THE INFLUENCES OF QUATERNARY PROCESSES ON NATIVE FRESHWATER DIVERSITY IN PATAGONIA: MOLECULAR INSIGHTS FROM THE GALAXIID FISHES

by

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DEDICATION PAGE

This thesis is dedicated to Gary Zemlak and Gail Rieschi. I am forever grateful for your patience, love and support as parents, best friends and mentors.

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ABSTRACT

Essential to our understanding of biological diversity are the influences exerted by historical changes in global landscapes. My research focuses on Patagonia, South America, where I concentrate on the influences of large-scale Quaternary processes in shaping the contemporary biological diversity and species distributions of endemic aquatic taxa. I begin by identifying the key biological implications of historical drainage evolution in Patagonia during Quaternary period by reviewing the relevant geological, climatic and phylogeographic/biogeographic literature. I then empirically test several hypotheses relating to the rise of the southern Andean mountain range (beginning *ca.* 23 MY BP) and the glacial cycles of the Pleistocene (2.6 MY – 10,000 yr BP) by reconstructing the phylogeographic history of two widespread fishes, the freshwater large puyen (*Galaxias platei*) and the diadromous puyen (*G. maculatus*). My work highlights the importance of the Andes mountain range in creating a strong, but penetrable barrier to dispersal between eastern and western Patagonia. For *G. platei*, the periodic switch in drainage direction of some river systems between the Atlantic and Pacific appear to have been a key facilitator of trans-Andean gene exchange between eastern and western populations. Gene flow between eastern and western populations of *G. maculatus*, however, was primarily through the marine realm and is best explained by a model of isolation-by-distance that circumscribes the southern Andes. Still, river drainage deflections appear to have exerted some influence in northern drainages of *G. maculatus*. Multilocus estimates of trans-Andean dispersal in *G. platei* suggest that rates of gene flow via river drainage deflections were low, bidirectional and likely available to aquatic taxa on multiple occasions during the transitionary periods between glacial and inter-glacial conditions of the mid- to late-Quaternary (1 MY BP – present). Finally, a comparative contrast of demographic histories between *G. platei* and *G. maculatus* reveals that the extensive glaciations of the past 1 MY played important roles in contracting range sizes of both species and purging genetic diversity, particularly those glaciations associated with the mid-Quaternary (1.1-0.6 MY BP). The negative influence on both freshwater and diadromous forms suggest that these glaciations had far-reaching consequences for cold-adapted aquatic organisms of Patagonia.

LIST OF ABBREVIATIONS USED

AFZ	Antarctic Frontal Zone
AMOVA	Analysis of Molecular Variance
BP	before present
ca.	*circa*
CPG	Coldest Patagonian Glaciation
GPG	Great Patagonian Glaciation
IBD	Isolation by Distance
Indel	Insertion or deletion
K2P	Kimura 2-Parameter
KY	thousand years
LGM	Last Glacial Maximum
MCMC	Markov Chain Monte Carlo
MST	Minimum Spanning Tree
mtDNA	Mitochondrial DNA
MY	million years
OIS	Oxygen Isotope Stage
SHWs	Southern Hemisphere Westerlies
SHEs	Southern Hemisphere Easterlies

GLOSSARY OF TERMS

Adiabatic forcing	The resultant increase of internal energy within rising parcels of air due to expansion at higher elevations and lower atmospheric pressures.
Argentinean rivers	Rivers that currently flow into the Atlantic and are completely contained within the eastern foothills and Patagonian Steppe (eastern Patagonia).
Chilean rivers	Pacific draining rivers which are completely restricted to the area west of the Andes.
Haplogroups	A group of closely related haplotypes that form a monophyletic cluster using phenetic or phylogenetic tree building algorithms.
O_2 *isotope ratios*	A temperature proxy which measures relative variations in the ratio of oxygen species (^{16}O *versus* ^{18}O) over time and is used to infer the chronology of historic climate cycles.
Palynological	The study of contemporary and fossilized distributions of plant grains and pollen spores.
Synthetic science	The creation of new perspectives or insights by integrating existing data, evidence and/or ideas from discrete sources/disciplines.
Terminal moraine	Deposits of glacially eroded sediment which mark the maximum extent of advance of a glacier.
Trans-Andean rivers	Rivers with headwaters originating on sides of the Andes opposite to the ocean they drain into. These rivers must bisect the Andes enroute to either the Pacific or Atlantic Oceans.

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CHAPTER 1: INTRODUCTION

This chapter was prepared as a preliminary exam essay by Tyler S. Zemlak and modified to serve as a general introduction for this thesis. Sandra J. Walde and Daniel E. Ruzzante edited this chapter. Don Stewart and Paul Bentzen also offered helpful advice during the exam (May 2010).

1.1 Abstract

Using GIS-based tools and a review of the relevant geological and climatic literature, I attempt to identify the key implications of Quaternary glacial cycles for drainage evolution in eastern Patagonia. In doing so, the stage is set for the proper integration of existing biogeographic and phylogeographic ideas to develop a suite of inferences aimed at elucidating how these processes influenced aquatic biodiversity of Patagonian Argentina. A primary finding of this research is that the southern mainland and/or Tierra del Fuego served as an important cryptic refuge for cold-adapted species, including aquatic taxa. At least one additional aquatic refuge is likely to have existed in either central or northern Patagonia. The low position of the Atlantic shoreline during glacial periods also revealed a much larger and inter-connected drainage network in southern Patagonia. During sea-levels stands below 100m, two new drainage coalescence points on the exposed continental shelf can be recognized among the Chico/Santa Cruz, Coyle and Gallegos river basins and between the Grande and Fuego rivers. Enhanced hydrological discharge during the deglaciation period of Late-Quaternary cycles is expected to have facilitated extensive inter-drainage connections within each of the northern and southern regions of eastern Patagonia via proglacial lake and/or stream coalescence. A large proglacial lake in the Nahuel Huapi Lake region is also recognized as the most likely temporary gateway for aquatic organisms to disperse between the Neuquen and Northern Patagonian Tablelands. I also recognize climate-induced drainage reversals as a bidirectional mechanism of trans-Andean dispersal and an important factor in determining the biogeography of widespread aquatic organisms in Patagonia.

1.2 Introduction

Phylogeographic investigations have been pivotal to our understanding of the degree to which Quaternary processes have influenced contemporary assemblages of species and populations. The physical and climatic environments of the Quaternary were particularly unstable. Orogeny and glacial cycles repeatedly perturbed flora and fauna globally to sunder, shift and shuffle species' ranges. Such dramatic rearrangements in the size and connectivity of suitable habitat left important genetic signatures in the genomes of modern descendents (Hewitt 2000). The combination of this genetic legacy with geographic signal forms the basis of phylogeographic research and has greatly improved our understanding of how landscapes have influenced biodiversity (Avise et al. 1987).

On a global scale, progress in phylogeography has not been uniform. Our current understanding of the biological implications of Quaternary processes is largely derived from a collection of literature that is dominated by the flora and fauna of North America and Europe (Beheregaray 2008). Developments have been comparatively slow in other parts of the globe, particularly for continental regions of the Southern Hemisphere (Beheregaray 2008). A more representative sample of global environments is required to formulate a comprehensive understanding of how biodiversity is created, maintained and distributed across global landscapes.

Patagonia, the southernmost and temperate region of South America is one location where the consequences of historical landscape changes for local biodiversity is understood only in broad terms and only for a limited number of (mostly terrestrial) species. A total of 32 phylogeographic studies have been conducted within this region (Table 1.1). The greatest proportion of this literature is devoted to the terrestrial flora (45% of studies), followed by terrestrial animals (40%) and then aquatic animals (15%). Cumulatively, these studies employ a variety of molecular tools to examine the geographic distribution and genetic diversity within 33 distinct species [some uncertainty due to species complexes, see Ruzzante et al. (2006), Avila et al. (2007), Morando et al. (2007)] at a variety of temporal and spatial scales (Table 1.1, Figure 1.1). One strong consistency among these studies points to Andean mountain orogeny [starting approximately 23 Ma (Ramos 1989)] as an important challenge to dispersal between eastern and western Patagonia, although estimated rates of trans-Andean gene flow vary greatly among taxa (Allnutt et al. 1999; Pearson and Smith 1999; Premoli et al. 2000; Smith et al. 2001; Bekessy et al. 2002; Allnutt et al. 2003; Marchelli and Gallo 2004; Muellner et al. 2005; Palma et al. 2005; Marchelli and Gallo 2006; Victoriano et al. 2008; Quiroga and Premoli 2010). The glacial cycles of the Quaternary (1.8 Ma – 10,000 yr BP) also appear to have grafted strong genetic structure within most taxa by isolating populations into different glacial refugia (Kim et al. 1998; Marchelli et al. 1998; Allnutt et al. 1999; Premoli et al. 2000; Bekessy et al. 2002; Marchelli and Gallo 2004; Muellner et al. 2005; Marchelli and Gallo 2006; Avila et al. 2007; Morando et al. 2007; Victoriano et al. 2008; Azpilicueta et al. 2009; Mathiasen and Premoli 2009; Pastorino and Gallo 2009; Pastorino et al. 2009; Tremetsberger et al. 2009; Xu et al. 2009; Cosacov et al. 2010; Quiroga and Premoli 2010). Recent post-glacial expansion into previously glaciated territory is also evident in several taxa. For example, populations currently located in glaciated regions have reputedly shown a relative paucity of genetic diversity with respect to closely allied populations that occupy non-glaciated territory (Pastorino and Gallo 2002; Pastorino et al. 2004; Ruzzante et al. 2006; Victoriano et al. 2008; Azpilicueta et al. 2009; Pastorino and Gallo 2009; Xu et al. 2009; Arana et al. 2010; Cosacov et al. 2010).

Few phylogeographic studies have addressed the role that Quaternary processes have played in shaping the aquatic diversity in lakes and rivers of Patagonia. Past focuses primarily concern a collection of widespread fishes: the temperate freshwater creole perch (*Percichthys trucha*), the cold-adapted freshwater puyen (*Galaxias platei*) and the diadromous whitebait species *Galaxias maculatus* (Ruzzante et al. 2006; Ruzzante et al. 2008; Zemlak et al. 2008; Zemlak et al. 2010). A detailed genetic study of the Chilean freshwater crab species *Aegla alacalufi* (Xu et al. 2009) is the only other aquatic taxon represented in the Patagonian phylogeoraphic literature. However, these studies have still managed to yield some important insights and hypotheses regarding the biological implications that Quaternary tectonics and climate held for freshwater diversity and biogeography.

The rise of the Patagonian Andes in the Miocene sundered the Patagonian aquatic landscape into separate Atlantic and Pacific draining systems and imposed an important barrier for dispersal between eastern and western Patagonia. Shallow, but well supported genetic structure between eastern and western populations of *G. platei*, *G. maculatus* and *P. trucha* suggests that the Andes have been a significant, but permeable barrier to dispersal (Ruzzante et al. 2006; Zemlak et al. 2008; Zemlak et al. 2010). Climate-induced drainage reversals have been recognized as important facilitators of recent trans-Andean dispersal for *G. platei* and *G. maculatus* (Zemlak et al. 2008; Zemlak et al. 2010). Drainage reversals occurred when ice dams collapsed during the retreat of glaciers following the Last Glacial Maximum (LGM) and forced shifts in drainage direction (from Atlantic to Pacific) in several basins. Also important was marine-mediated dispersal via the southernmost tip of South America for at least one species of diadromous fish, *G. maculatus* (Zemlak et al. 2010). Patterns of intra-specific genetic diversity of fishes and crabs also suggest that multiple refugia existed to the north and east of continental ice, as well as within glacial margins of the central-west (Ruzzante et al. 2006; Zemlak et al. 2008; Xu et al. 2009).

The purpose of this study is to advance Patagonian phylogeography by employing *synthetic science* as per Sidlauskas et al. (2010) to link clues from several separate sources to develop new perspectives about the possible implications that Quaternary drainage evolution in Patagonia held for aquatic biodiversity. The emphasis is on freshwater systems and their associated taxa, but there are also obvious implications for terrestrial organisms that are associated with lakes/rivers (i.e. those of temperate forests) or have limited dispersal ability and for which rivers may pose a barrier (e.g. lizards). I review relevant geological and climatic literature and employ GIS-based information and analysis software to estimate the range of possible forms that ocean-draining freshwater basins of Argentinean Patagonia could have taken over the past 2.6 MY. I then incorporate relevant findings from the phylogeographic and biogeographic literature to form a large-scale synthesis of how historical drainage evolution likely underpins contemporary freshwater biodiversity in Argentina. The result is a collection of testable inferences (Table 1.2) that predict how historical changes in freshwater habitat influenced the contemporary genetics (structure, diversity) and distributions of (primarily) aquatic species in Patagonia.

1.3 Present-Day Patagonian Landscape and Hydrology

Based on geological criteria, Patagonia can be recognized as the continental area extending from 37°S to Cape Horn at 56°S [Coronato et al. (2008), Figure 1.2]. Its landscape is primarily influenced by the southern Andes, an uplift which began approximately 23 MY BP and continues presently (Ramos 1989). The southern Andes extend continuously throughout Patagonia in a north-south orientation primarily paralleling the Pacific coastline (Patagonian Cordillera; north-south) but changes orientation at its southernmost reaches in Tierra del Fuego to trend east-west (Fueguian Cordilleras) and creates an orocline (Diraison et al. 2000). The result is an asymmetrical division of Patagonia into mostly eastern and western parts. The west (Chile) is a narrow strip of land characterized by a steep and rugged topography that quickly descends from the foothills of the Andes into the Pacific Ocean. Contrastingly, the eastern region (Argentina) is much larger and more gently sloping and contains the Patagonian Steppe which begins at the fringe of the Andean foothills and continues to the Atlantic coastline.

Contemporary river drainages in Patagonia can generally be classified into three types based on headwater position relative to the Andes and outflow direction, either Atlantic or Pacific. Almost all river drainages that currently flow into the Atlantic are completely contained within the eastern foothills and Patagonian Steppe (herein Argentinean) (Figure 1.3). Of similar regional containment are most Pacific draining systems which are completely restricted to the area west of the Andes (herein Chilean). The third group includes a handful of systems with headwaters originating on sides of the Andes opposite to the ocean they drain into (herein trans-Andean). The latter, trans-Andean systems, must bisect the Andes enroute to either the Pacific or Atlantic Oceans. Pacific draining, trans-Andean systems of the primary Patagonian Cordillera of the mainland (herein Pacific trans-Andean) include the Hua-Hum-Valdivia, Puelo, Futaleufu, Palena, Baker and Pascua rivers. Atlantic draining systems with headwaters in Chilean territory (herein Atlantic trans-Andean) include Rio Chico (Santa Cruz) and the Rio Grande (Tierra del Fuego) (Coronato et al. 2008), but it is important to note that the Rio Chico is at present mostly intermittent (Figure 1.3).

The hydrological dynamics of Patagonian lakes and rivers in Argentina is decided by climate and greatly influenced by the position of Andean mountain range. The north-south axis of the Patagonian Cordilleras creates a rain-shadowing effect whereby the humid westerlies are stripped of their moisture before reaching the Argentinean Steppe. This results in very dry and desert-like conditions for most parts of the year and primarily limits the location of lentic environments to glacial- and rain-fed locations at, or near, the Argentinean foothills (Figure 1.3). Lotic outlets that drain these lakes into the Atlantic (i.e. to complete the Argentinean systems) are mostly located throughout the Tablelands of Extra-Andean Argentina (Figures 1.2, 1.3). The Tablelands comprise large plateaus of arid flatlands limited by the Fueguian Cordillera to the south, the Patagonian Cordillera to the west and the Somun-Cura Massif to the north. The Deseado massif separates the tablelands into approximately equal parts known as the Northern and Southern Patagonian Tablelands. The remaining Atlantic flowing rivers in northern Patagonia are located north of the Somun-Cura massif and are contained within the Neuquen Basin which is bordered by the Brazilian Plateau to the north and north-east. The Patagonian Steppe also contains several endorheic basins that do not have a contemporary outlet to the sea. Both the Somun-Cura and Deseado Massifs comprise the largest collections of endorheic depressions in Extra-Andean Patagonia (Coronato et al. 2008).

1.4 Quaternary Glacial Cycles & Patagonian Drainage Evolution

Continental positioning and regular variations in the amount of solar radiation penetrating the atmosphere (Milankovitch cycles) interacted to drive climatic oscillations throughout the Quaternary (Berger 1988). This resulted in regular glacial cycles and predictable drops in global temperature, bringing major ice sheets onto the Earth's surface several times. The repeated glacial cycles are known to have been primary drivers of drainage evolution in high latitude environments throughout the Quaternary (Lemmen et al. 1994; Bernatchez and Wilson 1998; Mangerud et al. 2004). In this section, I explore the physical sciences literature to understand how the regular climatic cycles of the Quaternary could have influenced general drainage structure in Patagonia.

1.4.1 Timing, Frequency and Duration of Quaternary Patagonian Glaciations

This section is intended to provide an overview and summary of the frequency and duration of glaciations in Patagonia throughout the Quaternary. Several extensive articles have been published on this subject matter and I direct the reader to the following thorough resources for a more detailed account: Clapperton (1993), Coronato et al. (2004), Singer et al. (2004), Rabassa et al. (2005), Ehlers and Gibbard (2007), Rabassa (2008), Rabassa et al. (2011).

The most extensive geological evidence of Quaternary glaciations exists for two glacial periods, the Last Glaciation and the Largest Glaciation. The Last Glacial is likely to have taken place between 90-10 KY BP and reached its maximum (LGM) between 23-18 KY BP (Clapperton 1993; McCulloch et al. 2000). The Largest Glaciation is known as the "Great Patagonian Glaciation" (GPG) and is thought to have taken place between 1.168 and 1.016 MY BP (Rabassa 2008). Physical evidence from other glacial periods is fragmented and minimal because of disturbance from these two glacial cycles. However, by consolidating the results from a collection of studies that have dated residual terminal moraines throughout Patagonia (Mercer 1976; Clapperton 1993), 14-16 glacial periods have been inferred to have taken place in southern South America throughout the past 1 MY (Singer et al. 2004; Rabassa et al. 2005).

In the absence of suitable physical evidence, Ruzzante et al. (2008) used the predictive power of oxygen isotope ratio cycles available in a stacked benthic oxygen isotope 18 (δ^{18}) record from 57 globally distributed marine sediment cores (Lisiecki and Raymo 2005) as the best available representation of the timing and duration of Patagonian glaciations. Ocean sediments exceeding concentrations of $4.3^{o}/_{oo}$ δ^{18} were considered a suitable threshold for recognizing transitions between cold and warm cycles, and *vice versa*. This approach recognizes a clear transition to longer glacial cycles that is initiated around 0.9 MY BP, but glacial and inter-glacial durations still remain irregular. In comparison with the aforementioned synthesis of physical evidence by Rabassa (2005), more glacial periods are identified using this approach (total of 19), 10 of which occurred following the mid-Quaternary transition in cycle periodicity. One more important difference between inference methods is the timing of the longest glacial, or GPG, which according to the data of Lisiecki & Raymo (2005) is estimated to have occurred between 876,000 and 938,000 yr BP.

1.4.2 Physical Extent of Quaternary Patagonian Ice Sheets

Glacial ice most heavily influenced the Andes and areas to the west. It is likely that ice reached the Pacific coast on several occasions during the Quaternary and expanded as far north as 35°S. The direct impact of glaciers was much less severe to the east, but did descend from the Andean peaks to consume much of the eastern foothills and extend slightly onto the Patagonian Steppe. The period for which we have the most physical evidence is the Last Glaciation. During the period of maximum ice coverage (LGM 23-18 KY BP), a continuous sheet existed along the Andes for over 1800 km, extending west to the Pacific Ocean south of 42°S [(Clapperton 1993; McCulloch et al. 2000) (Figure 1.3)]. The LGM ice volume was approximately 500,000 km^3, roughly equivalent to 1.2 m of global sea-level (Hulton et al. 1994; Hulton et al. 2002). Substantial physical signatures remain also for the GPG, approximately 1 MY BP (Clapperton 1993; Coronato et al. 2004; Rabassa et al. 2005; Rabassa 2008). This glaciation shared similar characteristics with the LGM, although the magnitude of eastern and northern glacial advance were fractionally larger (Figure 1.3).

The advance of glacial ice onto the Argentinean foothills would have had important consequences for any lakes and/or rivers occupying this region prior to the onset of glacial cycles. All contemporary lakes in this region represent post-glacial formations and the vast majority were only revealed, and possibly formed following the LGM, the exceptions being some lakes currently situated in areas adjacent to the glacial limits. Therefore, advancing glaciers would have dramatically influenced the position of any pre-existing lakes, or removed them entirely. Similar evictions of freshwater habitat likely occurred on a regular basis during the onset of each glacial cycle.

1.4.3 Paleoclimate of Eastern Patagonia During Glacial Maxima

Present-day Patagonia is almost entirely influenced by a band of westerlies that extends from the basins of the Rio Colorado and Rio Negro (Figure 1.3) at approximately 38^{o}S-39^{o}S to the southernmost tip of Tierra del Fuego (Mancini et al. 2008). Consequently, most of eastern Patagonia is situated within the rainshadow of the Patagonian Cordillera and therefore receives little annual precipitation. Most annual rainfall occurs at the Argentinean foothills (*circa.* 750-1000 mm/yr) and this rate sharply drops eastward at the foothill/Steppe boundary with the Steppe experiencing less than 250mm/yr (Clapperton 1993). Dry winds and seasonal heat also create very high evaporation rates to create high evaporation/precipitation rates and unfavourable conditions for aquatic habitat. Significant lentic environments in Extra-Andean Patagonia are few and restricted to the Senguer River (Musters, Collhue Huapi) (Figure 1.3) and Lake Cardiel which is an isolated lake in Santa Cruz province. The Negro River currently includes several lakes (Los Barreales, Mari-Menuco, Pellegrini, Ramos Mejia), but these are man-made reservoirs.

The aridity of Extra-Andean Patagonia is expected to have been enhanced during glacial periods. Indeed, several proxies of terrestrial paleoclimate provide evidence of such conditions for eastern Patagonia (Clapperton 1993; Iriondo 2000; Wainer et al. 2005) which are reinforced by global geological dust records (Kohfeld and Harrison 2001). The reason(s) for a drier eastern Patagonia, however, is still unclear. One explanation is that there was a general increase in wind intensity during glacial periods that increased evaporation rates (Bard and Rickaby 2009). Another hypothesis instead attributes this to an overall decrease in precipitation rates as the band of westerlies destabilized and fragmentated into smaller, isolated and lower energy units (Compagnucci 2011). Regardless of the mechanism, there is a concensus that climate was generally drier in eastern Patagonia during glacial periods.

Another important unknown is whether all of Patagonia was influenced by the westerlies during glacial periods. Of particular importance was the position of the band of the Southern Hemisphere Westerlies (SHWs) throughout the Quaternary. The northern zonal maximum is currently located between the basins of the Colorado and Negro rivers (38-39^{o}S). To the north of 39^{o}S is the southeast trade circulation (herein Southern Hemisphere Easterlies; SHEs) associated with the Atlantic subtropical anticyclone which carries abundant moisture to the central region of the continent east of the Andes (Mancini et al. 2008). The southern zonal maximum of the dry SHWs is bordered in the south by the moist easterlies of the Antarctic Frontal Zone (AFZ), a boundary which is presently located just 3-5° south of the South American continent (Kaplan et al. 2008).

Several lines of evidence suggest that the band of SHWs shifted towards the equator by several degrees during cold periods because of a displacement of Southern Hemisphere air circulation boundaries by the growing Antarctic ice sheet. This is corroborated in part by an 800 KY ocean sediment core record from the coast of South Africa. Temperature proxies suggest that a severe glacial stadial centered around 340-420 KY BP caused a northward shift in the zonal maximum of the band of SHWs by as much as 7° (Bard and Rickaby 2009). Similar, equator-directed migration of the SHWs has also been detected for South America during the last glacial as per pollen frequency records and neighbouring marine records from the Brazilian Atlantic rainforest region (Ledru et al. 2005). Furthermore, modeling efforts suggest that a northward shift in SHWs by at least 5° is required to explain the glacier extent and morphology during the last glaciation (Hulton et al. 1994; McCulloch et al. 2000; Hulton et al. 2002). However, Compagnucci (2011) suggests that a simple decrease in the intensity of the SHWs could induce a similar effect as the hypothesized equatorial shift of the southern margin of the southern westerlies. The impact of a northward shift in the influence of the band of SHWs would have had important implications for unglaciated parts of southern Patagonia, particularly Tierra del Fuego, the region directly bordered by the moist AFZ to the south. Depending on the magnitude and duration of this shift, parts of Tierra del Fuego could have been exposed to periods of Antarctic easterly wind circulation and therefore could have been significantly wetter during one or more glacial advances of the Quaternary (Table 1.2A).

Other evidence suggests that climate-induced changes in SHW circulation were more complex. A model used by Markgraf (1989) suggests that the band of SHWs reaching Patagonia was not shifted, but instead focused and restricted to fewer latitudes during cold periods. The important difference in this climate model is that the zonal maximum of the SHWs (northernmost boundary) is expected to have shifted in an opposing and converging direction towards the pole during the same time in which the zonal minimum (southernmost boundary) was forced towards the equator by growing Antarctic sea ice. Support for a poleward shift in the zonal maximum is evidenced by paleoenvironmental pollen records from South America which indicate a southward range expansion of non-xeric floral species from mid-latitude South America into northern Patagonia territory (Markgraf 1987, 1989, 1993; Markgraf et al. 1995). Similar palynological findings have been reported from Australia (Harrison and Dodson 1993). The underlying climate model used to explain this idea indicates that the SHWs were compressed along its northern margins by an imposing transequatorial influence from the Northern Hemisphere. The much larger extent of Arctic ice, which extended throughout the palearctic during the LGM, is expected to have forced a cascading shift in Northern Hemisphere air circulation that would have been great enough to introduce opposing transequatorial influence on the SHWs. This model has important implications for northern Patagonian climate during glacials of the past. The Neuquen basin, the region directly below the contemporary SHE boundary, could have been exposed to moister periods during strong glacial advances in the Northern Hemisphere in which the southward depression of the northern boarder of the SHW allowed the introduction of the tropical SHEs from the north (Table 1.2A).

