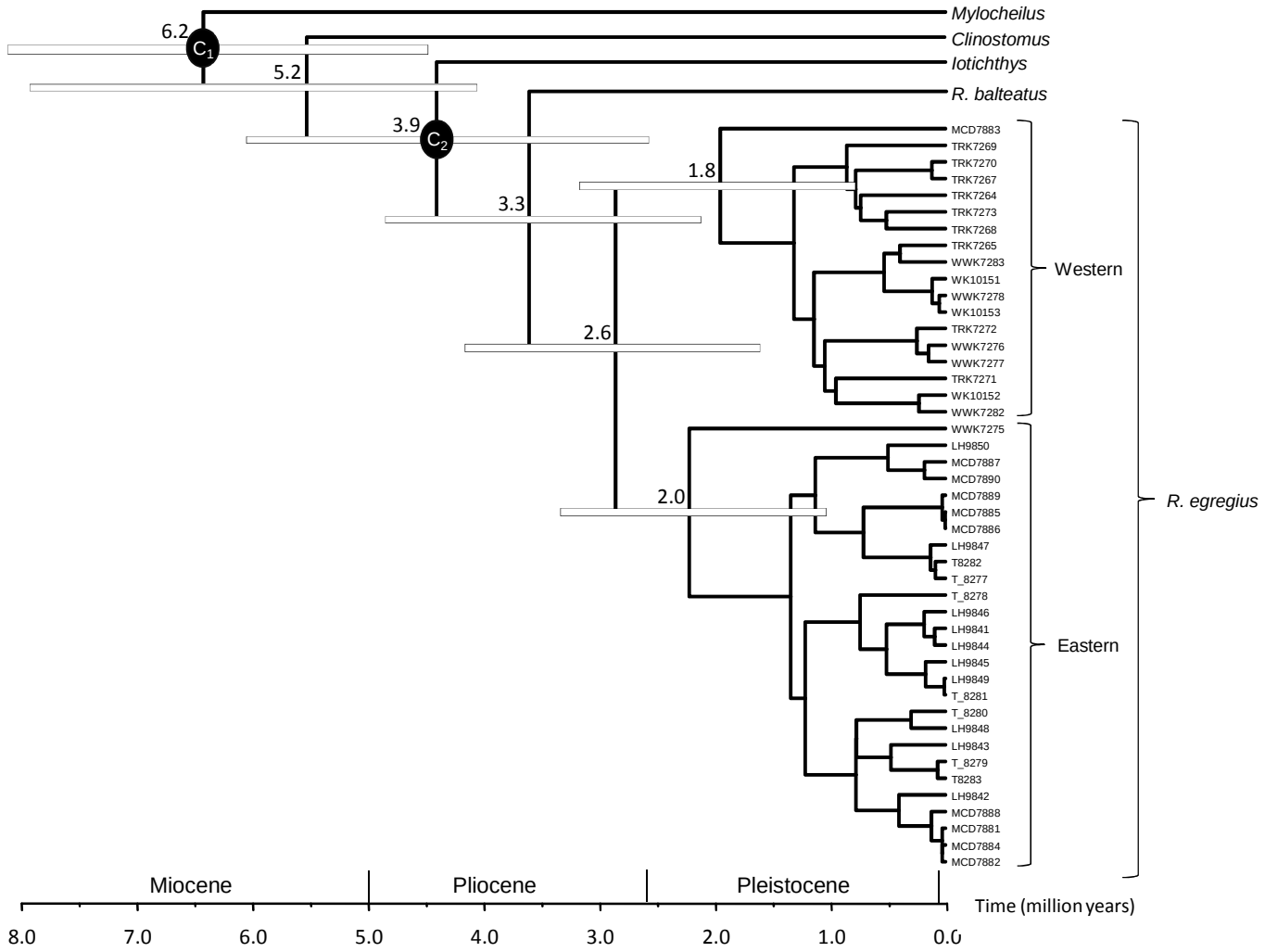

Clinostomus funduloides
Clinostomus elongatus
Iotichthys phlegethontis
Outgroup
90 / 86
1.00
Richardsonius balteatus
Richardsonius balteatus
Richardsonius balteatus
100 / 100
1.00
100 / 100
1.00
81 / 74 / <0.90
MC 7883 *
TK 7265
TK 7271
WK 7283
WK 10152
WK 7282
TK 7272
WK 7276
WK 7277
WK 7278
WK 10151
WK 10153
TK 7269
TK 7264
TK 7267
TK 7268
TK 7270
TK 7273
Western Clade
100 / 97
1.00
WK 7275 *
LH 9847
T 8282
T 8277
87 / 88
1.00
LH 9850
MC 7887
MC 7890
MC 7889
MC 7885
MC 7886
T 8278
LH 9841
LH 9844
LH 9845
LH 9846
LH 9849
T 8281
LH 9843
LH 9848
T 8280
T 8283
T 8279
LH 9842
MC 7881
MC 7882
MC 7884
MC 7888
Eastern Clade
R. egregius
0.02