1.4.4 The Dynamic Atlantic Shoreline of Patagonia

The frequency and amplitude of positional changes in marine shorelines can carry important changes for continental environments, particularly freshwater systems. A dynamic shoreline can change the connectivity of adjacent watersheds by exposing or hiding hydrological contours during rising (transgressing) or falling (regressing) sea-levels. This is particularly true of eastern Patagonia where the gently sloping relief of the Atlantic shelf would have resulted in dramatic changes on the exposed surface area even with small changes in sea-level.

To realize the potential influences of exposed shelf margins on drainage structure, I used geomorphological surface features of continental South America to reconstruct drainage boundaries at varying sea-levels. Geomorphological characteristics were derived from surface contours available from the ETOPO1 1-minute Global Relief database at the National Geophysical Data Centre (Amante and Eakins 2009). Geomorphological analysis of drainage basins was conducted using standard tools available in the software program RiverTools 3.0. First, the RiverTools application was used to extract the contemporary catchment boundaries of eastern Patagonia by restricting catchment outflows to present-day sea-level (Figure 1.4). The estimates of contemporary drainage structure recovered by the RiverTools application accurately reflected catchment boundaries estimated by independent mapping procedures (data not shown). Next, drainage structure of a fully exposed shelf was extracted by lowering catchment outflows to as low as -300m (Figure 1.4). Lastly, relief contours were used to visualize the influences of variable positions in the Atlantic shorelines on drainage structure using regular altitudes of 0m, -50m, -100m and -150m (measured with respect to the present-day Atlantic shoreline, Figure 1.5).

The sea-levels used in Figure 1.5 are arbitrary and used for illustration purposes only, but the range is fully justified. Localized estimates of historical sea-levels on the Argentinean coast are few and limited to Holocene and Late-Pleistocene time periods. In the absence of suitable local measurements of historical sea-levels, I used a composite sea-level curve that depicts a maximum variability interval of global sea-levels throughout the past 500 KY [Figure 8 in Rabineau et al. (2006)]. The curve is derived from 17 concatenated data sources and represents a variety of sampling techniques and geographic regions. The lowest sea-level (i.e. -150m) is a value corrected for isostatic rebound and represents the greatest drop in sea-level over the past 500 KY. It is important to note that this measurement only incorporates a generalized correction for tectonic subsidence and it does not account for any idiosyncratic tectonic response that may be specific to South America.

Figure 1.4 illustrates the hypothesized drainage basins recovered with the RiverTools application using a fully exposed Atlantic shelf and assuming that drainage discharge volumes are similar or less than present day. In the north, the Colorado and Negro rivers appear to remain strongly isolated from each other (despite their close proximity and shared easterly trajectory) as well as from all other eastern Patagonian drainages to the south. Oppositely, watersheds in central Patagonia, i.e. Chubut/Senguer and Deseado, do appear to coalesce within close proximity of the easterly boundary of the continental shelf. Similarly, to the south, the Chico/Santa Cruz, Coyle, Gallegos, Grande, and other small drainages in Tierra del Fuego (collectively the Fuego River) also coalesce at varying altitudes. Overall, four drainage basins can be recognized on a fully exposed shelf: 1) Colorado, 2) Negro, 3) Chubut/Senguer and Deseado, and 4) Chico/Santa Cruz, Coyle, Gallegos, Grande and Fuego. However, stream coalescences become a lot less likely when reasonable sea-levels are considered (Figure 1.5). At -150m, only two stream coalescences can be recognized and they are both limited to the south: 1)

Chico/Santa Cruz, Coyle and Gallegos and 2) Grande and Fuego. These stream coalescences remain possible until 100m below current sea level (Table 1.2C).

1.4.5 Deglaciation Events

As temperatures rebounded following periods of glacial maxima, continental ice gradually melted and deposited large amounts of meltwater onto surrounding landscapes. At times it is likely that the fluvial load created by melting glaciers was so great that water levels swelled beyond the capacity of contemporary drainage footprints and developed unrealized inter-drainage connections.

The most comprehensive insight regarding deglaciation of the former Patagonian Ice Sheet(s) is available in Glasser et al. (2008) where Late-Quaternary history of South America between 38-56 °S was reconstructed by geomorphological mapping subglacial and ice-marginal landforms using high resolution remotely sensed images. A total of 66 main outlet glaciers were recognized, 42 of which terminated along the eastern margins of the ice sheet in eastern Patagonia. Three sections of the ice sheet were recognized: north (38-44°S), central (44-51°S) and south (51-56°S). The northern outlet glaciers (N=20; 38-44°S) were recognized as unique with respect to other outlet glaciers in that they were restricted in extent and formed "alpine-style" valley glaciers. The topographical control of these outlet glaciers left limited opportunity for interactions between individual lobes (Rabassa et al. 2011). Furthermore, most glacial streams in this area are expected to have been minor, barring the basin occupied by contemporary Lake Icalma and the Biobio River of Chile [located immediately northwest of Lake Alumine (Figure 1.3)] which could have supported major glacial streams, but still would have been subject to the same topological constraints (Table 1.2D). Only 7 outlet glaciers were recognized in the central region (44-51°S). However, the lateral connections between the glacial outflows in this region are expected to be high. For example, a continuous deposit of glacial sediment (sandur) links Lakes Palena and Buenos Aires/General Carrera and suggests that this area of more than 280 km (or *ca.* 3° of latitude) was formerly connected by a single major drainage route linked by intersecting streams. Also increasing the likelihood of inter-drainage connections were several large moraine-dammed lakes which dominated this region, including Lake Buenos Aires/General Carrera, Lake Cochrane/Pueyrredon, Lake O'Higgins/San Martin, Lake Viedma and Lake Argentino. This finding is also reinforced by geological evidence for extensive post-LGM paleolakes throughout this region (Clapperton 1993; Tatur et al. 2002; Turner et al. 2005). In the southern region (51-56^{o}S), a total of 15 outlet glaciers were identified. Of particular importance in this region were the numerous marine outlets which now separate the southern mainland from Tierra del Fuego; glaciers repeatedly occupied these low-lying topographic depressions and contained several outlets. In combination with other adjacent outlet glaciers, the unique topography of this region created lateral and marginal meltwater channels and other channels in Tierra del Fuego that were sub-parallel to the former glacial margins and allowed the merger of streams into moraine complexes (Table 1.2E).

Higher drainage discharges expected during deglaciation periods may have also violated the key assumption of inference C (Table 1.2) and formed temporary, but unrealized stream coalescences on the exposed shelf via floodplain formations created by breaches in drainage boundaries. GIS-inferred drainage boundaries illustrated in the previous section were based on the assumption that discharge volumes of eastern drainages were similar to present day and found that stream coalescence was limited to specific areas of the southeast during sea-level stands between approximately 100 and 150 m below present. However, early periods of deglaciation provide an interesting exception in which sea-levels would have not yet transgressed and river discharge volumes would have been significantly elevated. In the event of network breaches, the shallow relief of the exposed continental shelf (Figure 1.6) would have provided a strong possibility for other stream mergers via floodplain formation. These coalescences seem most likely for central and southern parts of eastern Patagonia (i.e. upper and lower southern hypothesized drainage basins, Figure 1.4) in light of the expectedly high glacial melt water output expected for these areas in combination with the shallow relief and close proximity of drainage boundaries (Table 1.2E; Figures 1.4-1.6).

1.4.6 Aquatic Habitat During Glacial Maxima

Varying regional properties in the eastern terminus of the Patagonian ice sheet could have played an important role in supporting aquatic habitat during harsher climates surrounding the LGM. The basal layer of glaciers tends to be distinct from ice that makes up the rest of the glacier and can be quite variable spatially and temporally. Within temperate regions, but depending on topography, the basal layer can fully or partially consist of melt water (wet-based glaciers) because of the intense pressures from overhead ice and the entrapment of geothermal heat (Benn and Evans 1998). Signatures of wet-based glaciers, in the form of ice-scoured bedrock, are limited to locations south of approximately 44^{o}S (Glasser et al. 2008). The implications are that areas to the north are expected to be cold-based glaciers and devoid of basal melt-water. Oppositely, areas to the south likely hosted an abundance of wet-based glaciers and likely served as important sources of exogenous water for aquatic habitat within periglacial margins during glacial maxima of the past.

Recent work on the formation and deposition of Patagonia gravels (Rodados Patagonicos) throughout eastern Patagonia also supports the likelihood of aquatic environments persisting throughout glacial maxima (Martinez and Kutschker 2011). This work revealed that the deposition of Patagonian gravels occurred during a continous period of high fluvial energy in Late Cenozoic which persisted throughout several glacial cycles. Stream outflow during this time was determined by a host of factors (e.g. tectonics, sea-level, seasonality) that made it possible to maintain strong outflow even throughout peak glacial advances.

1.4.7 Trans-Andean Drainage Reversals

The formidable presence of glacial ice and its continuous position along Andes during the LGM carried important consequences for drainage patterns of trans-Andean systems. During the period surrounding the LGM it is believed that the headwaters of many, if not all, trans-Andean systems were blocked by a western barrier of ice and forced to drain into the Atlantic. The contemporary drainage direction of trans-Andean systems into the Pacific was only made available following the collapse of glacial dams that formerly bridged Andean peaks. For example, the Rio Baker which currently drains Lakes General Carerra/Buenos Aires and Pueyrredon/Cochrane (Lat. 46-47^{0}S) formed after the LGM when discharge for these two lakes abruptly changed direction and began to flow west when the ice barrier that had formed the western margin of a large paleolake broke *circa* 12,000 BP (Turner et al. 2005). Similar post-LGM headwater diversion between the Atlantic and Pacific during climatic oscillations could have also occurred in parallel in other parts of Patagonia involving other trans-Andean drainages (Hua-Hum, Puelo-Manso, Futaleufu, Palena, Pascua) (Table 1.2F). Furthermore, it is possible that similar diversions were occurring at these sites during more ancient glacial cycles considering that all trans-Andean basins are expected to have been in place since the beginning of Andean uplift during the Miocene (Coronato et al. 2008).

1.4.8 Summary of Climatic Influences on Historical Drainage Structure

It is clear that present-day lake and river drainage networks of eastern Patagonia represent only a snapshot in time and are no way representative of the variety of forms that freshwater catchments in this region are expected to have assumed throughout the Quaternary. The advancing ice and enhanced aridity during glacial periods is generally thought to have stressed aquatic habitat in eastern Patagonia. However, evidence from Patagonian gravel deposition suggests that aquatic habitats were plentiful during the Late Cenozoic. Additional run-off during glacial periods could also have derived from wet-based glaciers,or latitudinal shifts in the SHWs. The position of the Atlantic shoreline was also of importance. Sea-level is currently located at the very high end of the altitudinal spectrum and has fragmented a few large freshwater catchment basins into many smaller and more numerous watersheds by blanketing enormous surfaces of the continental shelf under seawater. A historically regressing shoreline is expected to have connected many sub-basins of the Southern Patagonian Tablelands. Glacial-induced marine regression within a range of -150m and -100m is expected to have created stream coalescence on an exposed continental shelf within two regions of south-eastern Patagonia: 1) the linking of the Chico/Santa Cruz, Coyle and Gallegos river basins of the southern mainland, and 2) the fusion of the Grande and Fuego river basins of Tierra del Fugeo. Subsequent periods of deglaciation brought immense hydrological discharge which also altered aquatic landscapes. This period is thought to be particularly important for eastern landscapes that were again located below 44°S. Central- and south-eastern Patagonia experienced immense opportunities for inter-drainage connections in periglacial territory via stream collisions and paleolake formation, in addition to enhanced opportunity for distal stream coalescence on an exposed shelf. Finally, late glacials would have also experienced the reversal of several trans-Andean headwaters from Atlantic → Pacific. This likely occurred on a regular basis throughout the Quaternary. Overall, the transition between glacial and inter-glacial would have dramatically influenced the sizes, connectivity and availability of aquatic habitat within, and between eastern- and western- parts of Patagonia throughout the Quaternary and would have had very important implications for the demographic and phylogeographic history of native species of Argentina.

1.5 Biological Significance of Climate-Induced Drainage Evolution

Patagonian phylogeography still remains in its infancy, particularly for freshwater taxa. The most comprehensive phylogeographic studies that cover the regions of interest are limited to the freshwater fishes *P. trucha* and *G. platei*, although some information can likely be gleaned from a study of the diadromous fish *G. maculatus* which included several Argentinean locations in the analysis (Ruzzante et al. 2006, 2008; Zemlak et al. 2008, 2010). Therefore, to supplement the signal available for fishes, I drew from the other phylogeographic literature available for terrestrial animals and plants. I believe that inclusion of information from temperate forest tree species is particularly safe and useful considering the close association between present day forests and lakes in Argentina and the very similar thermal and moisture requirements shared by freshwater and temperate forest taxa.

1.5.1 Freshwater Refugia in Eastern Patagonia

Mid- to low-latitude regions of previously glaciated continents have repeatedly been recognized as general areas of refuge for cold- temperate species (Stewart et al. 2010). For example, the mid- to high-latitude areas of North America and Europe were frequently inundated by extensive ice sheets during the Quaternary. Fossil pollen records and many phylogeographic studies from these regions show concordant evidence that the many temperate species were forced by advancing glaciers to shift their ranges to lower latitudes to occupy unglaciated regions, termed ‘southern’ refugia (see Stewart et al. 2010 and references therein). The mirror opposite of this pattern is expected for areas of the Southern Hemisphere influenced by ice sheets, i.e. glacial refugia were probably available to the north and many species ranges were likely shifted toward lower latitudes (Stewart et al. 2010).

Within this context, ‘northern’ refugia in Patagonia were probably most important for species and populations west of the Andes where the ratio of glacial ice to exposed surface area was highest. Indeed, several others authors have recognized the northern parts of Chilean Patagonia and south-central Chile as physically and climatically stable over long evolutionary time based on reports of high population- and species-level biodiversity (Dyer 2000; Premoli et al. 2000; Muellner et al. 2005; Ruzzante et al. 2006). At least one northern refuge is also expected at similar latitudes on the eastern side of the Andes according to the genetic signal available in some lineages of trees (discussed in more detail below) and at least one fish species, *P. trucha* (Ruzzante et al. 2006; Ruzzante et al. 2008). Cryptic refugia at higher latitudes are also likely in other parts of Patagonia on both eastern and western sides of the Andes. Corroborative signals from two freshwater species (*Aegla alacalufi*, *G. platei*) and *Nothofagus* trees suggest that the area surrounding Lake Riesco of southwestern Patagonia is likely to have served as an ice-free refuge in the past (Markgraf et al. 1996; Zemlak et al. 2008; Xu et al. 2009). Accumulating evidence also suggests that cryptic refugia could have also existed east of the Andes at higher latitudes (Zemlak et al. 2008; Mathiasen and Premoli 2009; Pastorino et al. 2009; Tremetsberger et al. 2009; Cosacov et al. 2010). The following focuses on consolidating and refining what we know about refugia east of the Andes. I specifically discuss the likelihood of cryptic refugia at higher latitudes and speculate about the possible number and location(s) of eastern refugia.

One overriding pattern that arises from the phylogeographic literature is the fragmented genetic structure of broadly distributed Argentinean species. This suggests that eastern taxa survived in more than one refuge in the past. Strong genetic structure has been reported for multiple tree species, including: *Hypochaeris incana*, *Calcelaria polyrhiza*, *Nothofagus pumilio* and *N. antarctica* (Mathiasen and Premoli 2009; Pastorino et al. 2009; Tremetsberger et al. 2009; Cosacov et al. 2010). The freshwater puyen, *G. platei*, also shows evidence of strong genetic structure within its eastern lineage suggesting the historical segregation of ancestral populations into two refugia (Zemlak et al. 2008). Several terrestrial animals also show evidence of allopatric separation, including the rodent *Oligoryzomys longicaudatus* (Kim et al. 1998) and several lizard species of the genus *Liolaemus* (Avila et al. 2007; Morando et al. 2007). Cumulatively, these patterns suggest that one or more southern cryptic refugia existed in parallel to a northern refuge in eastern Patagonia.

Several taxa also show genetic evidence that is consistent with long-term *in situ* habitation of eastern Patagonia without altered distributions and/or gene flow. For example, Jakob et al. (2009) suggested that several plant species of the genus *Hordeum* were able to survive on the Patagonian Steppe throughout the LGM without changes in their extant distribution ranges and without demographic consequence. The phylogeographic signal even suggested the survival of large populations of *Hordeum* species throughout southernmost Patagonia and Tierra del Fuego despite contradicting expectations from ecoclimatic niche modeling (Jakob et al. 2009). The rodent species *Abrothrix olivaceus* and *Phyllotis xanthopygus* also have very broad distributions throughout Argentina and Chile, but demonstrate very little genetic structure over their entire ranges according to the mitochondrial locus cytochrome *b* (Pearson and Smith 1999; Smith et al. 2001). The minor disruption imposed by the Last Glacial on the aforementioned species suggests that the Steppe region is likely to have remained amenable to life throughout one or more glacials.

The impressive scope of phylogeographic studies concerning several species of temperate forest trees provides the best starting point for the speculation about the possible locations of refuges in eastern Patagonia. Tremetsberger et al. (2009) recognized three distinct geographic lineages within the eastern Patagonian species *H. incana*: northern Patagonia (~38-44°S), central Patagonia (~46-50°S) and southern Patagonia and Tierra del Fuego (~50-54 °S). Argentinean populations of *C. polyrhiza* are also genetically fragmented (Cosacov et al. 2010). Two relict lineages are spatially restricted to high Andean habitat between ~30-37°S, with a structural break at approximately 35°S. To the south, and distributed throughout Patagonian territory, are two additional lineages with ranges highly overlapping between 42-48°S suggesting that this area represents an area of secondary contact and was re-colonized from separate refugia located to the north and south . Similar patterns are also found in the native beeches. Mathiasen & Premoli (2009) recognized two distinct lineages of *N. pumilio* that likely represent separate northern (~35-42°S) and southern (~42°S-54°S) ranges. This pattern was also revealed in the closely allied and co-distributed *N. antarctica* which also revealed a break at ~42°S (Pastorino et al. 2009). Paleoclimate reconstructions based on the pollen of *Nothofagus* species also led Markgraf et al. (1996) to recognize three vegetative zones within Patagonia: south of 51°S, between 51°S and 43°S and north of 43°S.

Overall, a strong trend is present in comparisons of co-distributed forest tree species. The range of most eastern tree species can be divided into 2 or 3 major refugial lineages, each dominating a certain latitudinal zone along a north ←→ south orientation. North, central and southern lineages are recognized, but the most consistent division is between north and central locations.

An important concordance between many forest trees and *G. platei* is the presence of a common southern lineage east of the Andes. The eastern lineage of *G. platei* shows a strong sub-division in which a separate southern lineage (haplogroup 4) is distributed between (~47-54°S) and forms a zone of secondary contact at 47°S (Lake Pueyrredon/Cochrane) with the aforementioned and centrally located haplogroup 3 [Zemlak et al. (2008), Figure 1.7]. Although post-glacial expansion has made it difficult to gauge the exact whereabouts of potential refugia for aquatic species in the central and northern parts of eastern Patagonia, it does appear that there is at least partial concordance between forest trees and *G. platei* haplogroup 3 to suggest that several species were able to survive in the same general area in the southernmost part of Patagonia during previous glacials. Similar territory is also thought to have been shared by at least one Steppe plant species (Jakob et al. 2009) and a rodent (Smith et al. 2001) throughout the LGM. Therefore, it seems very likely that a cryptic refuge existed in the southern most part of eastern Patagonia which likely served as suitable area for several cold-adapted species and may represent a location similar to that which was available to North American species in eastern Beringia (Table 1.2G).

The strong divide between north and central populations is not revealed in fishes. *Percichthys trucha* reveals minimal structure over its entire distribution throughout Argentinean Patagonia which covers both northern and central territory (33-47°S) [Figure 1.7, Ruzzante et al. (2006)]. The more southerly distributed freshwater puyen, *G. platei*, also shows a seamless connection at the very northern extent of its range. Several samples from Lake Espejo of the Neuquen Basin share mitochondrial control region haplotypes with Lakes Puelo and Rivadavia of the Northern Patagonian Tablelands (Zemlak et al. 2008). The aforementioned locations also share very strong genetic similarities with two other centrally located lakes, including Lake La Plata and Lake Pueyrredon, all of which derive from the northeastern haplogroup lineage of *G. platei* [haplogroup 3 in Zemlak et al. (2008)]. At this point it is uncertain whether this lineage shared a common refuge with *P. trucha*. It is possible that each *P. trucha* and *G. platei* haplogroup 3 existed in separate central and northern refuges and only recently became co-distributed following strong post-glacial dispersal. It is also possible that each occupied a similar refuge in either central or northern parts of eastern Patagonia.

1.5.2 Historical Drainage Connections

Proglacial lakes have been recognized as important corridors of dispersal for many freshwater organisms inhabiting the previously glaciated regions of the Northern Hemisphere. For example, the accumulations of meltwater that tracked the terminus of the Laurentide Glacier across North America also translocated fishes thousands of kilometers and helped establish extensive present-day ranges. Similar processes are also likely to have been operating in Patagonia but on a comparatively reduced scale and influence (Rabassa et al. 2011). Two phylogeographic studies on fishes and crabs suggest that proglacial lakes were important mechanisms of southward post-glacial expansion in western Patagonia (Zemlak et al. 2008; Xu et al. 2009). The importance of proglacial lakes is also invoked east of the Andes to help explain the broad distribution of unstructured populations of *G. platei* within the Southern and Northern Patagonian Tablelands (Zemlak et al. 2008). This review reports independent evidence for extensive paleolakes in central and southern regions of eastern Patagonia and reinforces the idea that moraine-damned meltwater has played important roles in redistributing aquatic life in this region.

Another important pattern in fishes arises that can only be explained by a recent connection between the Neuquen Basin and the Northern Patagonian Tablelands via proglacial lake(s). *Percichthys trucha* has minimal structure over its entire distribution throughout Argentinean Patagonia which covers both northern and central territory (33-47^{o}S) (Ruzzante et al. 2006). *Galaxias platei* is also devoid of structure in the northernmost extent of its range which reaches into the Neuquen Basin (i.e. Lake Espejo) (Zemlak et al. 2008) (Figure 1.7). The coalescence between the aforementioned basins on an exposed shelf is highly unlikely (Figures 1.4, 1.5). The only likely explanation is a temporary connection between basins to the east of the Somun-Cura Massif at the only shared boarder between these two catchments. I suggest that the paleolake which formerly occupied the Nahuel Huapi Lake region was an important connection between the Neuquen and Northern Patagonian Tablelands in recent history (Table 1.2H).

The contribution of distal stream coalescences was also likely an important contributor of post-glacial expansion for aquatic organisms in central and southern parts of eastern Patagonia. This essay identified two coalescent points in southern Patagonia that are expected to have created inter-drainage connections among presently isolated drainages of both the southern mainland and Tierra del Fuego (Table 1.2C). Furthermore, inference 2E predicts that introduction of one or more floodplains via melt water fluxes during the early periods of deglaciations could have created other unrealized connections within and/or between drainages of the Northern and Southern Patagonian Tablelands. Floodwaters associated with the deposition of Patagonian gravels in the Late Cenozoic could have also created connections on an exposed continental shelf during glacial periods (Martinez and Kutschker 2011; Ponce et al. 2011). More importantly, however, the genetic structure of *P. trucha* throughout eastern Patagonia is best explained by inter-drainage connections on the Patagonian Steppe. Therefore, it still remains uncertain to what degree proglacial lakes and associated streams *versus* distal stream coalescences have contributed to the impressive present-day distributions of freshwater fishes. It is likely that both played important roles, but the influence of each mechanism varies according access (i.e. latitude, species ecology).

1.5.3 Drainage Reversals

Contemporary species- and population-level diversity in South America implies that glacial-induced drainage reversals of the Quaternary played important roles in shaping the contemporary distributions of freshwater fishes of Patagonia. For example, a recent phylogeographic study of the freshwater fish *G. platei* concluded that drainage reversals were an important mechanism of dispersal across the Andean divide from east → west at least once in the past (Zemlak et al. 2008). The study revealed 4 well-structured mtDNA control region haplotype groups in which 2 were exclusively limited to western Patagonia (Chile) and 2 were primarily limited to the east (Argentina). However, several Argentinean haplotypes were also recovered from, or near, Chilean lakes and rivers belonging to trans-Andean systems. Analysis of Molecular Variation Analysis (AMOVA) tests also revealed that the inhabitants of trans-Andean systems are more closely allied with those of Argentinean systems than to those occupying Chilean catchments, a result which showed that historical drainage patterns prior to the LGM is a better predictor of genetic structure than present day drainage connections. These results imply that drainage reversals were important potential mechanisms of trans-Andean dispersal for several native fishes and were likely available to other aquatic taxa at multiple locations in Patagonia.

Another phylogeographic study involving the closely related, but diadromous species *G. maculatus* also showed evidence that population structure was at least partially influenced by drainage reversals (Zemlak et al. 2010). Although the contemporary distribution of *G. maculatus* is best explained by marine dispersal from Chile to Argentina via the southernmost tip of South America, drainage reversals do appear to have exerted at least some influence on the genetic structure involving northern drainages. Some members of, or near, the Hua-Hum river and located in the Valdivia basin (trans-Andean) appear to have been exchanged from either side of the Andean mountain range, implying bidirectional gene flow. The translocation of individuals from the east → west is concordant with patterns for *G. platei* and can be explained by the reversal of drainage direction of the Hua-Hum headwaters from Atlantic to Pacific when glaciers retreated. However, it is unclear how western haplotypes of *G. maculatus* ended up in the east.

The phylogeographic pattern in the creole perch, *P. trucha*, also implies that trans-Andean dispersal occurred in both directions, although these events were likely isolated. A rooted phylogenetic tree of haplotypes reveals 3 distinct clusters, two of which are located west of the Andes and another which is widespread east of the Andes (Ruzzante et al. 2006). The most basal and well supported group was recovered from only a single location (Itata River) in the west. The largest and most widespread cluster is found primarily throughout the central and northern parts of eastern Patagonia. The third and least supported is geographically located in western Patagonia (Andalien and Bio Bio rivers) but is genetically more closely allied to those haplotypes of Argentina. This structure is most consistent with an initial dispersal from west → east followed, by population expansion before a subsequent return of some individuals back into the west.

Although climate-induced drainage reversals which ensued following the LGM can explain the transfer of fishes from east → west, it is still uncertain how trans-Andean dispersal could have been facilitated in the opposite direction. I have created a new model, which I have called the *headwater diversion hypothesis*, which could explain how climate-induced drainage reversals could be a bidirectional mechanism of gene flow for aquatic organisms. The primary assumption of the *headwater diversion hypothesis* is that trans-Andean catchments existed prior to at least one glaciation, which seems reasonable in light of the expected ancient age of these systems and the similar patterns of ice advance throughout the Quaternary. I therefore construct the model based on the premise that gross continental drainage patterns were similar to contemporary drainage patterns of Patagonia in that Chilean, Argentinean and trans-Andean systems were all present prior to the last glaciation (Figure 1.8A). As temperatures dropped and ice began to descend from the peaks of the Andes, encroaching glaciers would have likely severed the eastern headwaters of trans-Andean systems from other Pacific draining components and created a diversion of headwaters (and their contents) towards the Atlantic (Figure 1.8B). This would have eliminated trans-Andean drainage patterns, increased the number of Argentinean systems, and served as the first opportunity for dispersal from west → east. Dispersal from west → east would have been dependent on the organism occupying headwaters during this time. The Atlantic drainage of trans-Andean headwaters would have persisted for long periods of time as glaciers grew to their maximum extent (Figure 1.8C). During subsequent deglaciation events, it is likely that the pooling of meltwater created the formation of large proglacial lakes that would have created the opportunity for inter-drainage mixing between eastern drainages (Figure 1.8D). The pooling of meltwater would have simultaneously placed a tremendous amount of pressure on the thinning glacial dams located to the west. The collapse of Andean glacial dams to the west would have initiated the beginning of what would be a drainage reversal of trans-Andean headwaters towards the Pacific and presented the second opportunity for dispersal from east → west (Figure 1.8E). Depending on the age of trans-Andean systems, the diversion of trans-Andean headwaters could have been an important mechanism of trans-Andean dispersal for aquatic organisms on multiple occasions as climates oscillated throughout the Quaternary (Table 1.2I).

1.6 Conclusion

Climatic oscillations of the past 1.8 MY interacted with major topographic features of Patagonia to create very important changes in the aquatic landscapes that certainly held important implications for native diversity and biogeography. This synthesis presents several inferences concerning the likely influences that Quaternary processes imparted on aquatic species of eastern Patagonia (summarized in Table 1.2). Periods of glacial advance played an important role in grafting additional genetic structure within eastern lineages by separating populations of a variety of taxa into multiple refugia that are distributed in a north to south orientation along the primary Cordillera. It is uncertain whether a separate northern and central refuge existed for aquatic organisms, but it appears very likely that a common refuge was available in the south for several cold-adapted taxa, including at least one species of freshwater fish. Northern and southern aquatic refugia appear feasible from a paleoclimate perspective. The dominant position of the band of SHWs is expected to have shifted either to the north or south during glacial maxima, a change which could have introduced important sources of moisture from adjacent easterly wind circulation cells. Wet-based glaciers are also likely to have fed peri-glacial environments at various locations throughout latitudes south of 44°S and supported aquatic refugia in central and southern regions. Lowered sea-levels during peak glacials are also expected to have created a limited number of opportunities for inter-drainage stream coalescence on an exposed continental shelf, especially during the Late Cenozoic when mountain runoff is expected to have remained high throughout several glacial cycles. Temporary connections among adjacent drainages within, but not between, the southern mainland and Tierra del Fuego were likely formed during sea-level low-stands and could have contributed to the low genetic structure seen in the cold-adapted freshwater fish, *G. platei*. However, subsequent periods of deglaciation would have provided the opportunity for inter-drainage mixing. The eastern margin of the Patagonian icesheet is expected to have expelled enormous amounts of melt water towards the Atlantic, particularly at central and southern latitudes. It is very likely that additional stream mergers occurred on the exposed shelf to the east of the Patagonian Tablelands via floodplains caused by breaches in boundaries of drainage networks. Furthermore, several moraine-dammed paleolakes and stream connections are expected to have joined the headwaters of several adjacent catchments of the Northern and Southern Patagonian Tablelands. Although paleolake formation is considered to have been more substantial in central and southern locations, it does appear that at least one large paleolake was likely in Nahuel Huapi Lake region to explain a former connection between the Nequen Basin and the Northern Patagonian Tablelands. Finally, a cascade of events associated with transitions between glacial and inter-glacial periods appear to have interacted with the topography of trans-Andean river drainages to create an effective mechanism of bidirectional dispersal between eastern and western Patagonia for aquatic organisms and were available in several parallel sites on multiple occasions throughout the Quaternary.

Table 1.1 A summary of phylogeographic studies conducted in Patagonia. Species targeted by each study are grouped according to taxonomy and habitat. Also specified is the genetic tool(s) employed, the total number of locations and individuals sampled and the corresponding map window in Figure 1.1 for which the geographic scope of the study is illustrated.

Habitat grouping	Taxa group	Species	Citation	Method(s)	No. Loc.	No. Ind.	Fig. Pos.
Aquatic Animal	Freshwater Crab	*Aegla alacalufi*	Xu et al. 2009	DNA sequence	14	183	A
	Freshwater Fish	*Percichthys trucha*	Ruzzante et al. 2006	DNA sequence	14	355	A
			Ruzzante et al. 2008	DNA sequence	36	552	A
		Percilia spp. complex	Ruzzante et al. 2006	DNA sequence	7	18	A
		Galaxias platei	Ruzzante et al. 2008	DNA sequence	25	166	A
			Zemlak et al. 2008	DNA sequence	25	212	A
	Diadromous Fish	*Galaxias maculatus*	Zemlak et al. 2010	DNA sequence	36	299	A
Terrestrial Animal	Lizard	*Liolaemus melanops* complex	Avila et al. 2007	DNA sequence	22	74	B
		Liolaemus fitzingerii complex	Avila et al. 2007	DNA sequence	23	69	B
		Liolaemus donosobarrosi group	Avila et al. 2007	DNA sequence	7	18	B
		Liolaemus boulengeri complex	Avila et al. 2007	DNA sequence	12	31	B
		Liolaemus gracilis	Morando et al. 2007	DNA sequence	21	32	B
		Liolaemus bibronii complex	Morando et al. 2007	DNA sequence	41	105	B
		Liolaemus tenuis	Victoriano et al. 2008	DNA sequence	41	144	B
		Liolaemus lemniscatus	Victoriano et al. 2008	DNA sequence	14	52	B
		Liolaemus pictus	Victoriano et al. 2008	DNA sequence	15	82	B
	Mammal	*Abrothrix olivaceus*	Pearson & Smith 1999	DNA sequence	13	26	B
			Pearson & Smith 1999	Allozyme	9	18	B
			Smith et al. 2001	DNA sequence	29	39	B
		Ctenomys sociabilis	Chan et al. 2005	DNA sequence	5^	53^	B
		Oligoryzomys longicaudatus	Kim et al. 1998	DNA sequence	31	33	B
		Phyllotis xanthopygus	Palma et al. 2005	DNA sequence	10	58	B

Table 1.1 continued

Habitat grouping	Taxa group	Species	Citation	Method(s)	No. Loc.	No. Ind.	Fig. Pos.
Terrestrial Plant		*Araucaria araucana*	Bekessy et al. 2002	RAPD	13	192	C
		Austrocedrus chilensis	Pastorino et al. 2002	Isozyme	15	387	D
			Pastorino et al. 2004	Isozyme	15	387	D
			Pastorino & Gallo 2009	Isozyme	27	746	D
			Arana et al. 2010	Microsatellite	14	30-32/loc.	D
		Calceolaria polyrhiza	Cosacov et al. 2010	DNA sequence	68	590	C
		Fitzroya cupressoides	Allnutt et al. 1999	RAPD	12	89	C
			Premoli et al. 2000	Isozyme	24	720	D
		Hordeum patagonicum	Jakob et al. 2009	DNA sequence	184*	164	D
		Hordeum pubiflorum	Jakob et al. 2009	DNA sequence	184*	286	D
		Hordeum comosum	Jakob et al. 2009	DNA sequence	184*	472	D
		Hypochaeris incana	Tremetsberger et al. 2009	AFLP	28	580	C
			Tremetsberger et al. 2009	DNA sequence	32	2-3/loc.	C
		Hypochaeris palustris	Muellner et al. 2005	AFLP	21	206	D
		Nothofagus antarctica	Pastorino et al. 2009	RFLP	12	60	D
			Pastorino et al. 2009	Isozyme	12	674	D
		Nothofagus nervosa	Marchelli et al. 1998	RAPD	11	22	C
			Marchelli & Gallo 2004	Isozyme	20	Not Avail.	C
			Marchelli & Gallo 2006	RFLP	26	133	C
		Nothofagus obliqua	Azpilicueta et al. 2009	RFLP	27	154	C
		Nothofagus pumilio	Mathiasen & Premoli 2009	DNA sequence	85	194	C
				Isozyme	41	1183	C
		Pilgerodendron uviferum	Allnutt et al. 2003	RAPD	16	192	D
		Podocarpus nubigena	Quiroga & Premoli 2010	Isozyme	14	354	C

^ *Sample sizes do not include 33 partial sequences collected from fossil teeth using ancient DNA techniques.*

* *Total number of sample locations in Jakob et al. 2009; an individual species breakdown is not available in the published version.*

Table 1.2 A summary of inferences presented in the Chapter 1 review concerning Quaternary drainage evolution in Patagonia and the associated implications for aquatic taxa.

Drainage evolution

A. Changes in the position and/or shape of the band of SHWs over Patagonia during glacial periods could have relieved parts of eastern Patagonia from the drier climates expected during full glacials. Parts of the Nequen Basin (north), and the southernmost areas of Tierra del Fuego (south), could have instead been under the influence of bordering easterly wind circulation which would have carried more precipitation and potentially supported aquatic environments via enhanced precipitation.

B. Wet-based glaciers were likely prevalent south of 44°S in eastern Patagonia and important sources of exogenous water during glacial periods.

C. Lowered sea-levels within a range of approximately -150m and -100m are expected to have created stream coalescence within two regions of south-eastern Patagonia: 1) southern mainland (Chico/Santa Cruz, Coyle and Gallegos) and 2) Tierra del Fugeo (Grande, Fuego). No coalescence is expected to have taken place between the Colorado and Negro drainages, nor between the Chubut/Senguerr and Deseado rivers within this range of lowered sea-level. This hypothesis hinges on the assumption that river flow volumes did not increase during periods of low sea stands.

D. River drainages at the eastern foothills of northern Patagonia (i.e. 38-44°S) are expected to have remained highly fragmented with low meltwater discharge.

E. Periods of deglaciation likely created temporary connections between the headwaters of several adjacent drainage basins within each of central (44-51°S) and southern (51-56°S) parts of eastern Patagonia. Early in periods of deglaciation, and prior to significant marine transgression, the increased river discharge in central and southern parts of eastern Patagonia may have increased chances of inter-stream coalescence on an exposed shelf through stream swelling over the limits of inferred drainage networks (but see hypothesis C).

F. The diversion of the Hua-Hum, Puelo, Futaleufu, Palena, Baker and Pascua (trans-Andean) river headwaters from Atlantic to Pacific is likely to have occurred repeatedly throughout the Quaternary.

Table 1.2 continued

Biological Implications

G. A cryptic refuge in south-eastern Patagonia is likely to have existed for cold-adapted species during one or more glacial periods of the Quaternary.

H. The southernmost part of the Negro basin (i.e. Limay River) was temporarily connected with the Chubut river following the LGM through a large paleolake occupying the Nahuel Huapi Lake region. This formed an important gateway of dispersal for aquatic organisms between the Nequen Basin and Northern Patagonian Tablelands.

I. Trans-Andean river systems of Patagonia played an important role in the bidirectional dispersal of aquatic organisms between the east and west. Advancing ice sheets first divert the Pacific-oriented headwaters and its biological contents towards the Atlantic to create an opportunity for east to west dispersal. The subsequent period of glacial retraction allows mixing of eastern populations within proglacial lakes before the headwaters are again allowed to return to its original orientation of draining into the Pacific, thus completing west to east dispersal.

A) Aquatic animals

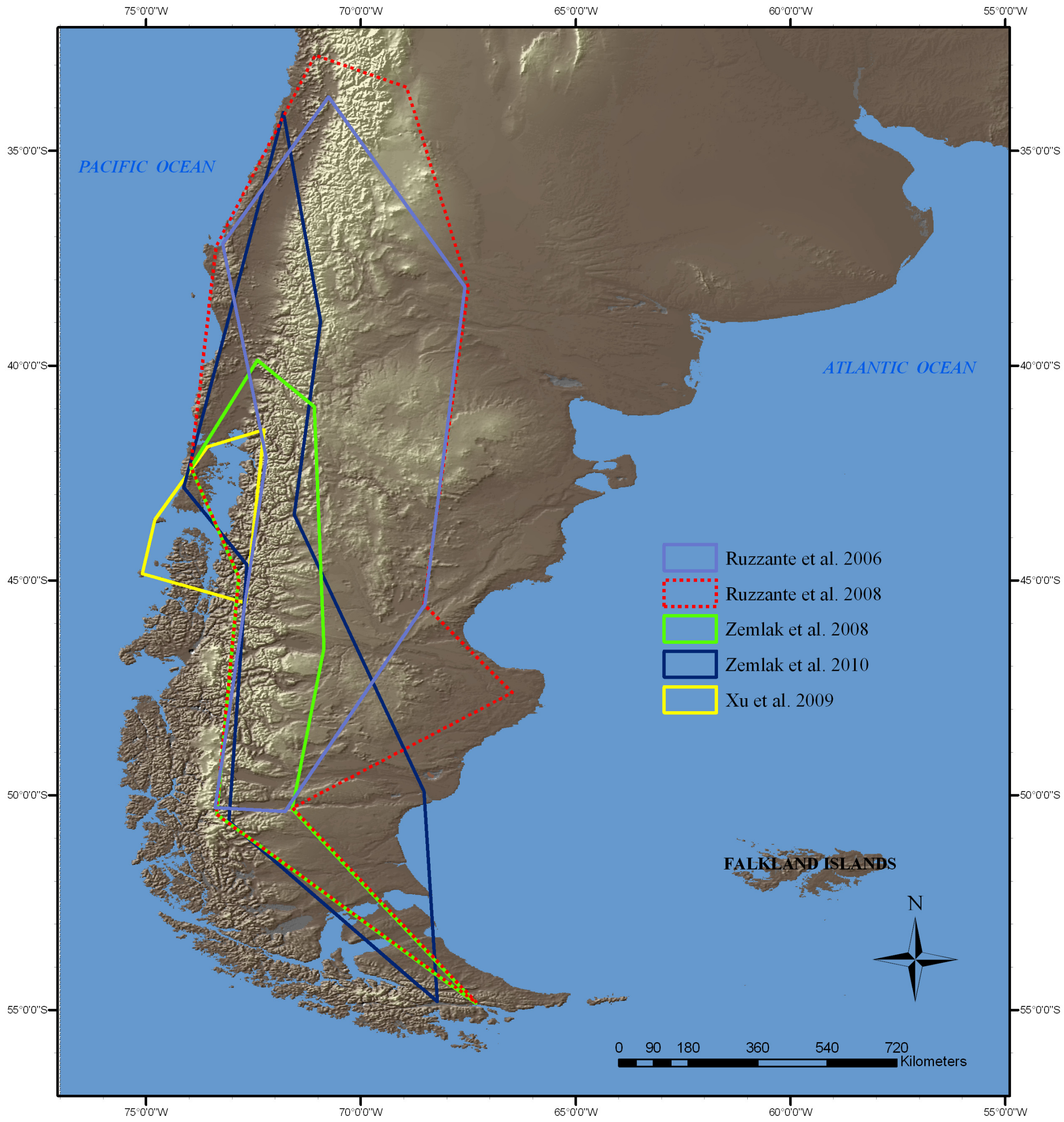

Figure 1.1 The geographic scope of phylogeographic studies conducted in southern South America. The polygons used to represent the geographic range of each study (summarized in Table 1.1) were created by plotting the peripheral sampling locations and therefore represent an approximateion. The figure is divided into quadrants based on taxonomy and habitat: A) aquatic animals, B) terrestrial animals, C) and D) terrestrial plants.