Figure 3.5


Mylocheilus
Clinostomus
Iotichthys
R. balteatus
6.2
C1
5.2
3.9
C2
1.8
3.3
2.6
2.0
MCD7883
TRK7269
TRK7270
TRK7267
TRK7264
TRK7273
TRK7268
TRK7265
WWK7283
WK10151
WWK7278
WK10153
TRK7272
WWK7276
WWK7277
TRK7271
WK10152
WWK7282
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MCD7887
MCD7890
MCD7889
MCD7885
MCD7886
LH9847
T8282
T_8277
T_8278
LH9846
LH9841
LH9844
LH9845
LH9849
T_8281
T_8280
LH9848
LH9843
T_8279
T8283
LH9842
MCD7888
MCD7881
MCD7884
MCD7882
Western
Eastern
R. egregius
Miocene
Pliocene
Pleistocene
Time (million years)
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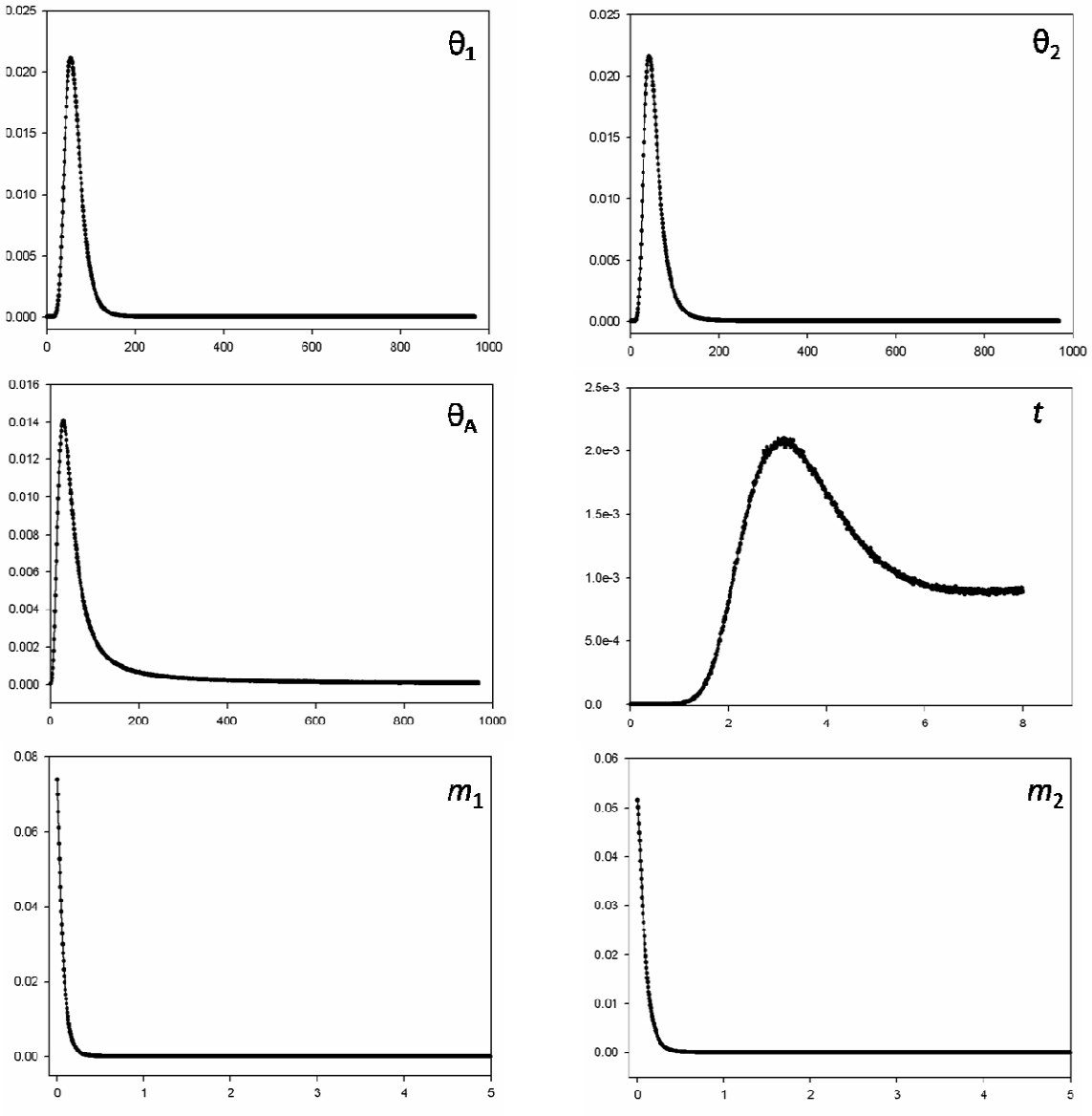
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

Figure 3.6



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Belk, M.C., Johnson, J. B., Wilson, K. W., Smith, M. E., Houston, D. D., 2005. Variation in intrinsic individual growth rate among populations of leatherside chub: adaptation to length of growing season? Ecology of Freshwater Fish 14, 177-184.

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