B) Terrestrial animals

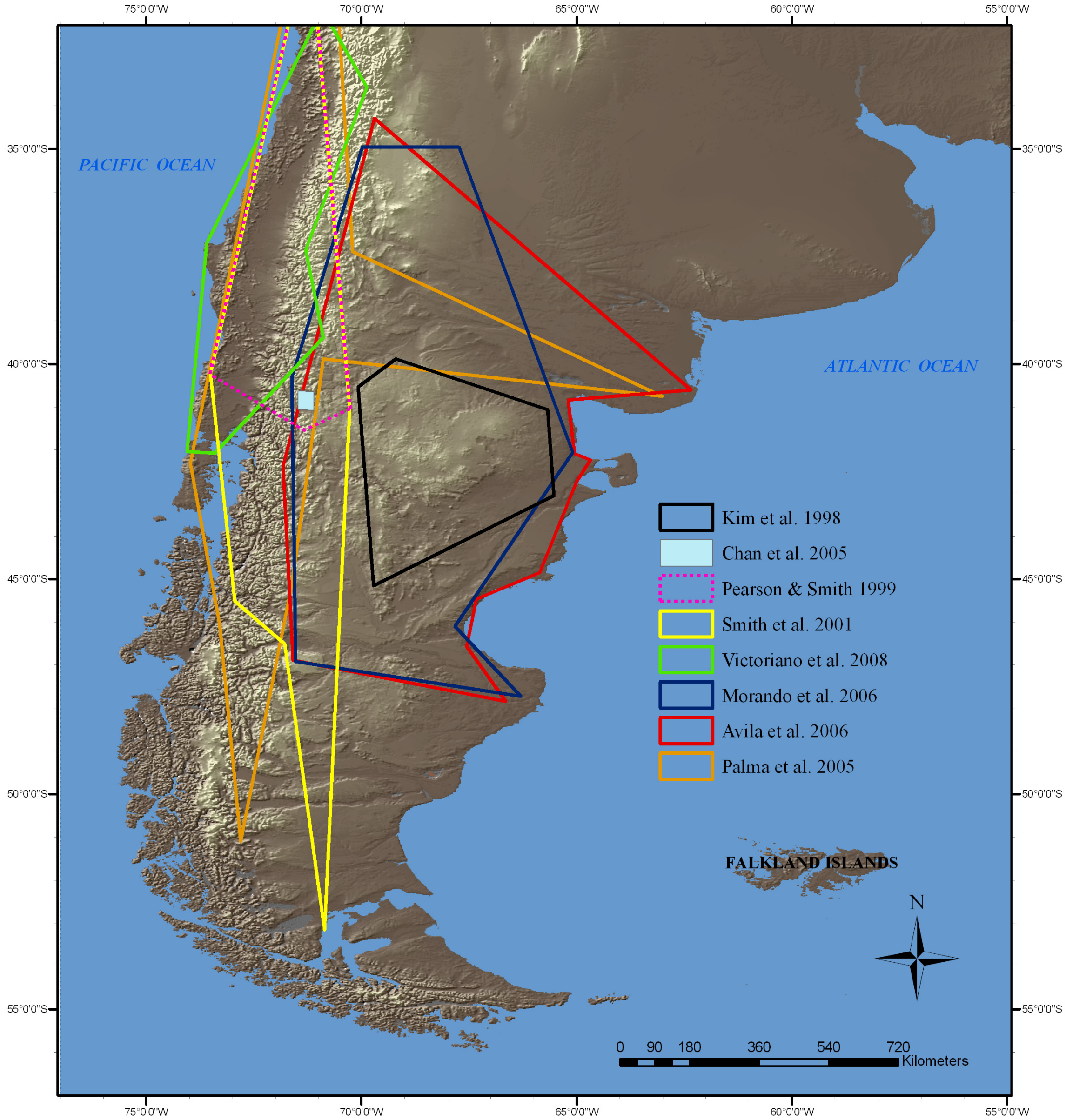

Figure 1.2 continued

C) Terrestrial plants

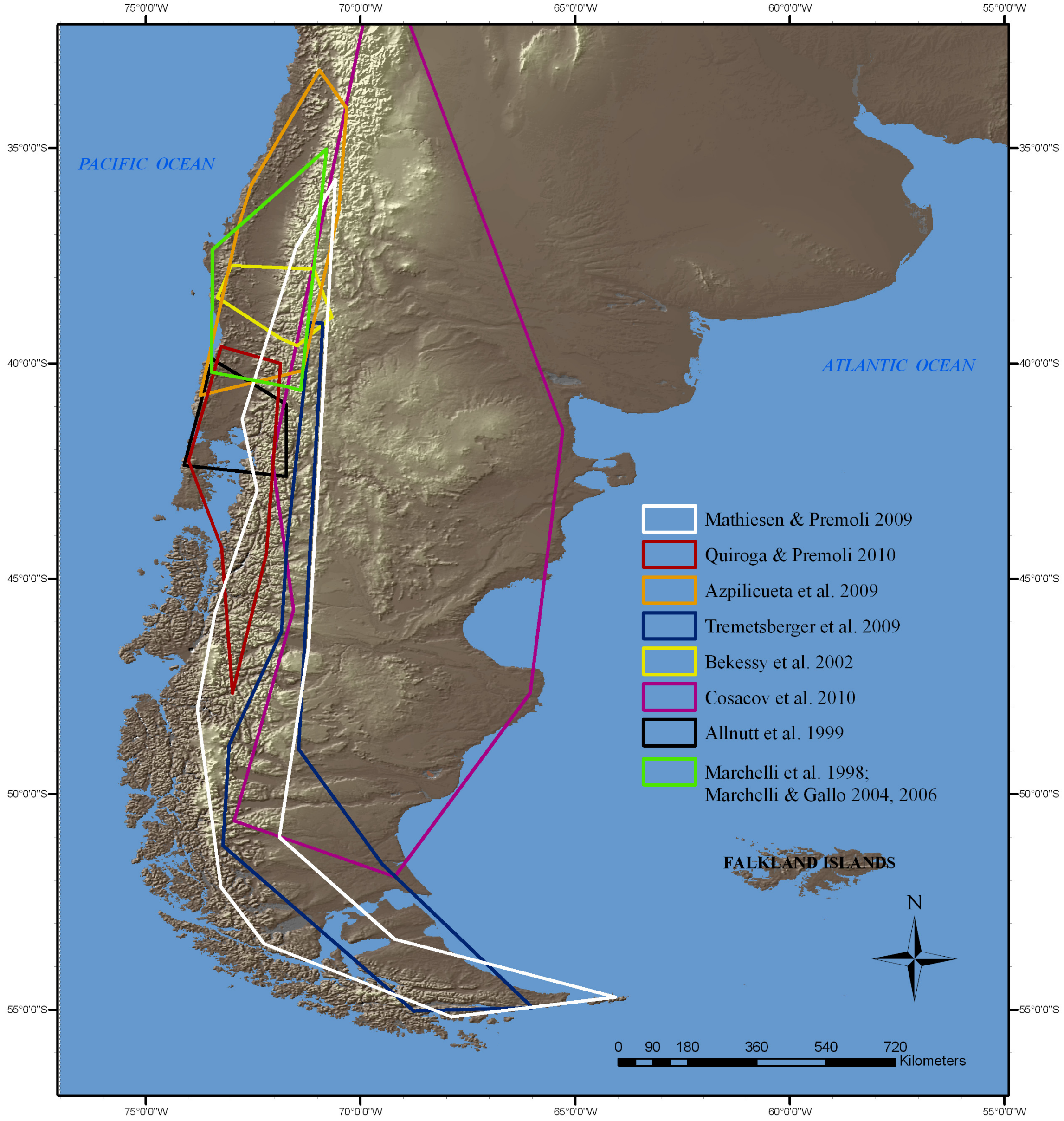

Figure 1.2 continued

D) Terrestrial plants

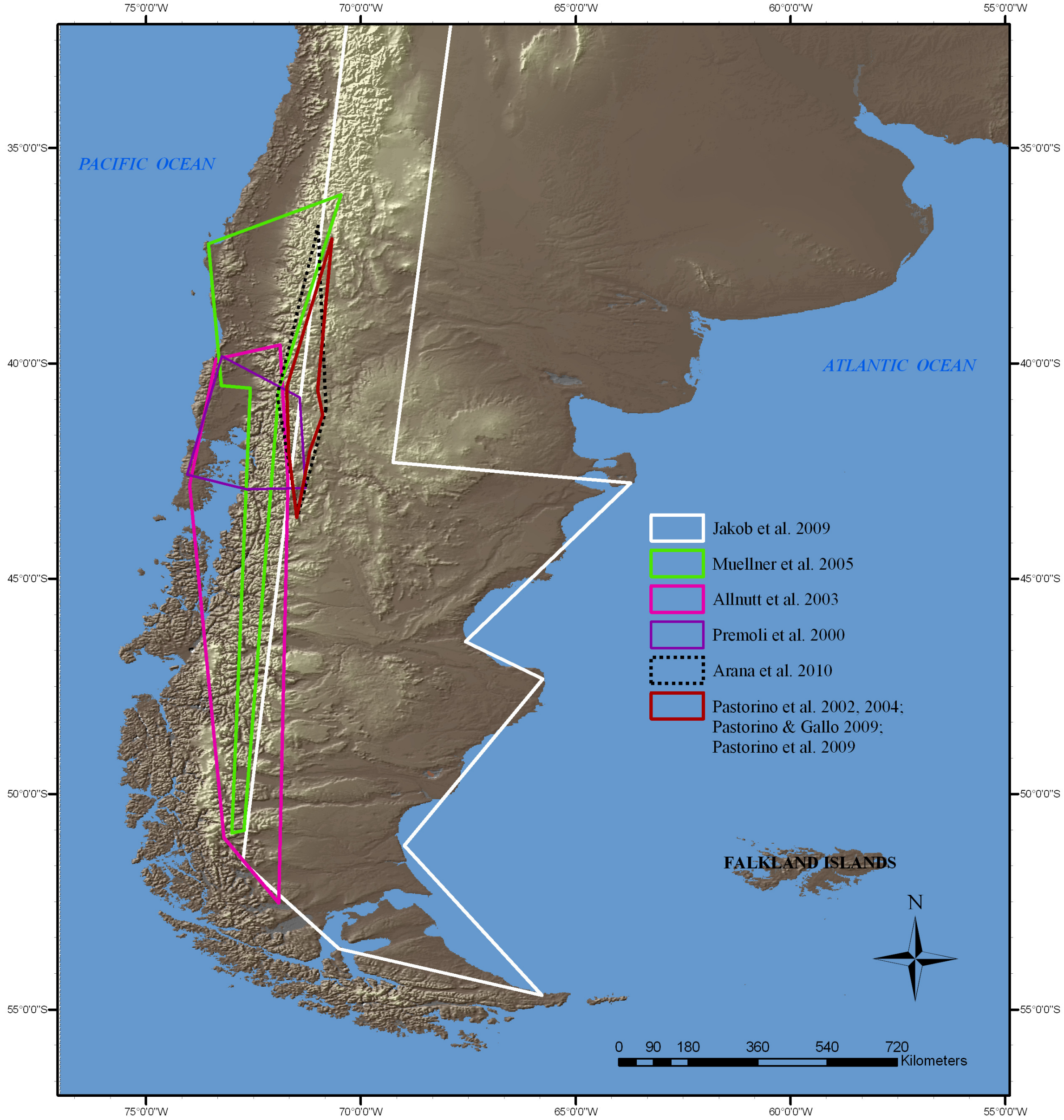

Figure 1.2 continued

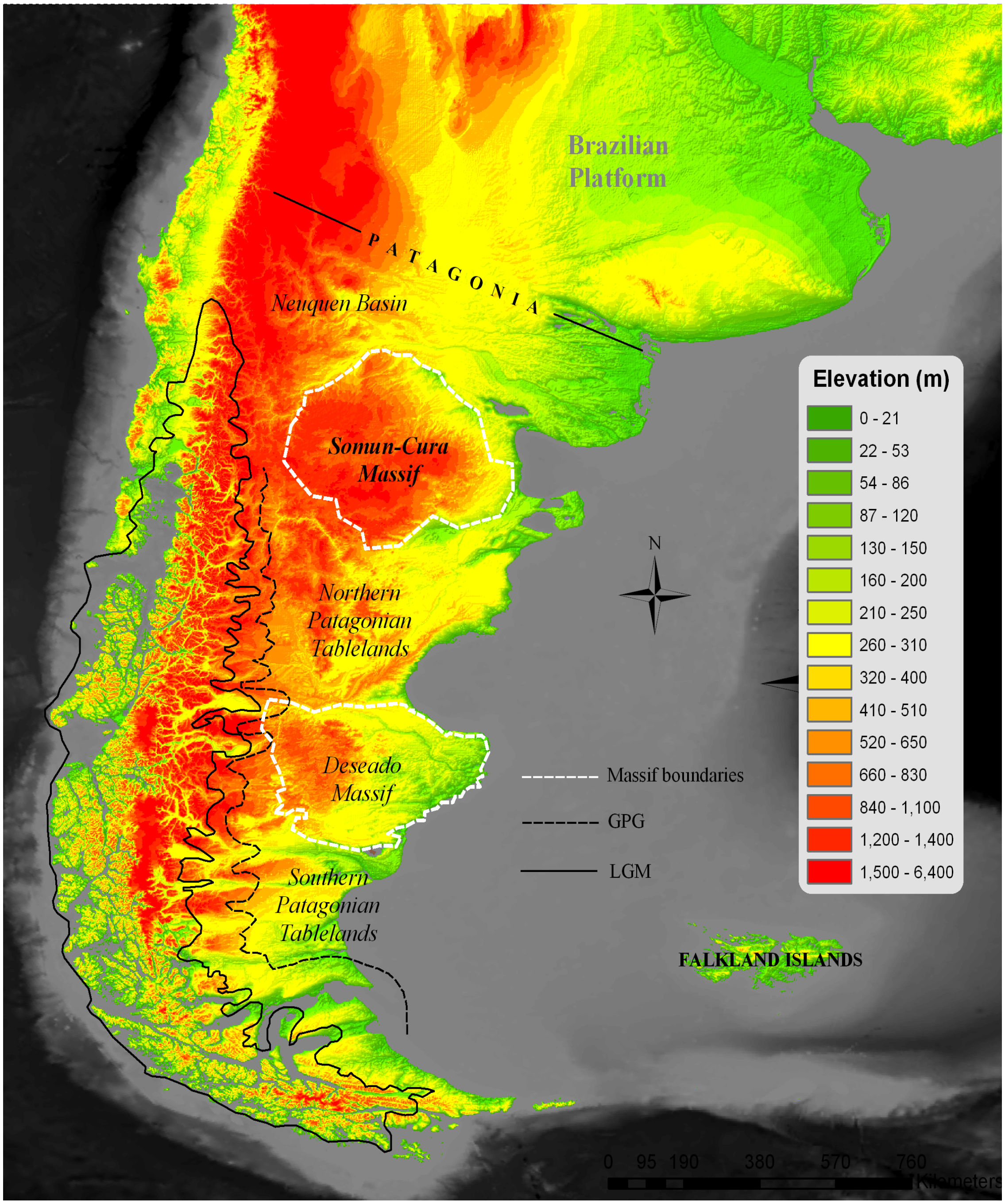

Figure 1.2 A digital elevation model of southernmost South America illustrating the major physical structures comprising the Patagonian landscape. Also indicated is the geopolitical border separating Chile and Argentina and the geological border which defines Patagonia.

Figure 1.3 The major lakes and rivers of contemporary Argentinean Patagonia. Features illustrated in yellow represent trans-Andean lakes and rivers that are shared with Chile and represent Pacific draining catchments which extend into Argentina. Blue features are lakes and rivers which are completely confined within Argentinean borders and form catchments which drain into the Atlantic. Also illustrated are the present-day icefields, in addition to the historical extent of the icefields during the Last Glacial Maximum (LGM) and Great Patagonian Glaciation (GPG) (Clapperton 1993; McCulloch et al. 2000).

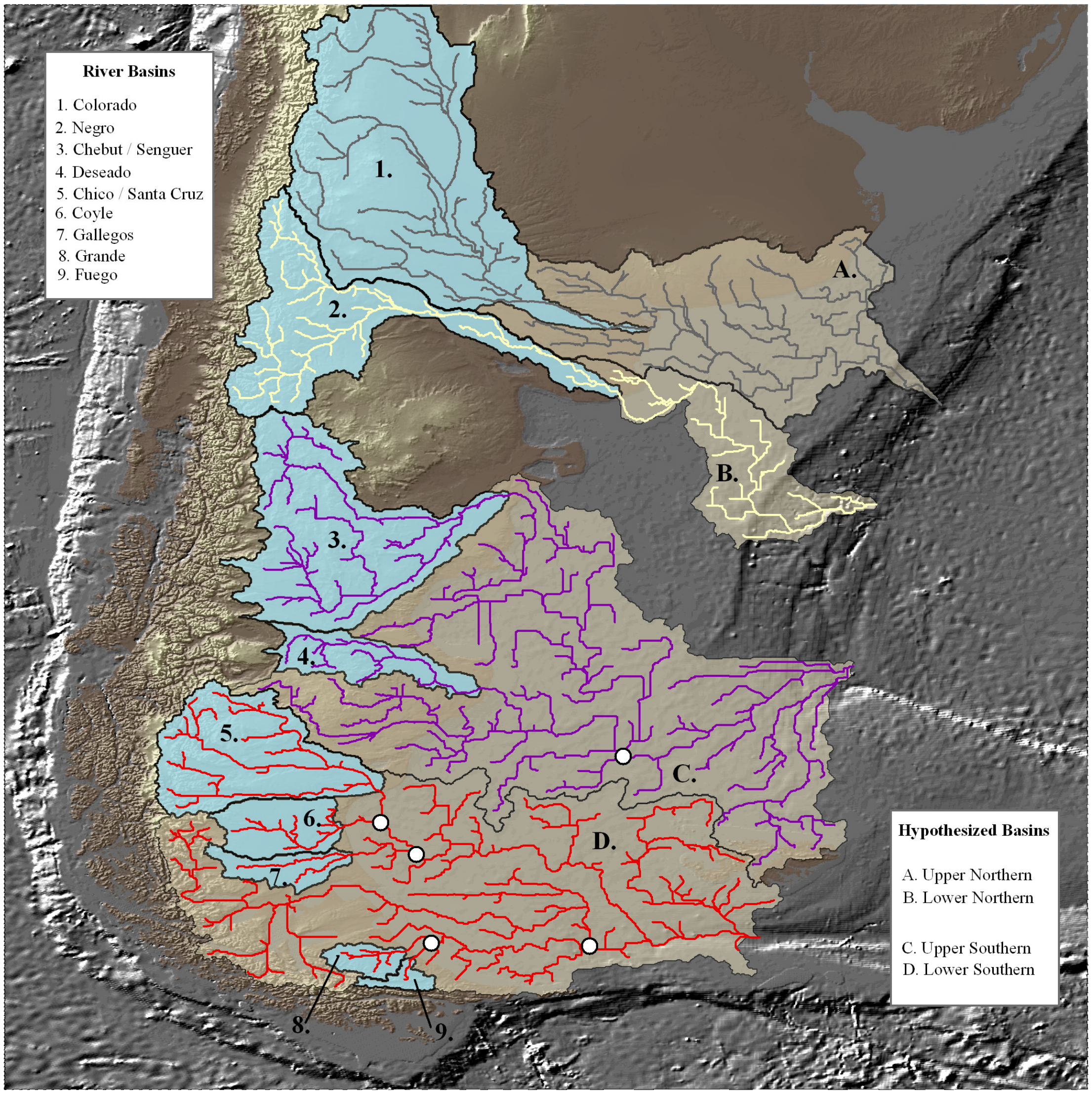

Figure 1.4 Inferred drainage boundaries of a fully exposed Argentinean shelf. Catchments 1-9 represent contemporary boundaries of several drainages in eastern Patagonia. Catchments A-D represent hypothesized boundaries with a completely exposed shelf. White circles represent coalescent points between any contemporary catchments (1-9) on the exposed shelf. All drainage boundary limits were inferred using the RiverTools 3.0 software from surface contours available from the ETOPO1 1-minute Global Relief Database (Amante and Eakins 2009).

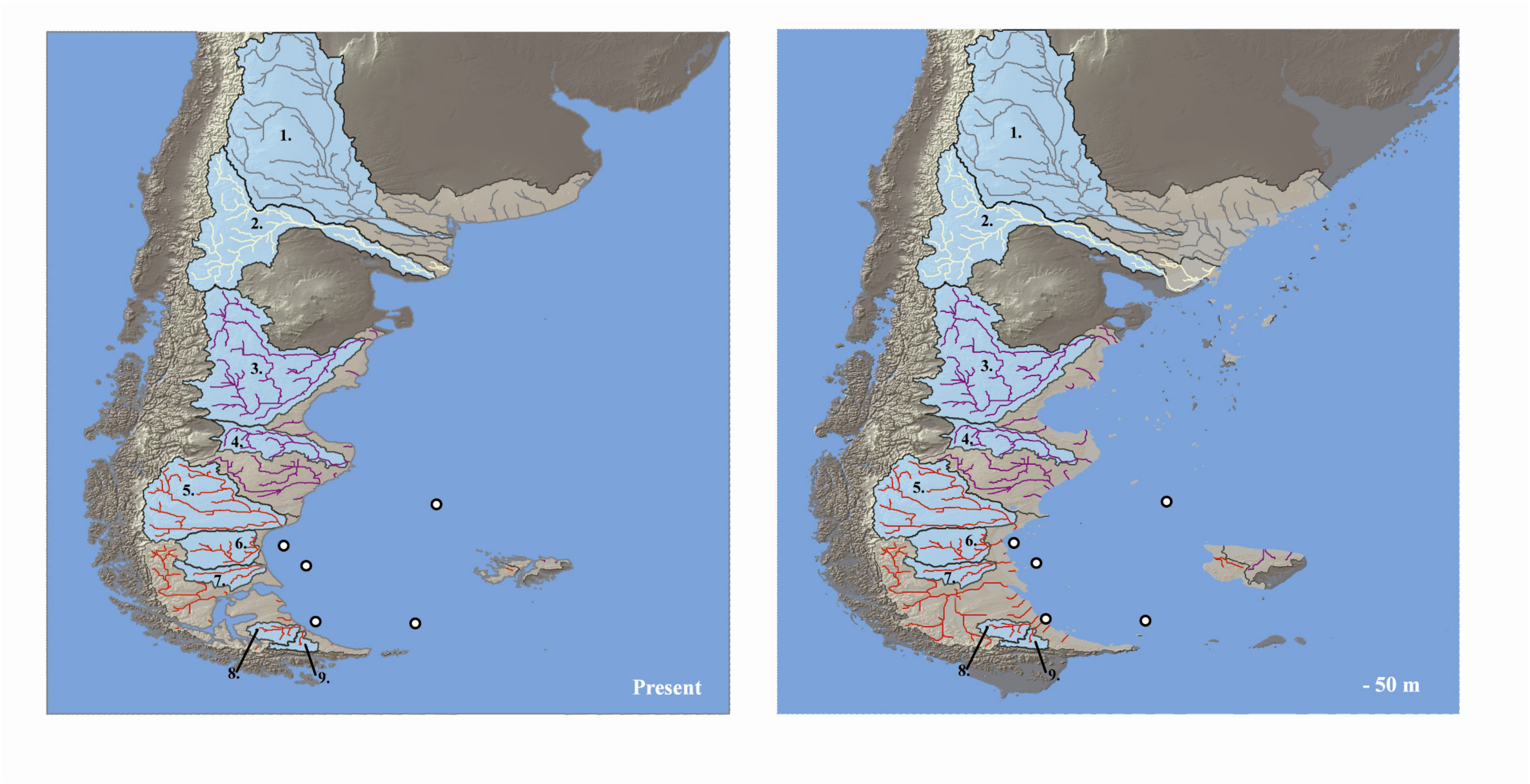

Figure 1.5 The influence of sea-level change (0 to -150 m) on inferred drainage boundaries that are illustrated in Figure 1.4.

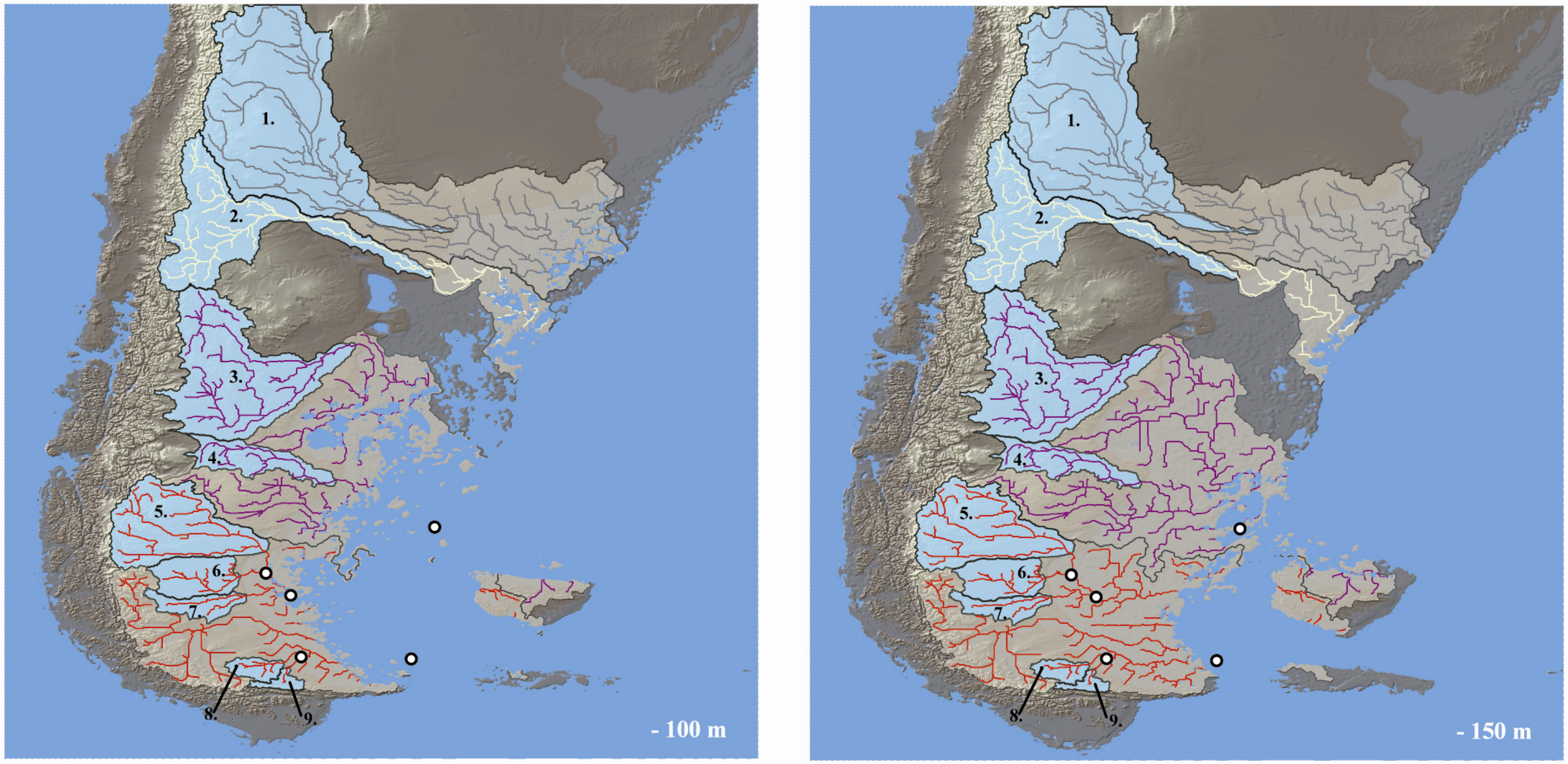

Figure 1.5 continued

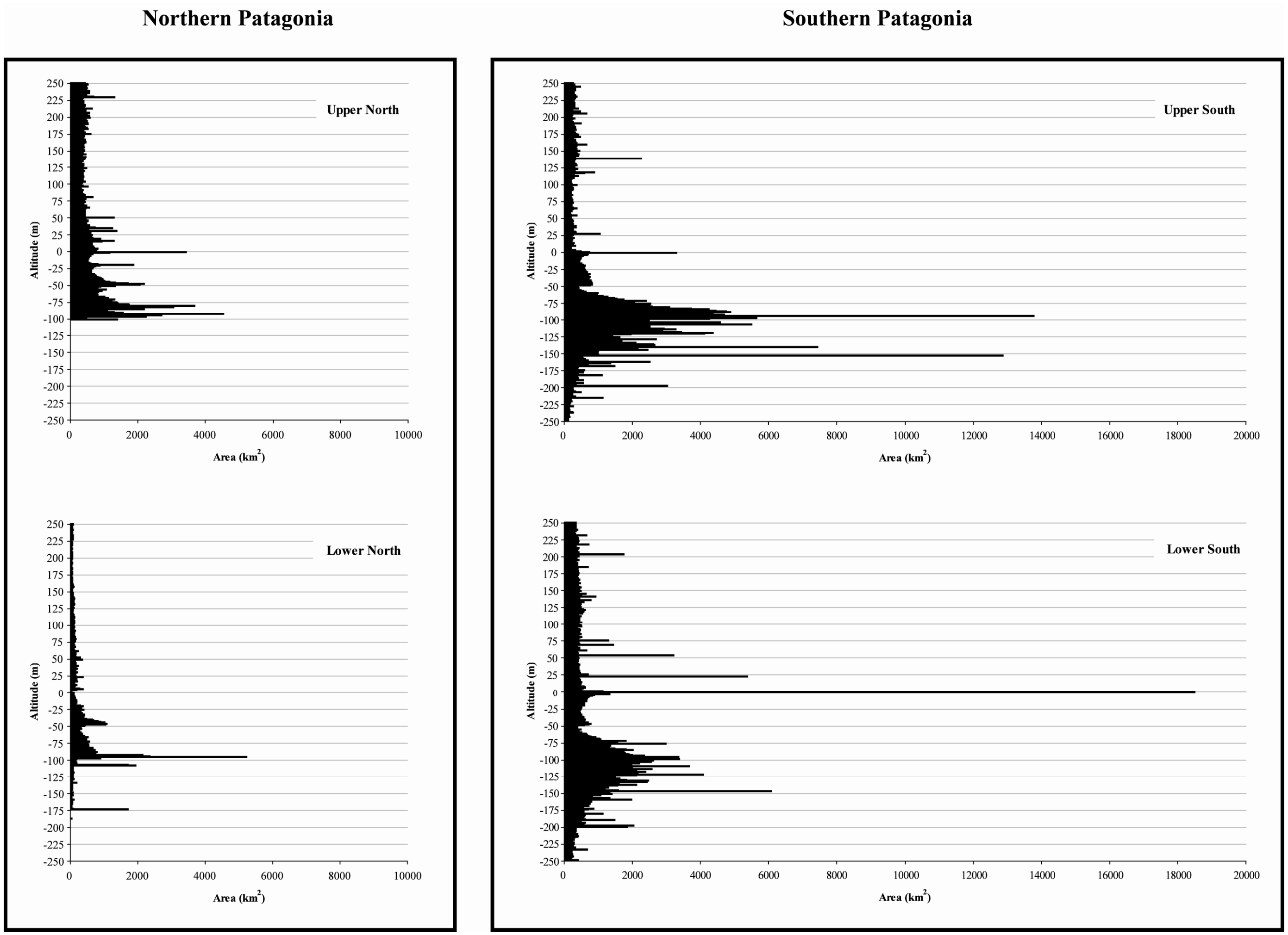

Figure 1.6 Area-elevation histograms for each hypothesized drainage basin during a fully exposed Argentinean shelf (A-D) in Figure 4. Area-elevation plots were constructed using data extracted from the ETOPO1 1-minute Global Relief Database (Amante and Eakins 2009) using the software RiverTools 3.0.

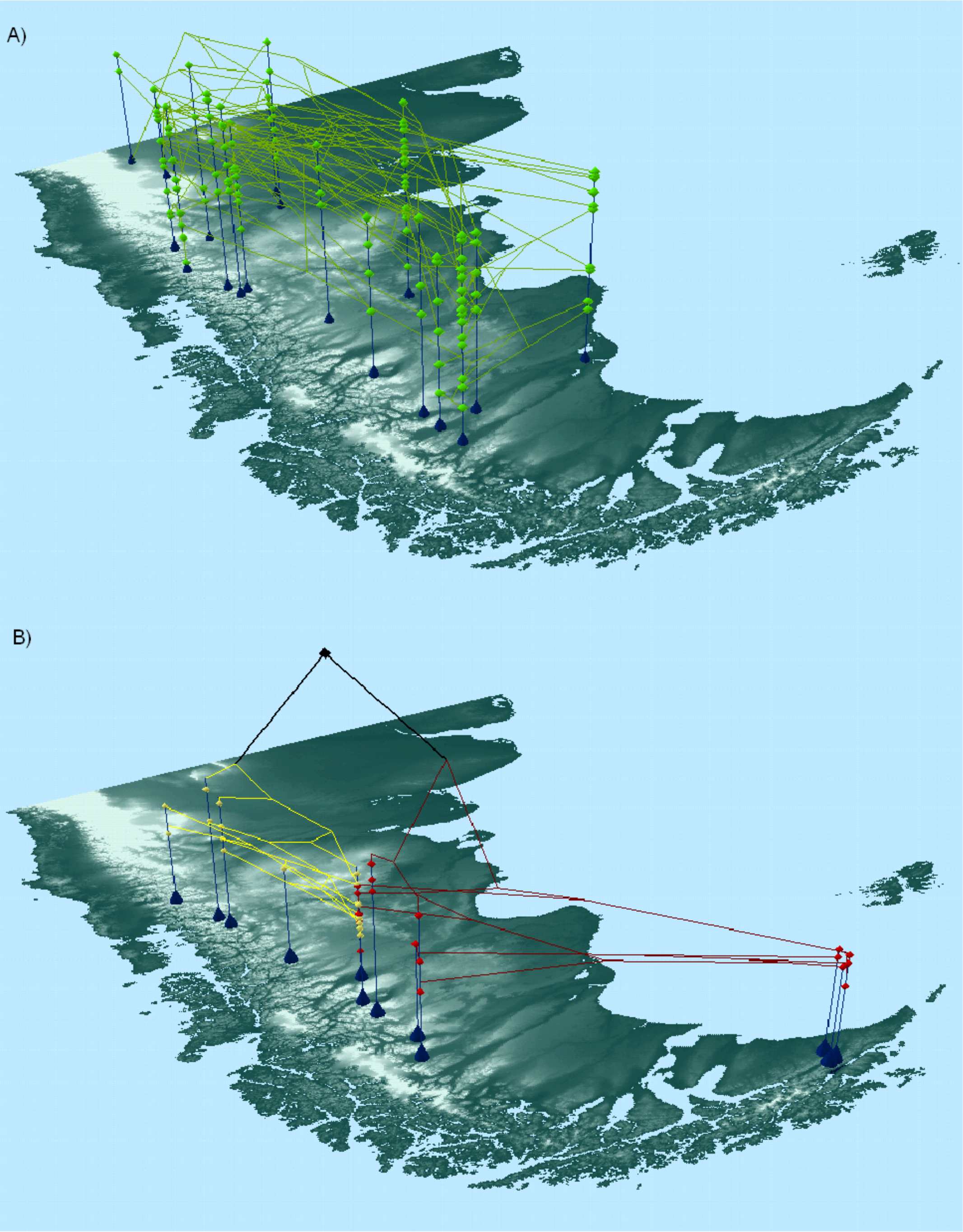

Figure 1.7 Phylogeographic structure of A*) Percichthys trucha* and B) *Galaxias platei* throughout their natural ranges in eastern Patagonia. Genetic structure was calculated using a Neighbour-joining algorithm assuming a Kimura-2-Parameter (K2P) model of sequence evolution using the software MEGA 4.0 (Tamura et al. 2007) and plotted using the software geophylobuilder 1.0 (Kidd and Xianhua 2008).

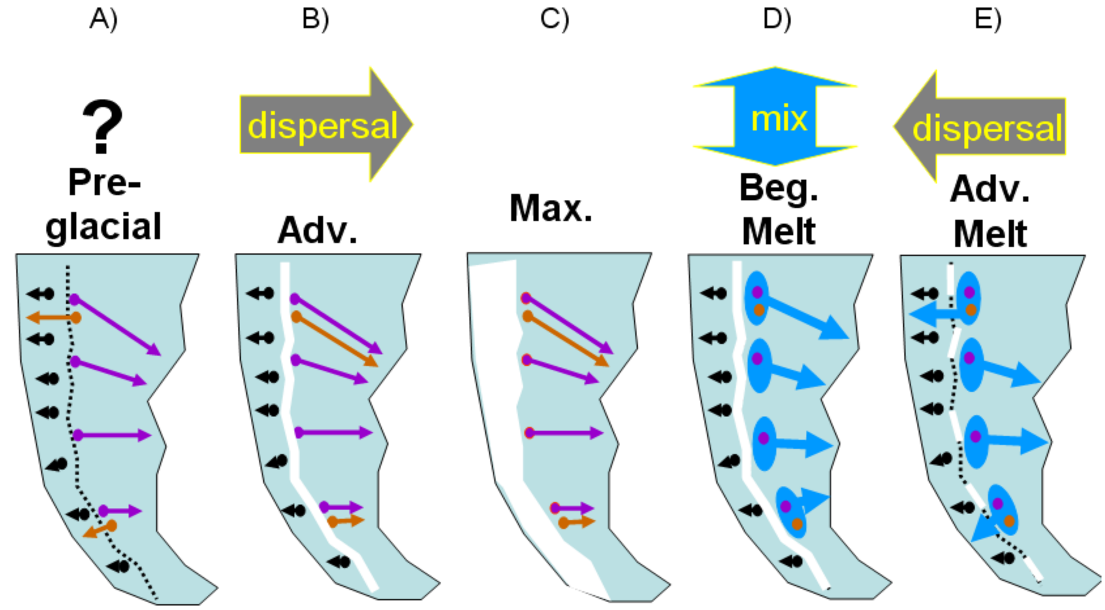

Figure 1.8 The hypothesized mechanism by which climate-induced drainage reversals facilitated bidirection and trans-Andean dispersal in aquatic organisms in Patagonia.

CHAPTER 2

ACROSS THE SOUTHERN ANDES ON FIN: GLACIAL REFUGIA, DRAINAGE REVERSALS AND A SECONDARY CONTACT ZONE REVEALED BY THE PHYLOGEOGRAPHICAL SIGNAL OF *GALAXIAS PLATEI* IN PATAGONIA

This chapter was published December 2008 as an *Original Article* in the journal of *Molecular Ecology* (Volume 17, Issue 3, pages 5049-5061). The primary author of the article is Tyler S. Zemlak. The article is co-authored by Evelyn M. Habit, Sandra J. Walde, Miguel A. Battini, Emily D.M. Adams and Daniel E. Ruzzante. All authors contributed to the conception and design of the study, and all participated in sample collection. TSZ generated the molecular data, and TSZ and DER were responsible for data analysis. TSZ drafted the initial version of the manuscript, and DER and SJW were involved in subsequent versions of the manuscript. All authors reviewed it critically. Permission to reproduce the article as a thesis chapter was obtained by the publisher *Wiley* using *Rightslink Copyright Clearance Center, Inc* (License number: 2610900332811, see Appendix 1.1).

2.1 Abstract

I employed DNA sequence variation at two mitochondrial (control region, COI) regions from 212 individuals of *Galaxias platei* (Pisces, Galaxiidae) collected throughout Patagonia (25 lakes/rivers) to examine how Andean orogeny and the climatic cycles throughout the Quaternary affected the genetic diversity and phylogeography of this species. Phylogenetic analyses revealed 4 deep genealogical lineages which likely represent the initial division of *G. platei* into eastern and western lineages by Andean uplift, followed by further sub-division of each lineage into separate glacial refugia by repeated Pleistocene glacial cycles. West of the Andes, refugia were likely restricted to the northern region of Patagonia with small relicts in the south, whereas eastern refugia appear to have been much larger and widespread, consisting of separate northern and southern regions that collectively spanned most of Argentinean Patagonia. The retreat of glacial ice following the LGM allowed re-colonization of central Chile from non-local refugia from the north and east, representing a region of secondary contact between all four glacial lineages. Northwestern glacial relicts likely followed pro-glacial lakes into central Chilean Patagonia, whereas catastrophic changes in drainage direction (Atlantic → Pacific) for several eastern paleolakes were the likely avenues for invasions from the east. These mechanisms, combined with evidence for recent, rapid and widespread population growth could explain the extensive contemporary distribution of *G. platei* throughout Patagonia.

2.2 Introduction

Compared with North America and Europe, little is known about the influence of Quaternary geological and climatic events on the evolutionary history of South American flora and fauna. This is particularly true for Patagonia, the southernmost region of Chile and Argentina, where the consequences of the Andean mountain uplift and of the Pleistocene climatic cycles for biodiversity are understood only in broad general terms and only for a limited number of (mostly) terrestrial species. Patterns of post-glacial dispersal have been described for some plants (Allnutt et al. 1999; Premoli et al. 2000; Pastorino and Gallo 2002; Muellner et al. 2005), lizards (Victoriano et al. 2008), rodents (Smith et al. 2001; Palma et al. 2005) and a relict marsupial (Himes et al. 2008); the results indicate the presence of terrestrial Pleistocene glacial refugia west and east of the Andes in northern Patagonia. Patterns of genetic diversity described for several terrestrial vertebrates inhabiting the Patagonian Steppe (lizards: Ávila et al. 2006; Morando et al. 2004, 2007; rodents: Kim et al. 1998) have provided valuable insights regarding localized effects of late Quaternary processes on intra-specific diversity. Studies on freshwater crabs (Perez-Losada et al. 2004) have linked phylogenies to events such as drainage changes and marine incursions that occurred several to many millions of years ago in northern Patagonia and regions to the northeast. Recent work on freshwater fish, however, suggests that events that have occurred during the past 1 to 3 million years may be most relevant to explaining current patterns of genetic diversity within Patagonian freshwater fauna (Ruzzante et al. 2006, 2008).

In the present study I examine in detail the patterns of genetic diversity of one of the most widespread fish species endemic to Patagonia, *Galaxias platei*, in relation to the geological and climate changes of the Pleistocene. The contemporary distribution of *G. platei* is exclusively within post-glacial lakes and rivers (Ruzzante et al. 2008). Tolerance of low temperatures and low oxygen environments, adaptations to low light (e.g. retinal modifications, cephalic lateral line), and highly protected gill epithelial tissues are characteristics that are thought to reflect adaptation to a benthic existence in the silty, dark environment of Patagonian lakes and rivers (see Cussac et al. 2004 and references therein) and likely influenced *G. platei* survival and dispersal during the repeated glacial cycles of the Quaternary.

2.2.1 The Rise of the Andes and the Glacial Cycles of the Quaternary

In some cases continental divides are formidable barriers to gene flow, not only for terrestrial species, but also for aquatic taxa, limiting exchange to valleys which transverse the range (e.g. Soltis et al. 2006). Deep and ancient genetic structuring might be expected for aquatic species east and west of the Andes, as the southern part of the range began to rise in the early Miocene [*ca.* 23 MY BP (Ramos 1989)].

Patterns of genetic diversity within Patagonian South America are also likely to reflect the climatic fluctuations of the Pleistocene [1.8 MY – 10,000 years MY BP (Clapperton 1993)]. At the Last Glacial Maximum (LGM), 23,000-25,000 BP, a continuous ice sheet existed along the Andes for over 1800 km, extending west to the Pacific Ocean south of 42°S [(Clapperton 1993; McCulloch et al. 2000) Figure 2.1]. Glacial events influence the distribution of genetic diversity directly through reduction of population size (producing genetic bottlenecks) and displacement of entire populations and indirectly through their effects on landscapes, e.g. the rearrangement of watersheds. The many glacial advances and retreats during the Pleistocene differed in extent and duration and thus probably also in their effects on the abundance and distribution of local biota. The extent to which aquatic taxa survived the glacial cycles within watersheds is not known. Refugia certainly existed north of continental ice (*ca.* 43°S) west of the Andes, and probably also in some eastern watersheds (Ruzzante et al. 2006). Refugia may also have been present west of the ice sheet on areas of continental shelf exposed by lowered sea levels. Pleistocene watershed rearrangement and its effects on patterns of genetic diversity have been extensively documented for New Zealand systems (Waters and Wallis 2000; Waters et al. 2001, 2006; Burridge et al. 2006, 2007; Craw et al. 2007; Craw and Waters 2007). Two effects of glacial cycles on Patagonian landscapes were likely important for aquatic organisms in southern South America. First, the large paleolakes that formed east of the

Andes during periods of glacial retreat probably allowed mixing of populations from adjacent watersheds (Ruzzante et al. 2008). Second, several drainages changed direction from the Atlantic to the Pacific during the melting of the ice following the LGM, including Lakes General Carrera/Buenos Aires and Cochrane/Pueyrredón, which began to drain west about 12 KY BP (Turner et al. 2005); reversals are likely responsible for all basins east of the Andes that currently drain into the Pacific.

2.2.2 The Galaxiidae

Galaxias platei is one of 6 species of the family Galaxiidae in South America. Galaxiids are confined to the Southern Hemisphere with representatives on all continents (and nearby islands), barring India and Antarctica (Croizat et al. 1974; Rosen 1978). Three genera are found in South America: *Galaxias* (3 species – *G. platei*, *G. maculatus*, *G. globiceps*), *Aplochiton* (2 species – *A. taeniatus*, *A. zebra*) and *Brachygalaxias* (*B. bullocki*) (McDowall 2006). All except *G. maculatus*, are endemic to South America and all are temperate species, restricted to latitudes south of 34^{o}S. All six species are present in Chile, but only three species are found east of the Andean mountain range (*G. platei, G. maculatus* and *A. zebra*) (Ringuelet 1955; Cussac et al. 2004).

In the present study I surveyed the genetic diversity (mtDNA control region and COI) of *G. platei* collected from across the species' latitudinal (39^{o}S to 54 ^{o}S) and altitudinal (sea level to *ca.* 1000 m) ranges on both sides of the Andes. I tested for patterns of diversity that would reflect the impacts of the Andean orogeny, the influence of glacial cycles on population size (bottlenecks and population expansion) and the effects of landscape rearrangement during the Pleistocene. My study provides genetic evidence consistent with (*a*) a relatively recent split (1.5 MY BP) between eastern and western lineages of *G. platei*, *(b)* the existence of Pleistocene glacial refugia west and east of the Andean mountains in both northern and southern Patagonia, and (*c*) the existence of a zone of secondary contact between lineages from the eastern and western refugia, probably as a result of post-glacial drainage reversals.

2.3 Methods and Materials

2.3.1 Samples

Samples were collected using gill nets and electrofishing between 1998-2007 from 25 lakes and rivers throughout Argentinean and Chilean Patagonia (Figure 2.1). For the present study a total of 212 individuals were examined for their DNA composition; of these, 112 (53%) were from 13 Chilean lakes, 31 (15%) from 3 cross-border lakes, and 69 (32%) from 9 Argentinean lakes (Table 2.1). Whole samples were retained and stored at several institutions within Chile and Argentina (details available upon request). Sub-samples (gill, muscle, fin, blood) for molecular analyses were fixed in 95% ethanol and transported to Dalhousie University for storage at -20°C.

2.3.2 Sequence Data

Prior to DNA extraction all tissue samples were dried of ethanol by exposure to ambient temperature for approximately 120 min. Total genomic DNA was isolated from each sub-sample (10 µL of blood or 2x2 mm^2 tissue) using the glassmilk procedure described in Elphinstone et al. (2003) with slight modifications for execution using a MultiPROBE® II HT PLUS EX robotic liquid handling system (PerkinElmer). Approximately 800 bp of the mitochondrial control region were amplified from each individual using the primer combination *S-phe* [(5’-GCT TTA GTT AAG CTA CG-3’; (Nielsen *et al.* 1994)] and *P3* [5’-AAC TTC CAT CCT CAA CTC CCA AAG -3’; (Sang et al. 1994)] with a Mastercycler® EP Gradient (Eppendorf) thermal cycler. Mitochondrial cytochrome *c* oxidase subunit I (COI; ~640 bp) was also amplified for a subset of 8 individuals, two from each of four *post-hoc* haplotype clusters (herein *haplogroups 1-4*) recovered by phenetic (minimum spanning tree; Figure 2) and phylogenetic (Bayesian inference; Figure 3a) analyses of control region data using the following primer sequences from Ward et al. 2005: *FishF1* (5’-TCA ACC AAC CAC AAA GAC ATT GGC AC -3’) with *FishR2* (5’-ACT TCA GGG TGA CCG AAG AAT CAG AA -3’). The thermal cycler was operated under the following conditions for control region: an initial denaturing temperature of 94ºC for 5 min, followed by 35 cycles of 94ºC for 1 minute, 50ºC for 1 min 30, and 72ºC for 1 minute 30, and a final extension at 72ºC for 5 min. The thermal regime used for COI was identical to that used for control region, but with a higher annealing temperature (i.e. 54ºC). All PCRs were 25 μL: 2.5 μL 10X Reaction Buffer [100mM KCl, 100mM $(NH_4)_2SO_4$, 200mM Tris HCl (pH 8.75, 22°C), 1% Triton X-100, 1mg/ml BSA], 2.5 μL dNTPs (2 μM each), 3.75 μL $MgSO_4$ (20 mM), 13 μL ddH_2O, 0.25 μL of each 10 μM primer, 1 U of Tsg DNA Polymerase (Bio Basic Inc.) and 2.0-2.5 μL of DNA template. PCRs were visualized using 1.0% agarose gels and sent off site for bidirectional DNA sequencing to Génome Québec Innovation Centre, Quebec, Canada or Macrogen Inc., Seoul, Korea.

Approximately 78% of the control region sequences and haplotypes used in this study were reported in an earlier study that examined the influence of climate on the demographic history of *G. platei* and *Percichthys trucha* (Ruzzante et al. 2008); haplotype sequences are available in GenBank under the accession numbers EU069832-EU069870. All newly collected control region and COI sequences were edited using Sequencher^{TM}. Subsequently, BioEdit Sequence Alignment Editor version 7.0.5.3 (Hall 1999) was used to form contigs between bidirectional sequences, align contigs using the accessory application ClustalW (Thompson et al. 1994) and identify haplotypes. Genbank accessions numbers for control region haplotypes are EU673457-EU673467; COI sequences have accessions FJ178348-FJ178355.

2.3.3 Data Analysis

Standard molecular diversity indices [haplotype diversity (h), nucleotide diversity (π), nucleotide frequencies, transition/transversion ratio, number of polymorphic sites] were calculated using ARLEQUIN ver. 3.1 (Excoffier et al. 2005). Estimates of haplotype and nucleotide diversities were first obtained for each lake/river individually, and then pooled to obtain an overall estimate of sequence diversity in *G. platei* over the entire region.

Additional *post-hoc* estimates of molecular diversity were also generated for each haplogroup.

Relationships among control region haplotypes were first explored using a minimum spanning tree (MST) based on uncorrected ‘p’ distances by MINSPNET, as implemented in ARLEQUIN ver. 3.1 (Excoffier et al. 2005). Subsequently, Bayesian inference was used to estimate phylogenetic relationships between haplotypes using MrBayes ver. 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), based on the HKY+G+I model of molecular evolution which was determined by MODELTEST (Posada and Crandall 1998) as the most appropriate model of DNA substitution. Parameter space was searched for 3,000,000 generations using two independent runs based on different random number seeds that each employed four heated chains with temperatures of 0.2 and swap rates of 1. Branch support and order were based on Bayesian posterior probability densities after discarding the first 750,000 generations as burnin and confirming convergence among independent runs by visual inspection of resultant tree topologies. The tree was rooted with *G. maculatus*. Phylogenetic analysis of COI data was conducted using identical parameter settings, but using the HKY+G model of sequence evolution, also determined by MODELTEST (Posada and Crandall 1998).

Corrected average pairwise divergences (controlling for within-cluster variation) between control region haplotype groups was calculated using ARLEQUIN ver. 3.1 (Excoffier et al. 2005) based on the Kimura 2-Parameter (K2P) model of molecular evolution to correct for multiple substitutions (Kimura 1980). Rough estimates of population divergence were first calculated for each haplogroup independently and then pooled into eastern and western groups to estimate Andean-induced divergence. The split between east and west was also estimated using COI sequence data in an attempt to account for the possible underestimation of ancient divergence because of multiple unaccounted hits (saturation) based on control region sequences. Similar calculations of within-lineage divergence using COI data seemed inappropriate considering sample sizes, and the paraphyly of eastern haplotypes. Thus, I only report divergence estimates, for the deepest split in the tree that was recovered by COI data. Rough estimates of time since divergence were subsequently calculated for both control region and COI using the "asymptotic" galaxiid mtDNA mutation rates (cyt b, control region) of 0.01876 changes/site/MY recently estimated by Burridge et al. (2008).

Hierarchical Analysis of Molecular Variance (AMOVA), executed in ARLEQUIN ver. 3.1, was used to determine how contemporary genetic variation is partitioned over the range of *G. platei*. Hierarchical groupings of samples included: (A) the lake/river from which individuals were collected (B) the freshwater drainage that each lake or river belongs to (C) the present day ocean drainage (Pacific or Atlantic) and (D) the historical or ancient ocean drainage [i.e. ancient refers to conditions prior to the LGM approximately 23,000-25,000 BP (Clapperton 1993; McCulloch et al. 2000; Tatur et al. 2002; Turner et al. 2005)]. Ancient drainage patterns are expected to have been very similar to contemporary drainage patterns with the exception of those post-glacial lakes located east of the Andes that currently drain into the Pacific. At the height of the LGM, ice was present to the west of these lakes. This ice disappeared during the melting of the glaciers that followed the LGM leading to chatastrophic drainage reversals from the Atlantic to the Pacific Ocean. Although this phenomenon is well documented for only some of the systems located east of the Andes and currently draining into the Pacific (Tatur et al. 2002; Turner et al. 2005), here I treat all sample locations east of the Andean mountain range currently draining into the Pacific as historically Atlantic draining. See Table 1 for details regarding hierarchical groupings.

2.3.4 Limitations

While the present case is an important first approach to understanding the neutral genetic structure and diversity of freshwater fishes in Patagonian lakes and rivers, I do realize the limitations of our approach and suggest caution when interpreting results. Inferences about populations are based solely on the analysis of organellar genes (i.e. control region, COI), which despite offering accurate depictions of mitochondrial history may not necessarily be representative of the overall genetic aspects of the organisms themselves. Several studies now recognize the importance of including at least one, or several unlinked nuclear loci to resolve sex-specific patterns and maximize the statistical power of divergence estimates by controlling for influences of ancestral polymorphisms and the stochastic nature of lineage sorting process (Wakeley and Hey 1997; Edwards and Beerli 2000; Hare 2001; Nichols 2001). Uncertainties about an appropriate molecular clock for control sequences further confound lineage divergence estimates and the timings of important demographic events. I employed the "asymptotic" rate of 1.88%/MY reported in Burridge et al. (2008) for galaxiid fishes, but consider it a very conservative estimate for the control region based on the range of rates reviewed by Bowen et al. (2006) for bony fishes. Considering the inverse relationship between substitution rate and divergence time, our estimates likely represent the upper bounds of time since lineage divergence, particularly considering that our single-gene based approach is susceptible to overestimating population divergence (Edwards and Beerli 2000). Despite the above uncertainties, I am confident that the mtDNA analyses presented here are appropriate for the questions presented and provide important new insights and hypotheses regarding the diversification and biogeography of freshwater fishes in the understudied region of Patagonian South America. Efforts involving multi-gene approaches for South American galaxiid fishes are encouraged to further test and refine hypotheses set forth in the present case and are currently underway in the Ruzzante laboratory.

2.4 Results

2.4.1 Intra-Specific Population Structure

A total of 50 mtDNA control region haplotypes were identified from 212 individuals of *G. platei* collected from throughout the species distribution (Appendix 2.1). Overall nucleotide frequencies were: C (22.34%), T (28.48%), A (29.78%), G (19.41%). The total number of polymorphic sites was 103, with a transition/transversion ratio of 2.15, and 37 observed indels.

A MST of 50 control region haplotypes recognized 4 major clusters (Figure 2.2), or haplogroups *1* (N= 12), *2* (N=3), *3* (N=15), and *4* (N=20), each identified as a single entity by several mutational steps: *1*↔*4*=49, *1*↔*2* =12 and *3*↔*4*=8. Phylogenetic reconstruction of those same haplotypes using Bayesian inference also recognized the four haplogroups, 1 and 2 receiving much greater branch support than groups 3 and 4 (Figure 2.3a). Additional strongly supported clusters were evident within each of the haplogroups, but were separated from other potential groups by only one or two mutational steps. Phylogenetic analysis of COI data provided further support for a deep split between eastern and western groups (1,2↔3,4) and recognized the distinct division between western haplogroups 1 and 2, but revealed a paraphyletic relationship between eastern haplogroups 3 and 4 (Figure 2.3b). Of the 212 individuals sequenced, haplogroups 1, 2, 3, and 4 had 40, 20, 101, and 51 individuals, respectively.

Haplotypes from groups 1 and 2 were found exclusively in locations west of the Andes, and were most abundant in northern drainages (Figure 2.4). Haplotypes from groups 3 and 4 were the most widely distributed, and were found in lakes and rivers on both sides of the Andes (Figure 2.4). Haplogroup 3 dominated northern drainages east of the Andes, while haplogroup 4 dominated southern drainages. All haplotype groups were present in the central-west drainages (see Appendix 2.1 for haplotype frequency by collection site).

The average pairwise divergence between haplotypes belonging to haplogroups 1 and 2 was 1.31%, similar to average divergence between 3 and 4 (1.26%) for control region sequence data. If haplogroups are pooled into the 2 major lineages (western: 1-2, eastern: 3-4), average pairwise divergence increases to 5.70%. For COI data, divergence between the eastern and western lineages was estimated to be 5.64%. Applying these divergence values to approximate per site mutation rates of approximately 1.88%/MY for control region and COI data (Burridge et al. 2008), I obtained the following rough estimates of times since divergence: 1-2 ↔ 3-4 (1.5 MY), 1↔2 (349 KY) and 3↔4 (337 KY).

Most of the contemporary genetic variation could be explained by grouping individuals by lake/river (91%), with very little variation associated with individual collection sites (8.98%) (1-way AMOVA, Table 2). Analysis of sequence variation of the control region using a hierarchical AMOVA showed that nearly half (40%) of the variation was associated with basin or watershed, while a similar amount of variation was associated with different locations within a basin (Table 2.2). I then nested collection sites within drainage direction (Pacific vs. Atlantic). Current drainage direction explained none of the variance (-0.49%). However, historical drainage direction (prior to the LGM) explained 35.91% of the total variance.

2.4.2 Demography

Haplotype diversity was similar for populations east (Argentina) and west (Chile) of the Andes, but eastern populations had lower nucleotide diversity (Table 2.1). Measures of haplotype and nucleotide diversity co-vary for most locations (i.e. lakes/rivers showing high haplotype diversity also show high nucleotide diversity and *vice versa*), but display no clear latitudinal clines (Table 2.1). The highest levels of haplotype and nucleotide diversity were seen in groups 1 ($h = 0.7859$, $\pi = 0.0041$) and 4 ($h = 0.8643$, $\pi = 0.0018$), followed by groups 3 ($h = 0.6438$, $\pi = 0.0009$) and then 2 ($h = 0.1947$, $\pi = 0.0004$) (Table 1). According to classifications by (Grant and Bowen 1998), haplogroup 2 qualified as category I (recent population bottleneck or founder event by single or a few mtDNA lineages; $h < 0.5$, $\pi < 0.5\%$), while haplogroups 1, 3 and 4 all placed within category II (population bottleneck followed by rapid population growth and accumulation of mutations; $h > 0.5$, $\pi < 0.5\%$).

2.5 Discussion

Of the various historical factors that have influenced the diversity, abundance and distribution of native temperate South America flora and fauna, both the orogeny of the southern Andes and the glacial cycles of the Pleistocene appear to have played important roles in shaping the contemporary distribution and intra-specific genetic diversity of *G. platei*. First, the Andes have separated *G. platei* into eastern and western lineages for at least 1.5 MY BP. The deep genetic structure detected within each of the eastern and western lineages was dated to approximately 330-350 KY BP, the timing of which suggests that glacial cycles may have been responsible, probably by displacement into separate refugia. Furthermore, the distribution of genetic variance reflects patterns of drainage evolution following the LGM, especially the catastrophic drainage shifts (from the Atlantic to the Pacific Ocean) in the north-central part of the latitudinal range of *G. platei*. I identify a zone of secondary contact between eastern and western lineages in western drainages (Lat 44 - 45^{o}S) and a zone of contact between the northern and southern clades of the eastern lineage at Lat ~ 47^{o}S.

2.5.1 The Andean divide

Mountain buildup is well supported as an important mechanism of genetic isolation; evidence is present at both the inter- and intra-species levels for diverse assemblages of taxa from regions all over the world (see Soltis et al. 2006). Here I found evidence that the Andean divide has played an important historical role in creating deep intra-specific structure within *G. platei*, effectively sundering its historical range into eastern (combined haplogroups 1&2) and western (combined haplogroups 3&4) lineages. Similar phylogeographic breaks are associated with the Andes for another Patagonian freshwater fish, *P. trucha* (Ruzzante et al. 2006), as well as a long-lived conifer (Allnutt et al. 1999). Biogeographic patterns at the species level coincide with intraspecific discontinuities, with numerous aquatic species found to the west but not east of the Andes (Dyer 2000). For example, only three of the six putative species of galaxiids currently recognized in South America have distributions which extend east of the Andes, two of which have a substantial presence (*G. platei and G. maculatus*) and one (*A. zebra*) is restricted to Pacific draining systems (Ringuelet 1955). These concordant patterns strongly indicate that the southern Andean uplift has historically presented an important barrier to dispersal for species like *G. platei* with distributions that span both sides of the Andes.

Molecular clock estimates suggest that the split between eastern and western lineages of *G. platei* occurred roughly 1.5 MY BP. As the southern Andes began their uplift much earlier, *ca.* 23 MY BP (Ramos 1989), the data imply gene flow across the divide. Trans-Andean gene flow has been indicated for terrestrial organisms, including two widespread species of Patagonian rodents (*Oligoryzomys longicaudatus* (Palma et al. 2005) and *Abrothrix olivaceus* (Smith et al. 2001), and the marsupial *Dromiciops gliroides* (Himes et al. 2008). These mammals are thought to have utilized the continuous forest habitat which exists in low-lying passes which effectively served as corridors for dispersal in the past and likely continue to do so in the present. Gene flow for aquatic taxa may have occurred through drainages that currently or previously traversed the Andes from the Argentinean side. The timing of the split coincides roughly with the first evidence that the Patagonian ice sheet was fully developed (Rabassa 2008). At this time *G. platei* may have been reduced to populations occupying northern drainages on both sides of the Andes, or the current split between the eastern and western lineages formed during re-colonization when the climate warmed.

2.5.2 Pleistocene Glaciations

Superimposed on the Andean-linked structure, a further sub-division was detected within each of the eastern and western lineages. Molecular clock estimates using control region data suggest the gene splits occurred in the east at ~337 KY BP and in the west at ~349 KY BP. While long term paleoclimatic data are not available for this time period in southern South America, Ruzzante et al. (2008) report on the likelihood of several glacial advances and retreats between 0.2-1.0 MY BP using ocean marine sediment records as proxies for glacial fluctuations on land. It is likely that the lineage splitting detected within eastern and western groups is the product of survival in separate refugia on both sides of the Andes during a similar glacial event in this period.

The number, size and location of potential Pleistocene refugia suitable for *G. platei* and/or other freshwater taxa in Patagonia are not known. West of the Andes, the northernmost region of Patagonia, north of 35^{o}S, is likely to have contained continuous aquatic habitats, since the region remained ice free during glacial maxima, and the climate remained humid, at least during the LGM (Heusser et al. 2000). This region has likely been key to the preservation of biodiversity in general through numerous glacial cycles, as indicated by the relatively deep intra-specific genetic diversity, as well as species-level biodiversity that it supports for various taxa (Dyer 2000; Ruzzante et al. 2006). The same general area has been suggested as the most likely western refuge for other Patagonian taxa, including: plants [*Fitzroya cupressoides* (Premoli et al. 2000), *Hypochaeris palustris* (Muellner et al. 2005)], rodents [*A. olivaceus* (Smith *et al.* 2001)] and fish [*P. trucha* (Ruzzante et al. 2006)]. However, my data suggest that *G. platei* also survived in a second western refuge. It is certainly conceivable that areas to the south could have been glacial refugia, perhaps within discontinuities of the ice field or on exposed portions of the Pacific continental shelf revealed by lowered sea levels. It seems likely that one group (haplogroup 1) of the western lineage utilized the ice free region of the Patagonian northwest, whereas the other (haplogroup 2) found refuge further south. The low frequency, diversity and restricted distribution of haplogroup 2 suggests that this refuge population was initially very small and/or that it endured repeated population bottlenecks in subsequent glacial cycles.

East of the Andes lies the Patagonian Steppe, a large flatland characterized by an arid environment with limited annual rainfall and limited suitable habitat for *G. platei*. During the glacial maxima this aridness increased, presumably reducing or eliminating available habitat for *G. platei* populations. However, my data suggest that the eastern lineage has been present for at least 3 million years, and that the northern and southern lineages were formed during a subsequent glacial period approximately 330 KY BP. Populations of *G. platei* must have survived in northern drainages, and there must also have been a southern refuge (haplogroup 4 is not found north of Lat 46^{0}S). Pastorino and Gallo (2002) suggest that a native conifer, the cypress (*Austrocedrus chilensis*), survived the LGM in several small terrestrial refugia directly east of the glaciers, where conditions were locally cold but moist in a narrow strip between the ice and the dry steppe. The steppe itself may have contained refugial populations of cypress (Pastorino & Gallo 2002), as well two rodents (Kim et al. 1998; Palma et al. 2005). The close association between present day cypress and lakes in Argentina suggests that at least some of the refugia may have been moist enough to contain water bodies with relict populations of *G. platei*.

Although populations in individual lakes and rivers differ genetically, and significant structure was detected within each of the four haplogroups, the amount of divergence was small, typically 1 or 2 mutation steps. In part, the low intra-lineage structure can likely be explained by one or more founder-flush cycles during Pleistocene glacial cycles that effectively purged much of the intra-specific variation. Evidence from molecular diversity indices in the present study, as well as coalescent approaches used by Ruzzante et al. (2008) suggest that a recent and widespread bottleneck for *G. platei* was likely. The large paleolakes that formed during glacial melt were also likely contributors to the low genetic structure observed. Geological evidence indicates the presence of a number of large paleolakes east of the Andes following the LGM, including Elpalafquen, Cari Lafquen, Caldenius, Fuegian and an unnamed lake which joined present day lakes General Carrera/Argentino, Pueyrredon/Cochrane, (Clapperton 1993; Tatur et al. 2002; Turner et al. 2005). Paleolake sizes dwarf those of present day lakes, and the large increase in habitat availability probably led to rapid population growth and/or increased the potential for gene flow among watersheds, resulting in the low genetic diversity and structure observed within present lineages (haplogroups 3, 4). Similar mechanisms have been suggested to explain low levels of genetic structure east of the Andes for another Patagonian fish, the perch, *P. trucha* (Ruzzante et al. 2006). The presence of paleolakes west of the Andes is less certain. Western slopes are much steeper than that of the very flat eastern steppe, reducing the opportunity for meltwater to accumulate. If proglacial lakes formed, they likely remained small.

The highest levels of genetic diversity within *G. platei* were found in central Chilean Patagonia; this is the only region where all four haplogroups were found. Glaciers are believed to have reached the Pacific Ocean south of Lat. 42^0S, and current populations are thus likely the result of colonization. The southern haplogroup of the western lineage (2) may have persisted in refugia near or within the region. The northern haplogroup (1) probably expanded south, perhaps utilizing proglacial lakes, as has been suggested for the colonization of post-glacial water bodies by North American freshwater fish (Bernatchez & Wilson 1998). Eastern groups, however, likely crossed the Andean divide with the extreme fluxes of water that accompanied catastrophic drainage shifts during glacial retreat. The Rio Baker which currently drains Lakes General Carerra/Buenos Aires and Pueyrredon/Cochrane (Lat. 46-47^0S) formed after the LGM when discharge for these two lakes abruptly changed direction and began to flow west when the ice barrier that had formed the western margin of a large paleolake broke *ca.* 12,000 yr BP (Turner et al. 2005). Our genetic data strongly suggests that drainage reversals brought eastern lineages into central Chilean Patagonia. Historical drainage patterns better explain geographic patterns of genetic variation than do current drainage patterns, and central Chilean Patagonia is the only region where eastern lineages are found west of the Andes. Less information is available for other Pacific-draining watersheds with headwater lakes situated east of the divide, but our hierarchical AMOVA suggests it is likely that they share a similar history of drainage reversal. Such widespread changes in drainage patterns would have created an efficient mechanism for asymmetrical gene flow across the Andes, increasing genetic diversity west of the Andes. Consequences of drainage rearrangements for patterns of genetic diversity have been particularly well documented for New Zealand galaxiids (Waters and Wallis 2000; Waters et al. 2001, 2006; Burridge et al. 2006, 2007; Craw et al. 2007; Craw and Waters 2007). Such an approach might eventually help attain more accurate substitution rates for South American galaxiids and effectively reduce uncertainties for applications that employ these metrics (e.g. timing of lineage splitting).

2.5.3 Taxonomy and Conservation

Species-level taxonomy for the Galaxiidae in South America has a tangled past that extends from the early parts of the 20th century until present. Most controversy concerns the genus *Galaxias* to which as many as 16 nominal taxa have been assigned, only three of which are currently recognized as good species (McDowall 1971, 2006). The source of this inflation is mainly attributed to poor records and the description of distinct juveniles as new species, but can also be blamed on the extreme variation in morphology observed in some widespread species, particularly *G. platei* (Milano et al. 2002, 2006).

The phylogeographic evidence presented here suggests *G. platei* constitutes one species over its entire range supporting the original conclusions of McDowall (1971). Future studies employing nuclear markers in central Chile (area of proposed secondary contact) are being planned to further test the species-level status of *G. platei*.

Below the species level, four distinct evolutionary lineages were detected by phylogeographic analyses. Each of these units likely represents an independent evolutionary trajectory, each contributing an important source of genetic variation to the species recognized as *G. platei*. Secondary contact between each of these lineages in central Chile has resulted in this region currently hosting the greatest genetic diversity of *G. platei* throughout its range in Patagonia. This has important implications for conservation and management of the species, particularly considering the hydro-electric dams intended for construction in this area for the near future. I suggest that, unless additional genetic analysis indicates otherwise, future conservation strategies consider *G. platei* as a single species, but recognize the deep genealogical lineages that are distributed throughout Patagonia which meet in central Chile to form what is potentially the richest source of historical neutral genetic variation.

Table 2.1 Collection site details for Patagonian lakes/rivers, categorized according to relative position with the Andes, either west (Chile), east (Argentina) or spanning the range (cross-border), and therein ordered in a north/south orientation with respect to latitude. Cross-border lakes have two names (Chilean/Argentinean), the region of the lake sampled as indicated by underlined text. Details include the number of individuals sampled, local drainage basin membership (basin), GPS coordinates (latitude/longitude) and expected direction of ocean drainage before (historical) and after (current) the Last Glacial Maximum (LGM). Haplotype (*h*) and nucleotide (*π*) are listed for each location and post-hoc haplogroup cluster identified by phenetic and phylogenetic analyses of control region sequences (see Figures 2.2 and 2.3 and text for details).

Collection Site	**# Indv.**	**Basin**	**Latitude**	**Longitude**	**Ocean Drainage**		**Sequence Diversity**	
			(dd mm ss)	**(dd mm ss)**	**Current**	**Ancient**	***h***	***π***
Chile								
L Rinihue	2	Valdivia	39 46 29 S	72 27 10 W	Pacific	Pacific	1.0000 +/- 0.5000	0.006098 +/- 0.006680
L Llanquihue	14	Maullin	41 15 44 S	72 59 40 W	Pacific	Pacific	0.7692 +/- 0.0895	0.007719 +/- 0.004372
R Cudil	14	Chiloe	42 22 29 S	73 48 22 W	Pacific	Pacific	0.2747 +/- 0.1484	0.000348 +/- 0.000441
L Risopatron	17	Palena	44 15 51 S	72 31 20 W	Pacific	Pacific	0.2279 +/- 0.1295	0.000287 +/- 0.000389
L Las Torres	9	Cisnes	44 48 05 S	72 12 23 W	Pacific	Pacific	0.6944 +/- 0.1470	0.001355 +/- 0.001103
L Los Palos	5	Aysen	45 19 00 S	72 43 00 W	Pacific	Pacific	0.9000 +/- 0.1610	0.026309 +/- 0.016426
L Riesco	8	Aysen	45 29 56 S	72 40 40 W	Pacific	Pacific	0.4286 +/- 0.1687	0.006264 +/- 0.003866
L Alta	1	Aysen	45 31 16 S	72 41 45 W	Pacific	Pacific	1.0000 +/- 0.0000	0
L Escondida	9	Aysen	45 31 26 S	71 49 04 W	Pacific	Pacific	0.2222 +/- 0.1662	0.000271 +/- 0.000398
L Thompson	10	Aysen	45 38 18 S	71 47 15 W	Pacific	Pacific	0	0
L Pollux	10	Aysen	45 39 02 S	71 50 25 W	Pacific	Pacific	0	0
L Azul	10	Aysen	45 52 12 S	72 01 15 W	Pacific	Pacific	0.6444 +/- 0.1012	0.000894 +/- 0.000817
L Jeinimeni	3	Aysen	46 51 14 S	72 01 35 W	Pacific	Pacific	1.0000 +/- 0.2722	0.012195 +/- 0.009620
Cross-border								
L General Carrera / Buenos Ai	18	Baker	46 17 52 S	71 56 14 W	Pacific	Atlantic	0.8954 +/- 0.0653	0.007118 +/- 0.003991
L Cochrane / Pueyrredon	12	Baker	47 18 00 S	71 55 00 W	Pacific	Atlantic	0.8182 +/- 0.0957	0.006098 +/- 0.003589
L O'Higgins / San Martin	1	Chico	49 01 55 S	72 14 40 W	Pacific	Atlantic	1	0

Table 2.1 continued

Collection Site	# Indv.	Basin	Latitude (dd mm ss)	Longitude (dd mm ss)	Ocean Drainage		Sequence Diversity	
					Current	Ancient	*h*	π
Argentina								
L Espejo	10	Limay	40 41 00 S	71 40 00 W	Atlantic	Atlantic	0.3556 +/- 0.1591	0.000434 +/- 0.000518
L Puelo	10	Puelo	42 05 55 S	71 37 03 W	Pacific	Atlantic	0.2000 +/- 0.1541	0.000488 +/- 0.000556
L Rivadavia	10	Futalaufquen	42 37 00 S	71 40 28 W	Pacific	Atlantic	0.7333 +/- 0.1005	0.001138 +/- 0.000963
L La Plata	7	Senguerr	44 52 58 S	71 50 55 W	Atlantic	Atlantic	0	0
L Belgrano	10	Nansen/Bravo	47 52 10 S	72 08 46 W	Pacific	Atlantic	0.5111 +/- 0.1643	0.000678 +/- 0.000682
L Viedma	4	Santa Cruz	49 35 28 S	72 15 53 W	Atlantic	Atlantic	1.0000 +/- 0.1768	0.004472 +/- 0.003398
L Yehuin	7	Azopardo	54 24 00 S	67 44 00 W	Pacific	Atlantic	0.2857 +/- 0.1964	0.000348 +/- 0.000476
L Margarita	2	Azopardo	54 40 00 S	67 50 00 W	Pacific	Atlantic	0	0
L Escondido	9	Azopardo	54 40 24 S	67 44 31 W	Pacific	Atlantic	0.5556 +/- 0.1653	0.000881 +/- 0.000819
Haplotype Group								
A	40	-	-	-	-	-	0.7859 +/- 0.0438	0.004138 +/- 0.002403
B	51	-	-	-	-	-	0.8643 +/- 0.0348	0.003024 +/- 0.001843
C	101	-	-	-	-	-	0.6438 +/- 0.0427	0.001132 +/- 0.000872
D	20	-	-	-	-	-	0.1947 +/- 0.1145	0.000244 +/- 0.000352
All	212	-	-	-	-	-	0.8975 +/- 0.0134	0.030968 +/- 0.015093

Table 2.2 Analysis of Molecular Variance (AMOVA) using mitochondrial control region sequences for 212 individuals of *Galaxias platei* collected over the study area. Sequences were grouped according to (A) lake/river from which individuals were collected, (B) the freshwater basin containing each lake/river, and ocean (Pacific or Atlantic) into which each basin (C) currently drains or (D) historically drained [i.e. ancient refers to conditions before the Last Glacial Maximum approximately 10,000-20,000 yr BP]. Variation is reported as a percentage of the total with degrees of freedom (d.f.).

	% of variation (df)		
Grouping	**Among groups**	**Among populations within groups**	**Within populations**
A. Lake/River	91.02 (24)	-	8.98 (187)
B. Current basin	40.28 (14)	51.02 (10)	8.71 (187)
C. Current basin drainage	-0.49 (1)	91.47 (23)	9.02 (187)
D. Ancient basin drainage	35.91 (1)	56.64 (23)	7.45 (187)

Data source: ESRI World Data & Digital Chart of the World

Figure 2.1 Collection locations for *Galaxias platei* throughout Patagonia, South America. Sampled locations are represented by arrows that indicate contemporary drainage direction, either Atlantic or Pacific. Pacific (rev) denotes a drainage reversal, from Atlantic to Pacific, which likely occurred following the retreat of the Pleistocene glaciers (Turner et al. 2005). The extent of the LGM and contour of the largest Patagonian glaciation were adapted from Clapperton (1993) and Turner et al. (2005).

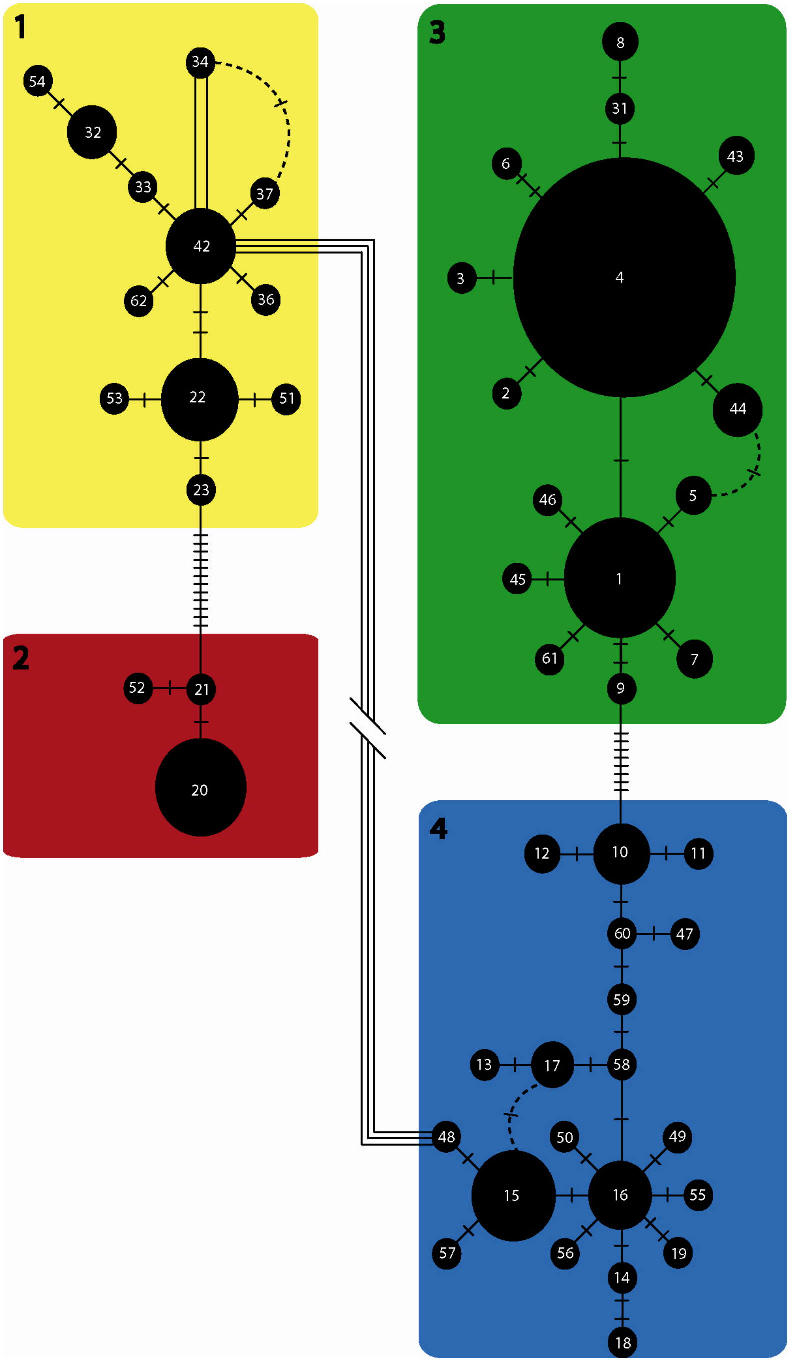

Figure 2.2 Minimum-spanning tree of mitochondrial control region haplotypes (n=50) for 212 individuals of *Galaxias platei*. Circles represent haplotypes (Appendix 2.1) and are scaled proportionally to the frequency of each haplotype in the sample. Single mutational steps between haplotypes are represented by a perpendicular line. The triple line connecting haplotype 42 with 48, and double line connecting 34 with 42, represent 49 and 33 mutational steps, respectively. Groups 1-4 are clusters of haplotypes, or haplogroups separated by several mutational steps.

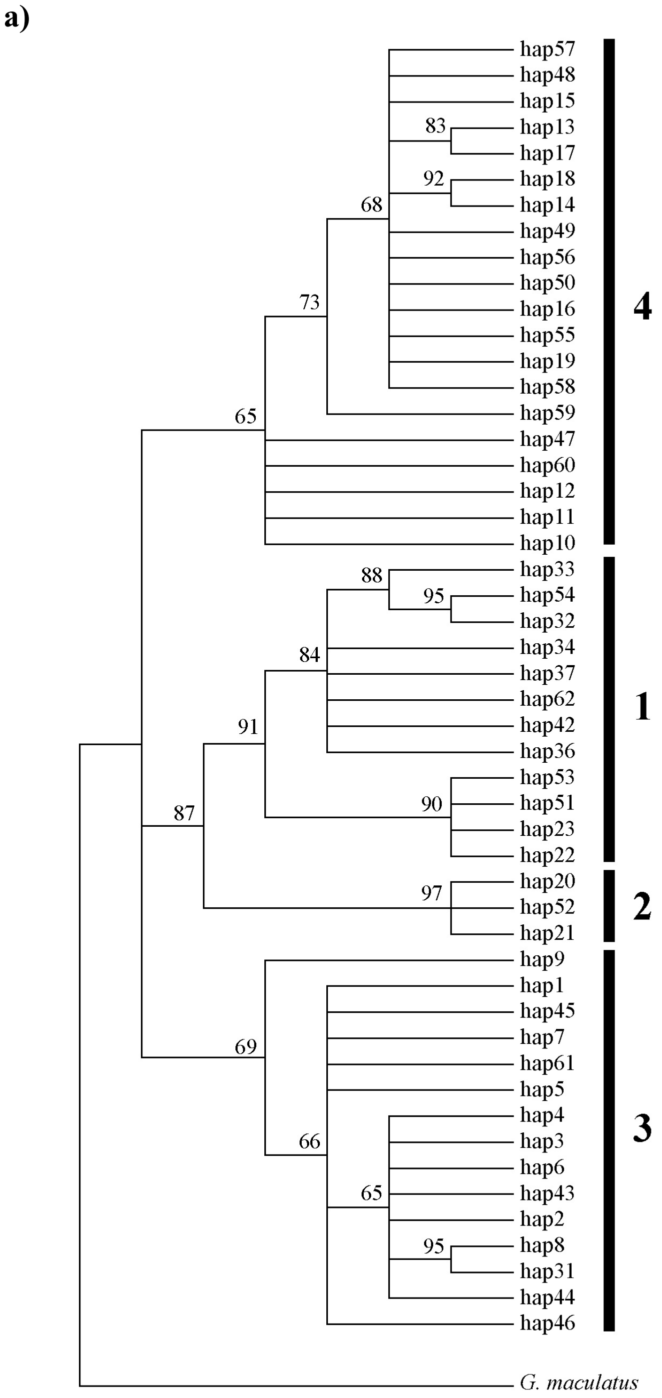

Figure 2.3 Phylogenetic majority consensus trees constructed using Bayesian inference employing (a) 50 control region haplotypes assuming an HKY + G + I model of molecular evolution, (b) eight COI sequences selected to represent the four *post-hoc* haplogroups (1-4) recovered by control region data. Analysis of COI data employed an HKY + G model of molecular evolution. Branch support for each analysis is based on Bayesian posterior probability densities.

b)

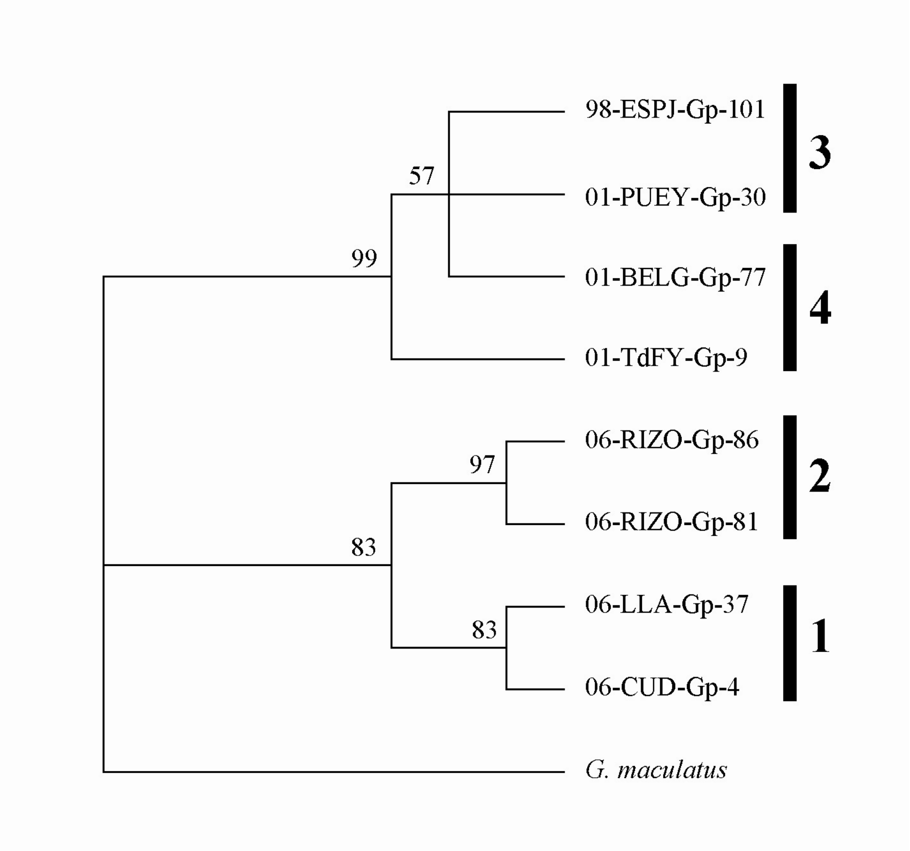

Figure 2.3 continued

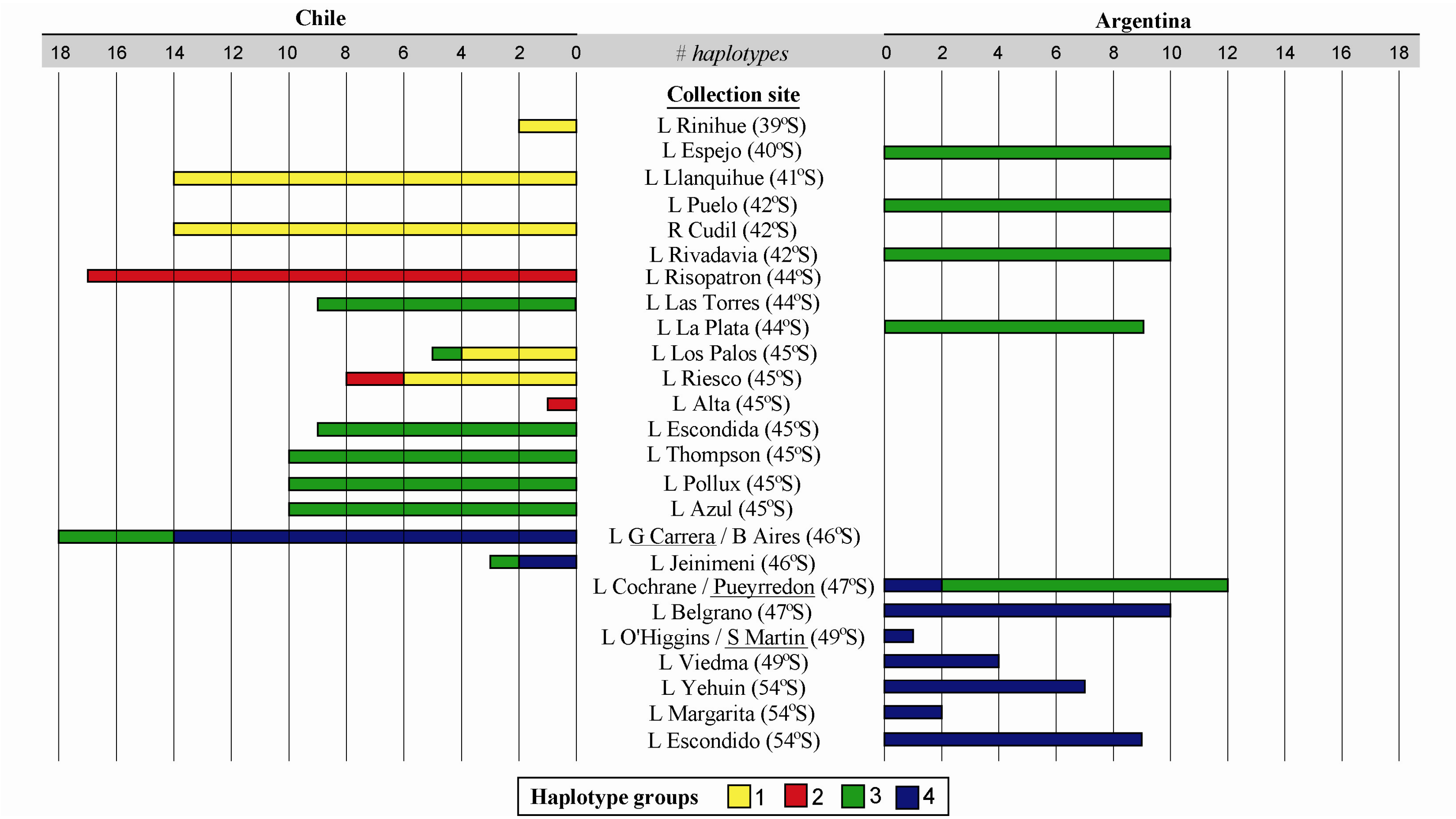

Figure 2.4 Control region haplotype frequency distribution based on haplogroup membership (number of individuals carrying a haplotype from a given haplogroup) for 212 individuals of Galaxias platei throughout the sample region. Lakes/rivers are organized according to latitude and therein categorized as Chilean or Argentinean. Cross-border lakes were categorized by the site in which samples were collected (Chile/Argentina), as indicated by the underlined text.

CHAPTER 3

SURVIVING HISTORICAL PATAGONIAN LANDSCAPES AND CLIMATE: MOLECULAR INSIGHTS FROM *GALAXIAS MACULATUS*

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3.1 Abstract

The dynamic geological and climatic histories of temperate South America have played important roles in shaping the contemporary distributions and genetic diversity of endemic freshwater species. I use mitochondrial and nuclear sequence variation to investigate the consequences of mountain barriers and Quaternary glacial cycles for patterns of genetic diversity in the diadromous fish *Galaxias maculatus* in Patagonia (~300 individuals from 36 locations). Contemporary populations of *G. maculatus,* east and west of the Andes in Patagonia, represent a monophyletic lineage comprising several well supported groups. First diversifying in the central/north-west region of Patagonia, *G. maculatus* extended its range into Argentina via the southern coastal regions that join the Pacific and Atlantic oceans. More recent gene flow between northern populations involved the most ancient and most derived lineages, and was likely facilitated by drainage reversal(s) during one or more cooling events of the late Pleistocene. Overall female effective population size represents the end result of a widespread and several hundred-fold increase over approximately 0.5 MY BP, spanning several climatic fluctuations of the Pleistocene. The minor influence of glacial cycles on the genetic structure and diversity of *G. maculatus* likely reflects the access to marine refugia during repeated bouts of global cooling. Evidence of genetic structure that was detected on a finer scale between lakes/rivers is most likely the result of both biological attributes (i.e. resident non-migratory behavior and/or landlocking and natal homing in diadromous populations) and the Coastal Cordillera as a dispersal barrier.

3.2 Introduction

The past two decades of phylogeographic research have tremendously increased our understanding of the evolutionary influences of Quaternary geological and climatic events on endemic biodiversity, but research thus far has been severely biased to regions of the Northern Hemisphere (Beheregaray 2008). Among the most neglected regions of the Southern Hemisphere is South America. This finding is surprising considering that the tropics of South America are expected to host an unmatched level of species richness and represent one of the best natural laboratories for studying speciation. Only recently has the Amazonian rainforest been the focus of large-scale surveys of intra-specific genetic variation aimed at understanding the potential mechanisms contributing to, and maintaining such high levels of species diversity (Lessa et al. 2003; Carnaval et al. 2009).

Phylogeographic patterns in temperate South America have also received relatively little attention, but recent studies point to the over-riding importance of two historic variables: the Andean mountain orogeny [starting approximately 23 MY BP (Ramos 1989)] and the glacial cycles of the Quaternary (2.5 MY - 10,000 yr BP). Estimated rates of trans-Andean gene flow vary greatly among taxa, including one species of plant (Allnutt et al. 1999), two fishes (Ruzzante et al. 2006; Zemlak et al. 2008), and several mammals (Smith et al. 2001; Palma et al. 2005; Himes et al. 2008), but the continental divide represents a barrier for all species examined to date. Post-glacial patterns of dispersal for several taxa indicate the existence of several independent Quaternary glacial refugia east of the Andes on the Patagonian Steppe (Kim et al. 1998; Premoli et al. 2000; Pastorino and Gallo 2002; Palma et al. 2005; Ruzzante et al. 2006; Zemlak et al. 2008) and west of the Andes, both within (Zemlak et al. 2008; Xu et al. 2009) and outside of northern and western limits of the glaciers (Premoli et al. 2000; Muellner et al. 2005; Ruzzante et al. 2006; Victoriano et al. 2008; Zemlak et al. 2008; Xu et al. 2009). Populations persisting in some of these refugia experienced repeated founder-flush cycles leading to the purging of genetic variance and resulting in the development of genetic structure (Ruzzante et al. 2008).

Phylogeographic patterns of aquatic organisms are largely determined by historical changes in hydrological landscapes. In Patagonia, starting approximately 23 MY BP (Ramos 1989), the rise of the southern Andes created a continental divide that separated eastern and western basins into primarily Atlantic and Pacific drainages, and studies of two freshwater species, *Percichthys trucha* (Ruzzante et al. 2006) and *Galaxias platei* (Zemlak et al. 2008) have shown that the uplift presented a significant barrier to gene flow. Glacial advances during periods of global cooling in the Quaternary eliminated much southern and higher altitude aquatic habitat (Clapperton 1993; McCulloch et al. 2000), reducing and/or displacing populations. Patterns of intraspecific genetic diversity of fishes and crabs suggest that multiple refugia existed to the north and east of continental ice, as well as within glacial margins of the central-west (Ruzzante et al. 2006; Zemlak et al. 2008; Xu et al. 2009). Important rearrangement of basins also occurred during the retreat of continental glaciers in Patagonia (Turner et al. 2005). The formation of temporary but large proglacial lakes at the fringes of melting glaciers, coupled with high altitude mountain run-off, probably allowed the exchange of aquatic organisms among drainage basins, and allowed the expansion of populations into unoccupied basins (Zemlak et al. 2008; Xu et al. 2009). Catastrophic shifts in drainage direction (from Atlantic to Pacific) in several basins, as ice dams collapsed during the retreat of glaciers, have been implicated as important vectors of gene flow from east → west across the Andean divide for *G. platei* (Turner et al. 2005; Zemlak et al. 2008).

In the present study, I examined the potential consequences that major Quaternary geological and climatic events in Patagonia had on the evolution of *Galaxias maculatus*, one of the most widespread diadromous fish species worldwide. I employed DNA sequence variation at both mitochondrial and nuclear loci from *G. maculatus* populations throughout their range in Patagonia to test for influences of mountain ranges as barriers to gene flow and the roles of repeated Pleistocene glacial cycles in further shaping the contemporary genetic diversity and structure of a diadromous fish. To the best of our knowledge, this study represents the first phylogeographic investigation of a diadromous fish species in temperate South America.

Galaxias maculatus is considered amphidromous, a special case of catadromy characterized by a shortened duration at sea. Disjunct populations of *G. maculatus* are found in the coastal systems of southern Australia, Tasmania, New Zealand and surrounding islands (e.g. Lord Howe, Chattam, New Caledonia), and in the temperate latitudes of South America and the Malvinas (Falkland) Islands (McDowall 1970, 1971; Croizat et al. 1974; Rosen 1978). Although both dispersal (McDowall 1969, 1978) and vicariance (Rosen 1974, 1978) hypotheses have been proposed to explain this fragmented distribution, most empirical evidence indicates that populations dispersed from Australia to other locations by ocean currents (West Wind Drift) (Berra et al. 1996; Waters and Burridge 1999; McDowall 2000; Waters et al. 2000a).

The influence of the Andes mountains in shaping the genetic structure of *G. maculatus* in Patagonia is uncertain. One possibility is that a widespread distribution was established prior to the onset of Andean orogeny and vicariance either temporarily, or permanently isolated eastern and western populations to create deep genetic structure (vicariance). Equally likely is the establishment a widespread distribution following Andean uprise. Ancestral lineages could have persisted in either eastern or western Patagonia and subsequently expanded into the adjacent region (dispersal, W→E or E→W). To better understand the colonization history of the surviving lineage(s) that comprise contemporary South American populations of *G. maculatus* I reconstructed the phylogenetic relationship of approximately 300 individuals with respect to a suitable outgroup. Vicariance assumes a widespread distribution prior to Andean uprise, followed by a simultaneous division of eastern and western locations to yield reciprocally monophyletic haplotype assemblages. Alternatively, dispersal suggests that *G. maculatus* attained its widespread distribution throughout Patagonia following Andean orogeny to imply that one location, either east or west (depending on dispersal direction), was colonized prior to the establishment populations on both sides of the Andes. Phylogenetically, this would be represented by the dispersing lineage being nested within the source lineage. Assuming that *G. maculatus* originally arrived in South America via WWD, a scenario involving E→W dispersal would suggest local extirpation of western populations followed by recolonization of an eastern lineage (Figure 3.1).

In the event that dispersal was an important component of colonization history, there are at least two mechanisms that could have facilitated connections between eastern and western Patagonia. First, the diadromous capacity of *G. maculatus* affords an indirect route of marine-mediated stepping-stone dispersal. Populations could have invaded from either eastern or western sources by accessing the connection between Atlantic and Pacific oceans at the southernmost tip of South America. The second mechanism involves drainage reversals; a climate-induced shift in which formerly Atlantic draining river systems catastrophically shifted to assume an opposite direction of outflow into the Pacific. Drainage reversals following the Last Glacial Maximum (LGM) are thought to have facilitated a direct route of dispersal across the Andean divide for the co-distributed freshwater species *G. platei* and could have had similar implications for *G. maculatus*. Assuming that colonization history reflects dispersal, I attempt to discern between the relative influences of direct (trans-Andean) versus indirect (marine) modes of dispersal by comparing genetic distance with geographic distance modeled under each scenario. Furthermore, I test for the specific influences of drainage reversals following the LGM by comparing partitions of genetic variation according to the expected pre- and post-LGM drainage patterns in Patagonia.

Finally, I examined the potential influence of repeated Pleistocene glacial cycles on long-term effective population size. The diadromous capacity of *G. maculatus* affords populations an additional and/or alternative form of refuge in the much more stable marine realm. The availability of marine refugia might have reduced or exempted populations from the founder-flush cycles that are typically experienced by freshwater-limited taxa (Bernatchez and Wilson 1998, 1999; McDowall 1999) and the reshuffling effects of drainage reversals. However, exceptions to an amphidromous life-cycle have been reported for several populations in South America in the form of physically landlocked and non-migratory populations that remain exclusively within freshwater throughout their life cycle. Depending on the duration and frequency with which some populations remained within freshwater in the past, glacial cycles could have introduced new genetic structure and reduced population sizes throughout parts of the range of *G.*

maculatus. Bayesian skyline plots are employed to reconstruct long-term historical effective population sizes over a period of repeated Pleistocene glacial advances.

3.3 Methods and Materials

3.3.1 Sample Collection and Preservation

A total of 299 individuals were collected using seine nets and electrofishing between 1998-2007 from 36 lakes and rivers throughout Argentina and Chile (Figure 3.2). Sample sizes varied according to location and region (Table 3.1). Ninety (30%) individuals derived from 10 Argentinean lakes, and the remaining 209 individuals were collected from 26 Chilean lakes/rivers. Sub-samples (gill, muscle, fin, blood) for molecular analyses were fixed in 95% ethanol.

3.3.2 Sequence Data

Prior to DNA extraction all tissue samples were dried of ethanol by exposure to ambient temperature for approximately 120 min. Total genomic DNA was isolated from the majority of sub-samples (10 μL of blood or 2x2 mm^2 tissue) using the glassmilk procedure described in Elphinstone et al. (2003) with slight modifications for execution using a MultiPROBE® II HT PLUS EX robotic liquid handling system (PerkinElmer). Sub-samples derived from two Argentinean locations (Quillén, Espejo) showed signs of tissue degradation and were extracted using standard phenol/chloroform protocols, and subsequently concentrated using ethanol precipitation (Sambrook and Russel 2001).

A section of approximately 800 bp of the mitochondrial control region was amplified from all Chilean samples and the majority of Argentinean individuals using the primer combination *S-phe* 5'-GCT TTA GTT AAG CTA CG-3' (Nielsen et al. 1994) and *P3* 5'-AAC TTC CAT CCT CAA CTC CCA AAG-3' (Sang et al. 1994). The full mitochondrial genome available in GenBank (NC_004594) was used to design species specific forms of both *S-phe* (5'-GCT TTA CTT AAG CTA CG-3') and *P3* (5'-AAC TCT CAC TCT TAA CTC CCA AAG-3') to amplify all individuals from six Argentinean locations (lakes Quillén, Espejo, Morenito, Hess, Martin, Steffen). PCRs were 25 µL: 2.5 µL 10X Reaction Buffer [100mM KCl, 100mM $(NH_4)_2SO_4$, 200mM Tris HCl (pH 8.75, 22°C), 1% Triton X-100, 1mg/ml BSA], 2.5 µL dNTPs (2 µM each), 3.75 µL $MgSO_4$ (20 mM), 13 µL ddH_2O, 0.25 µL of each 10 µM primer, 1 U of Tsg DNA Polymerase (Bio Basic Inc.) and 2.0-2.5 µL of DNA template. A Mastercycler® EP Gradient (Eppendorf) thermal cycler was used to conduct all reactions under the following thermal regime: an initial denaturing temperature of 94ºC for 5 min, followed by 35 cycles of 94ºC for 1 minute, 50ºC for 1 min 30, and 72ºC for 1 min 30, and a final extension at 72ºC for 5 min.

Nuclear DNA (approximately 600 bp) was also amplified from a subset of individuals (N=26) selected based on *post-hoc* information to represent each mitochondrial haplogroup. These individuals represented rivers Bueno (4), Valdivia (3), and Toltén (2) and lakes Llanquihue (4), Tarahuin (5), and Natri (3) in Chile, and lakes Argentino (4) and Martin (1) in Argentina. The target locus was isolated using unpublished primer sequences [ANL22 forward (5'-TGT TTG GCT TCT ATG CAG GA-3') and reverse (5'-TGC GAT CCA TCA TCA ACT TT-3')] which originated from a genomic library aimed at isolating anonymous nuclear loci from a closely allied species *G. platei* following protocols of Jennings and Edwards (2005) and Carstens and Knowles (2006). Except for an annealing temperature of 56ºC, the PCR conditions used to amplify the nuclear locus were identical to those reported above for control region.

Amplicons were visualized using 1.0% agarose gels and sent off site for bidirectional DNA sequencing at Macrogen Inc. locations in Korea and the USA. Each forward and reverse sequence was edited using Sequencher™. Sequences were aligned in ClustalX2 (Larkin et al. 2007) using default parameters and subsequently checked by eye. The complete alignment for control region data was compressed into haplotypes using DnaSP 3.0 (Rozas and Rozas 1999) and listed under the following accessions in GenBank: GQ180504-GQ180776. Ambiguous nuclear alleles, represented by sequences containing

2 or more heterozygous base calls, were separated with 100% probability using the program PHASE v2.1.1 (Stephens et al. 2001). All alleles were confirmed to be free of recombination using both RDP and MaxChi algorithms as executed in the program RDP3 (Martin and Rybicki 2000), and subsequently blasted in GenBank to ensure no matches with mitochondrial DNA. Nuclear allele sequences are available in GenBank under the following accessions: GQ180467-GQ180503.

3.3.3 Data Analysis (Control Region mtDNA)

Standard molecular diversity indices for control region haplotypes [haplotype diversity (h), nucleotide diversity (π), nucleotide frequencies, transition/transversion ratio, number of polymorphic sites] were calculated using Arlequin 3.1 (Excoffier et al. 2005). Phylogenetic relationships among haplotypes were reconstructed using maximum likelihood methods as implemented in PHYML (Guindon and Gascuel 2003) using the web based server PHYML-Online (Guindon et al. 2005). Modeltest (Posada and Crandall 1998) selected the GTR+G ($\gamma = 0.5483$) as the most appropriate model of molecular evolution for phylogenetic analysis with branch support based on 100 bootstrap replicates. The outgroup was based on a single Tasmanian *G. maculatus* control region haplotype (NC_004594) because it represented the only full length control region isolate available in GenBank that fully overlapped with Patagonian derived haplotypes. However, auxiliary phylogenetic analyses were conducted using partial isolates of control region sequences from (Waters et al. 2000a) (AF240184-AF240339) to justify the use of a single sequence as an outgroup. Maximum likelihood analysis of ~150 haplotypes derived from Tasmanian and New Zealand origins indicate close associations between all haplotypes derived from eastern Pacific sources and support Patagnonian haplotypes as a strongly supported monophyletic group. Supplemental trees are available in newick tree format (Appendix 3.1, 3.2).

Alternate dispersal scenarios were investigated using Mantel Tests as implemented in Arlequin 3.1 (Excoffier et al. 2005) using 1000 bootstrap replicates. Collection locations were grouped into regional categories based on both proximity and location with respect to the Andes (Table 3.1): north-west (NW), west 1 (W1), west 2 (W2), east (E) and south east (SE). Approximate pairwise distances (km) between regions were calculated from the central geographic point using Google Earth according to two models: first, a direct route across terrestrial landscape to represent trans-Andean dispersal; second, an indirect route to circumscribe the southern Andes via oceanic connections to represent marine-mediated dispersal (Appendix 3.3). Genetic distances between regions were based on standard pairwise estimates of F_{ST} calculated using Arlequin 3.1 (Excoffier et al. 2005).

Hierarchical Analysis of Molecular Variance (AMOVA), as executed in Arelquin 3.1 (Excoffier et al. 2005), was used to determine how contemporary genetic variation is partitioned over the range of *G. maculatus*. Groupings included (1A) lake/river of collection, (1B) river drainage, (1C) present-day and, (1D) ancient (pre LGM) ocean (Pacific or Atlantic) drainage. Further *post-hoc* regional analysis was conducted for a subset of collection locations in Chile between latitudes 34°S and 40 °S. This regional consideration was motivated by distributional trends of haplotype groups which suggested compositional differences between populations located near the Pacific coast versus those located within the continental interior of the Andean foothills (Figure 3.6). The analysis involved 17 collection sites from 10 river basins, all of which drain into the Pacific Ocean (Table 3.2, Figure 3.3). Running in a north → south orientation are two mountain ranges: the Coastal Cordillera located near the Pacific coast, and the Andean mountains to the east with the Central Valley in between the two mountain chains. Of the 10 river basins that were sampled, four originate in the Andean foothills and bisect the Central Valley and the Coastal Cordillera en route to draining into the Pacific (Tolten, Valdivia, Bueno, Maullin), two of which were represented by collection sites located on either side of the Coastal Cordillera (Valdivia, Maullin). The remaining six basins originate within the Coastal Cordillera and extend only a short distance to the coast (Topocalma, Nilahue, Reloca, Queule, Lingue, Contaco). I grouped each population as "coastal" or "Andean" for AMOVA analysis based on two criteria: (2A) location of headwaters and (2B) location of collection site (Table 3.1).

Historical population sizes of *G. maculatus* were estimated using the coalescent-based Bayesian skyline plot (Drummond et al. 2005) as implemented in the program BEAST 1.4 (Drummond and Rambaut 2007). Priors included the GTR+G+I model of molecular evolution with an estimated substitution rate matrix (A-C = 1.35, A-G = 7.83, A-T = 0.34, C-G = 1.02, C-T = 3.47, G-T = 1.0) as determined by Modeltest (Posada and Crandall 1998), and implemented a mutational timescale of 0.01876 substitutions/site/My appropriate for galaxiids (Waters et al. 2007). Starting operators were based on default settings and auto-optimized during searches of parameter space using Markov Chain Monte Carlo (MCMC) sampling procedures. Parameter estimates were based on posterior probability distributions constructed by sampling the stationary distribution for 40,000,000 generations, sampling every 1000 steps.

3.3.4 Data Analysis (Nuclear Locus)

Standard molecular indices were calculated as per the mtDNA control region. Unrooted phylogenetic relationships among alleles were reconstructed using maximum likelihood methods as implemented in PHYML (Guindon and Gascuel 2003) using the web based server PHYML-Online (Guindon et al. 2005). Modeltest (Posada and Crandall 1998) selected the K81uf+G ($\gamma = 0.5759$) as the most appropriate substitution model. Branch support was based on 100 bootstrap replicates.

3.4 Results

A total of 273 mtDNA control region haplotypes were identified from 299 individuals of *G. maculatus* collected from throughout the species distribution in South America (Appendix 3.4). Overall, control region nucleotide frequencies were: A (28.94%), C (24.30%), G (19.36%), T (27.41%). The total number of polymorphic sites was 250, with a transition/transversion ratio of 2.16, 92 observed indels, an average of 37.71 +/- 16.44 pairwise differences between sequences (4.6 +/- 2.0%), a nucleotide diversity (π) of

0.9989 +/- 0.0005 and haplotype diversity (*h*) of 0.046394 +/- 0.022376. Individual nucleotide and haplotype diversity estimates are listed for each location in Table 3.1, and do not vary according to latitude.

Nineteen unique alleles were recovered from the 26 individuals sequenced at the nuclear locus, and over half of the individuals (61%) were homozygous for a given allele. Overall nucleotide frequencies were: 32.7% (A), 17.7% (C), 21.3% (G) and 28.3% (T). The total number of polymorphic sites was 19, with a transition/transversion ratio of 0.58, no indels, an average of 2.35 +/- 0.002 pairwise differences between sequences, a nucleotide diversity (π) of 0.0039 +/- 0.0024 and haplotype diversity (*h*) of 0.8679 +/- 0.0495. Maximum likelihood analysis of alleles revealed a poorly supported gene tree which was paraphyletic with respect to mitochondrial-defined haplogroups (Figure 3.4).

3.4.1 Evidence of Marine Dispersal

Maximum likelihood analysis of mitochondrial control region haplotypes revealed four distinct clusters (Figure 3.5). The four clusters (herein referred to as haplogroups 1-4) included the following haplotypes: (1) 255-271, (2) 245-254, (3) 212-244, (4) 1-211. Smaller, well supported clusters were also recognized within haplogroups 3 and 4 (herein sub-groups): (3a) 233-243, (4a) 206-210, (4b) 103-118, (4c) 25-100. Haplotypes 272-273 formed a distinct, well-supported branch among basal lineages, but was not recognized as a separate sub-group because of low sampling density (i.e. only consisted of 2 haplotypes).

When the frequency of each haplogroup at each collection location was plotted by geographic location (Figure 3.6), I obtained a pattern reflective of dispersal from west→east. Northern Chilean Patagonia hosts the most diverse assemblage of haplotype groups. By contrast, populations in Argentina had mostly haplotypes from sub-groups nested within haplogroup 4; sub-group 4c dominated the northern Andean locations (Limay & Manso river basins) and haplogroup 4b was ubiquitous in southern Andean locations (lakes Roca and Argentino). Haplogroup 1 is primarily limited to the northwest coastal regions of Chile, except for very minor representation in the most northern drainage of Argentina. The most geographically widespread lineage, haplogroup 4, was found in both Chile and Argentina.

Mantel tests supported the marine realm as the most important route of dispersal into eastern Patagonia (Figure 3.7). A comparison of pairwise F_{ST} versus geographic distance yielded a strong positive relationship when distance was modeled according to a scenario of marine dispersal ($r = 0.69$, $P = 0.055$). By contrast, direct distance between regions was not correlated with genetic distance ($r = -0.05$, $P = 0.463$).

3.4.2 Drainage Reversals

I conducted several hierarchical AMOVAs using control region sequences. First, when all collection sites (lake or river) were considered independently, 61% of the total variance in sequences was explained by differences among collection sites and 39% by variation among individuals within sites (Table 3.2). Grouping the 36 lake/river collections into 17 basins explained nearly 30% of the total variance (Table 3.2) suggesting that populations from different lakes within river systems are more similar to each other than they are to populations in other river drainage systems. Thirdly, pooling samples according to their historical (pre-LGM) oceanic drainage (Pacific vs. Atlantic) explained approximately four times more variance than pooling them into present-day drainage (15.6% vs. 3.7%, Table 3.2) suggesting some of the systems that currently drain into the Pacific Ocean may historically have drained into the Atlantic Ocean.

3.4.3 The Coastal Cordillera

I conducted an AMOVA considering only coastal and Andean river basins within latitudes 34^{o}S and 40 ^{o}S in Chile. This analysis revealed that grouping samples according to collection site (coastal vs. interior, with coastal being west and interior being east of the Coastal Cordillera) explained approximately twice as much genetic variation (13.93%) than grouping samples according to where the headwaters lie (Andean vs. coastal) (6.64%). This finding suggests that downstream populations of Andean systems in this region of Chile are more similar to populations of other coastal rivers than to upstream populations within their own river systems and suggests that the Coastal Cordillera may represent a partial barrier to geneflow (Table 3.2).

There were marked differences between coastal and Andean populations in Chile, particularly at mid-latitudes. Western Andean populations (Valdivia basin) are much more closely allied with populations east of the Andes at the same latitude (Limay & Manso river basins) than with a western coastal collection site within the same basin (Rio Valdivia) (Fig 3.6).

3.4.4 Demography

Demographic reconstructions based on control region haplotypes revealed long-established maternal effective population sizes which coalesce at approximately 3.2 MY BP (Figure 3.8). Effective population size appears to have remained relatively constant until roughly 0.5 MY BP when population size rapidly increased two orders of magnitude [100X (60X-190X)] to reach contemporary levels.

3.5 Discussion

Of the potential vicariant processes associated with historical shifts in landscape and climate of ancient Patagonia, only mountains appear to have had significant influence on the distribution and genetic structure of *G. maculatus*. Effective population size increased through the past 0.5 MY BP despite repeated periods of glacial advance and retreat. The role of Quaternary climatic cycles appears restricted to northern drainages where river drainage diversion swapped representatives of two divergent lineages. Otherwise, genetic structure appears to be a consequence of the variations in the life history of *G. maculatus*, responding to isolation from the sea (landlocking) or to other factors limiting female dispersal.

3.5.1 Evolutionary Structure

Contemporary *G. maculatus* populations form several distinct matrilineal lineages whose phylogeographic signal suggests a probable pattern of dispersal and diversification within Patagonia. Extant populations are most likely derived from coastal populations in northern Chilean Patagonia. This region contains most of the current genetic diversity. One haplogroup (4) is common on the Atlantic and Pacific coasts, as well as in the Beagle Channel. Two sub-groups of haplogroup 4, sub-groups b and c, are almost entirely restricted to Andean locations, and the latter is more common in the north and found in both western and eastern drainages.

These patterns, combined with evidence of Isolation by Distance (IBD) under a scenario of marine dispersal (Mantel tests), suggest that *G. maculatus* began to diversify in systems of Northern Chilean Patagonia and subsequently spread south along the Pacific coast, colonizing adjacent coastal systems via stepping-stone dispersal, eventually reaching Atlantic systems via the southern tip of South America. On the Atlantic side, range expansion and diversification likely continued northward and westward. Haplogroup 4 was likely responsible for establishing the widespread distribution of *G. maculatus* on the eastern side of the Andes, given its current distribution and abundance. The pattern of expansion thus contrasts with those of other co-distributed, but freshwater-limited species such as *P. trucha* and the closely related *G. platei* whose widespread contemporary distributions more strongly reflect the effects of vicariant separation initiated by mountain uplift (Ruzzante et al. 2006; Zemlak et al. 2008). Within a more recent timeframe, the invasive, diadromous Chinook salmon (*Oncorhynchus* *tshawytscha*) has followed a route to Argentina similar to that of *G. maculatus* after escaping from aquaculture pens in Pacific Chilean waters (Becker et al. 2007).

The strong matrilineal structure found in control region sequences suggests limited female dispersal among populations, but does not indicate whether male dispersal has also been restricted or if haplogroups represent only female philopatry. Contrasting genetic signatures between nuclear and mitochondrial markers is commonly employed to evaluate alternate explanations, and disagreement between marker classes is taken as evidence for sex-biased dispersal. However, in the present case, supplemental analysis at a single variable nuclear locus failed to provide a clear answer. The resulting nuclear gene tree was paraphyletic with respect to each haplogroup (Figure 3.4), a result which could be explained by inter-lineage hybridization via male-mediated gene flow or by the persistence of ancestral polymorphisms in isolated populations because of incomplete lineage sorting. Male-biased dispersal has been recognized in several species of fishes (Hutchings and Gerber 2002; Bekkevold et al. 2004; Cano et al. 2008), and considering that polygamous mating strategies are thought to encourage male-biased dispersal (Perrin and Mazalov 2000), it seems plausible to consider that such a pattern could arise in a broadcast spawner such as *G. maculatus*. The large differences between coastal and Andean populations in Chile, however, is likely due to restricted dispersal by both sexes, as many of the Andean rivers and lakes are landlocked or are far from the sea with populations that are non-migratory. Physical isolation or landlocking has been recognized several times as an important mechanism of diversification within galaxiids at both population and species levels (Allibone and Wallis 1993; Waters et al. 2000b; Waters and Wallis 2001). Further investigation will be required to fully understand the biological explanations of genetic structure revealed by mitochondrial signal. In particular, several additional, independent nuclear loci are required to provide the statistical framework to confidently distinguish between instances of secondary contact and incomplete lineage sorting (Brito and Edwards 2009). Our laboratory is currently developing suitable primers to address this problem.

South American populations of *G. maculatus* appear to show stronger regional genetic structuring than do populations in the western Pacific. In addition to our findings, Waters and Burridge (1999) reported unexpectedly high estimates of genetic divergences at the cytochrome *b* locus between the Malvinas (Falkland) islands and mainland populations. Zattara & Premoli (2005) also found genetic signatures consistent with restricted geneflow between adjacent, landlocked populations in Argentina based on allozyme frequencies. By contrast, molecular studies in the western Pacific (i.e. New Zealand, Australia, Tasmania) suggest that *G. maculatus* is an uninhibited disperser capable of maintaining both regional and trans-oceanic gene flow. Barker and Lambert (1988) found no evidence for genetic structuring between four populations from the Bay of Plenty, New Zealand using measures of geneflow based on several allozyme loci. Further phylogeographic analysis generalized this finding by reporting very little genetic structure between several broadly distributed locations throughout New Zealand (Waters et al. 2000a). DNA sequence data and allozyme-based studies also support recent trans-ocean dispersal between the eastern-Pacific continents (Berra et al. 1996; Waters et al. 2000a). These contrasting patterns suggest that the genetic structuring observed in Patagonian populations of *G. maculatus* may be unusual for the species.

3.5.2 Drainage Reversals

The presence of the same haplogroups in eastern and western draining basins in the northern Patagonian Andean populations suggests that, in contrast to the coastal mountains in Chile, there has been substantial gene flow across the Andes. Two very divergent groups (1 and 4c) were found on both sides of the continental divide in this region. I suggest that rearrangement of the hydrological landscape during the Pleistocene, systems that experienced one or more drainage reversals between Pacific and Atlantic, is the mechanism most likely responsible for bidirectional, trans-Andean gene flow of *G. maculatus*, producing a distribution of genetic diversity that is better explained by ancient drainage direction than by current orientation. Along the latitudinal range of the Andes in Patagonia, there are several examples of river systems with headwaters originating in

Argentina that bisect the mountains to drain into the Pacific. Continental ice during the LGM imposed a western barrier to aquatic systems east of the Andes forcing their flow into the Atlantic (Turner et al. 2005). Subsequent periods of warming created large accumulations of melt water (paleolakes) at the eastern fringe of glacial mountain ice, dammed by terminal moraines in the east and the ice itself in the west. The collapse of the western barriers of these meltwater paleolakes as the ice melted resulted in a catastrophic drainage reversal from the Atlantic to the Pacific (Clapperton 1993; Tatur et al. 2002; Turner et al. 2005), with a significant flood of glacial melt water and any associated biota. For example, the Valdivia river system in Chile has its headwaters (Lago Lácar) in Argentina, and populations of *G. maculatus* in its Andean lakes are phylogenetically more closely allied with the northern populations of Argentina (Limay, Manso river basins) than with other nearby Chilean populations. The same mechanism is thought to be responsible for introducing eastern lineages of *G. platei* into the west (Zemlak et al. 2008). In the present study, the presence of haplotypes of haplogroup 1 east of the Andes in Lake Quillén (Figure 3.5) suggests that dispersal also occurred west to east. The geological mechanism(s) that could have facilitated this exchange is not known. Perhaps individuals of *G. maculatus* occupied the headwaters of an ancient Pacific drainage which historically originated on the eastern side and were diverted along with the headwaters toward the Atlantic as glacial ice formed. Pleistocene-induced alterations in freshwater hydrology, especially river capture, have been very important influences on contemporary diversity within the Galaxiidae in New Zealand as well (Waters and Wallis 2000; Waters et al. 2001; Burridge et al. 2006, 2007; Craw et al. 2007a; Craw et al. 2007b).

3.5.3 Historical Demography

Long-term and widespread increases in effective population size despite repeated bouts of glacial advance throughout the Pleistocene suggest that contemporary populations of *G. maculatus* inhabiting post-glacial regions may have retreated to coastal refugia during periods of global cooling. Bayesian skyline plots indicated only upward trends in female effective size over the past 0.5 MY BP. Unvarying estimates of high haplotype, but low nucleotide diversity across sample locations are also consistent with genetic signatures of widespread population growth (Grant and Bowen 1998). Access to stable marine environments would have released *G. maculatus* from the genetic purging via founder-flush cycles typically associated with freshwater refugia and would explain the contrasting patterns revealed in other co-distributed Patagonian freshwater fishes that showed evidence of at least one genetic bottleneck during the late Pleistocene (Ruzzante et al. 2008). A review of patterns of genetic diversity in the Palearctic and Nearctic revealed that this strategy appears to be widespread among diadromous fishes of the northern hemisphere (Bernatchez and Wilson 1998, 1999; McDowall 1999, 2007), and is likely extendible to *G. maculatus* in the southern hemisphere. The reason for constant increase, however, is less clear. Perhaps the concomitant period of dramatic cooling was responsible for creating a scenario of ecological release in which severe conditions evicted most inhabitants from several Patagonian systems and allowed the uninhibited invasion of *G. maculatus* from several healthy marine-based sources. Interestingly, the period during which this invasion appears to have taken place immediately follows the coldest Patagonian glaciation approximately 0.7 MY BP, which was responsible for dramatic declines in population sizes of the co-distributed freshwater galaxiid, *Galaxias platei* (Ruzzante et al. 2008). Perhaps similar glacial-induced extirpations affected other native species and provided the right circumstances for *G. maculatus* to invade the eastern systems of Argentina.

3.6 Conclusion

This study provides important insights into the potential mechanisms responsible for influencing the distribution and diversity of *G. maculatus* throughout Patagonia. Vicariant processes associated with mountain building and climate-induced drainage shifts appear to have been particularly influential in determining access routes of dispersal throughout Patagonia. Population sizes, however, remained very resilient in spite of repeated climatic shifts suggesting that marine environments played very important roles as glacial refugia on perhaps several occasions throughout the Quaternary. Several questions relating to the biology of *G. maculatus* also emerged based on the fine-scale phylogeographic structure, including the potential contributions of gender-specific dispersal and/or landlocking to population-level structuring. These broad-scale interpretations of genetic structure provide a much needed historical context within which studies of metapopulation dynamics can be properly interpreted, as well as *a priori* knowledge of regional diversity for phylogeographic and phylogenetic analyses.

Table 3.1 Collection site details for Patagonian lakes/rivers, categorized according to relative position with the Andes, either west (Chile) or east (Argentina), and therein ordered in a north/south orientation with respect to latitude. The northernmost Chilean locations are also classified according to relative position with the Coastal mountains, either west (Coastal) or east (Interior). Details include the number of individuals sampled, local drainage basin membership (basin), GPS coordinates (latitude/longitude), and expected direction of ocean drainage before (ancient) and after (current) the Last Glacial Maximum (LGM) (Clapperton 1993; Turner et al. 2005; Zemlak et al. 2008), and locatinon of headwaters as either coastal or Andean for a sub-set of Chilean locations used for post-hoc groupings for analyses of molecular variance (Table 2). Sequence diversity metrics [haplotype (h) and nucleotide (π)] based on mitochondrial control region sequence data are listed for each location.

Collection Site	# Indv.	Basin	Latitude (dd mm ss)	Longitude (dd mm ss)	Position wrt Coastal Range	Ocean Drainage		Sequence Diversity	
						Current	Ancient	*h*	*π*
Chile									
Estero Topocalma	1	Topocalma	34 17 51	71 57 46	Coastal	Pacific	Pacific	-	-
Estero Nilahue	4	Nilahue	34 29 01	72 00 58	Coastal	Pacific	Pacific	1.0000 +/- 0.1768	0.014429 +/- 0.009937
Estero Reloca	3	Reloca	35 37 49	72 33 45	Coastal	Pacific	Pacific	1.0000 +/- 0.2722	0.015038 +/- 0.011755
Rio Tolten	8	Tolten	38 59 09	72 37 10	Interior	Pacific	Pacific	0.8929 +/- 0.1113	0.028831 +/- 0.016213
Rio Queule	10	Queule	39 23 30	73 11 57	Coastal	Pacific	Pacific	1.0000 +/- 0.0447	0.016423 +/- 0.009146
Rio Lingue	10	Lingue	39 26 45	73 12 48	Coastal	Pacific	Pacific	1.0000 +/- 0.0447	0.030121 +/- 0.016386
Lago Calafquen	10	Valdivia	39 34 16	72 14 27	Interior	Pacific	Pacific	1.0000 +/- 0.0447	0.012898 +/- 0.007281
Lago Pangupulli	5	Valdivia	39 38 41	72 19 24	Interior	Pacific	Pacific	1.0000 +/- 0.1265	0.009045 +/- 0.005973
Lago Rinihue	10	Valdivia	39 46 29	72 27 10	Interior	Pacific	Pacific	0.9333 +/- 0.0773	0.033125 +/- 0.017973
Lago Neltume	10	Valdivia	39 48 36	71 59 40	Interior	Pacific	Pacific	0.9778 +/- 0.0540	0.009464 +/- 0.005462
Rio Valdivia	9	Valdivia	39 51 46	73 21 12	Coastal	Pacific	Pacific	1.0000 +/- 0.0524	0.004038 +/- 0.002605
Rio Bueno	10	Bueno	40 19 27	73 05 38	Interior	Pacific	Pacific	0.9333 +/- 0.0620	0.026235 +/- 0.014330
Rio Contaco	4	Contaco	40 34 44	73 41 53	Coastal	Pacific	Pacific	1.0000 +/- 0.1768	0.035297 +/- 0.023563
Lago Rupanco	8	Bueno	40 47 23	72 41 11	-	Pacific	Pacific	1.0000 +/- 0.0625	0.005026 +/- 0.003194
Lago Llanquihue	10	Maullin	41 15 43	72 59 40	-	Pacific	Pacific	1.0000 +/- 0.0447	0.029946 +/- 0.016294

Table 3.1 continued

Collection Site	# Indv.	Basin	Latitude (dd mm ss)	Longitude (dd mm ss)	Position wrt Coastal Range	Ocean Drainage Current	Ocean Drainage Ancient	Sequence Diversity *h*	Sequence Diversity π
Chile continued									
Rio Paredes	5	Maullin	41 23 14	73 11 16	-	Pacific	Pacific	1.0000 +/- 0.1265	0.027945 +/- 0.017431
Rio Maullin	10	Maullin	41 36 43	73 36 22	-	Pacific	Pacific	0.8667 +/- 0.1072	0.006099 +/- 0.003673
Lago Huillinco	7	Chiloe	42 40 16	73 54 11	-	Pacific	Pacific	1.0000 +/- 0.0764	0.031567 +/- 0.018129
Lago Tarahuin	10	Chiloe	42 43 01	73 45 01	-	Pacific	Pacific	1.0000 +/- 0.0447	0.013705 +/- 0.007706
Lago Natri	10	Chiloe	42 47 60	73 47 18	-	Pacific	Pacific	1.0000 +/- 0.0447	0.008104 +/- 0.004739
Rio Blanco	9	Blanco	42 56 04	72 43 28	-	Pacific	Pacific	1.0000 +/- 0.0524	0.018287 +/- 0.010272
Rio Negro	10	Yelcho	42 56 35	72 40 60	-	Pacific	Pacific	1.0000 +/- 0.0447	0.014248 +/- 0.007995
Rio Yelcho	7	Yelcho	42 57 18	72 45 08	-	Pacific	Pacific	1.0000 +/- 0.0764	0.030949 +/- 0.017784
Lago Yelcho	9	Yelcho	43 10 60	72 25 53	-	Pacific	Pacific	1.0000 +/- 0.0524	0.023038 +/- 0.012821
Rio Palena	10	Palena	43 48 27	72 57 45	-	Pacific	Pacific	1.0000 +/- 0.0447	0.015837 +/- 0.008836
Rio Cisnes	10	Cisnes	44 44 48	72 42 20	-	Pacific	Pacific	1.0000 +/- 0.0447	0.014202 +/- 0.007970
Argentina									
Lago Quillen	10	Limay	39 02 00	71 02 00	-	Atlantic	Atlantic	0.9778 +/- 0.0540	0.049779 +/- 0.026769
Lago Espejo	9	Limay	40 41 00	71 40 00	-	Atlantic	Atlantic	1.0000 +/- 0.0524	0.015038 +/- 0.008530
Lago Morenito	2	Limay	41 05 00	71 32 00	-	Atlantic	Atlantic	1.0000 +/- 0.5000	0.010025 +/- 0.010633
Lago Hess	9	Manso	41 22 02	71 44 23	-	Pacific	Atlantic	1.0000 +/- 0.0524	0.013506 +/- 0.007708
Lago Martin	9	Manso	41 30 00	71 40 00	-	Pacific	Atlantic	0.8889 +/- 0.0910	0.017648 +/- 0.009931
Lago Steffen	13	Manso	41 31 00	71 33 00	-	Pacific	Atlantic	0.9872 +/- 0.0354	0.010732 +/- 0.005965
Rio Chico	9	Rio Chico	49 47 00	68 38 00	-	Atlantic	Atlantic	1.0000 +/- 0.0524	0.029307 +/- 0.016181
Lago Argentino	10	Santa Cruz	50 02 05	72 04 00	-	Atlantic	Atlantic	0.8667 +/- 0.1072	0.010091 +/- 0.005795
Lago Roca	9	Santa Cruz	50 31 33	72 41 56	-	Atlantic	Atlantic	1.0000 +/- 0.0524	0.013033 +/- 0.007456
Beagle Channel	10	-	54 47 43	68 15 28	-	-	-	1.0000 +/- 0.0447	0.018296 +/- 0.010136
36	**299**	**19**						**0.9989 +/- 0.0005**	**0.046394 +/- 0.022376**

Table 3.2 Analysis of Molecular Variance (AMOVA) using mitochondrial mitochondrial control region sequences for 299 individuals of Galaxias maculatus collected over the study area (all samples) and a sub-set of locations from northern Chilean Patagonia (90 individuals). All samples were grouped according to (A) lake/river from which individuals were collected, (B) the freshwater basin containing each lake/river, and ocean (Pacific or Atlantic) into which each basin (C) currently drains or (D) historically drained [i.e. ancient refers to the conditions before the Last Glacial Maximum (LGM) approximately 10,000-20,000 yr BP]. Further post-hoc regional analysis was conducted for a subset of collection locations in Chile between latitudes 34°S and 40°S. I grouped each population as "coastal" or "Andean" for AMOVA analysis based on two criteria: (2A) location of headwaters and (2B) location of collection site (Table 3.1). Variation is reported as a percentage of the total with degrees of freedom (d.f.).

	% of variation *(df)*		
Grouping	**Among groups**	**Among populations within groups**	**Within populations**
All samples			
1A. Lake/River	61.00 *(35)*	-	39.00 *(263)*
1B. River drainage system	29.94 *(18)*	32.08 *(16)*	37.98 *(254)*
1C. Current ocean drainage	3.68 *(1)*	58.76 *(33)*	37.55 *(254)*
1D. Ancient ocean (pre-LGM) drainage	15.55 *(1)*	49.41 *(33)*	35.04 *(254)*
Chile			
2A. River basin headwaters	6.64 *(1)*	58.06 *(15)*	35.3 *(110)*
2B. Collection location	13.93 *(1)*	51.73 *(15)*	34.34 *(110)*

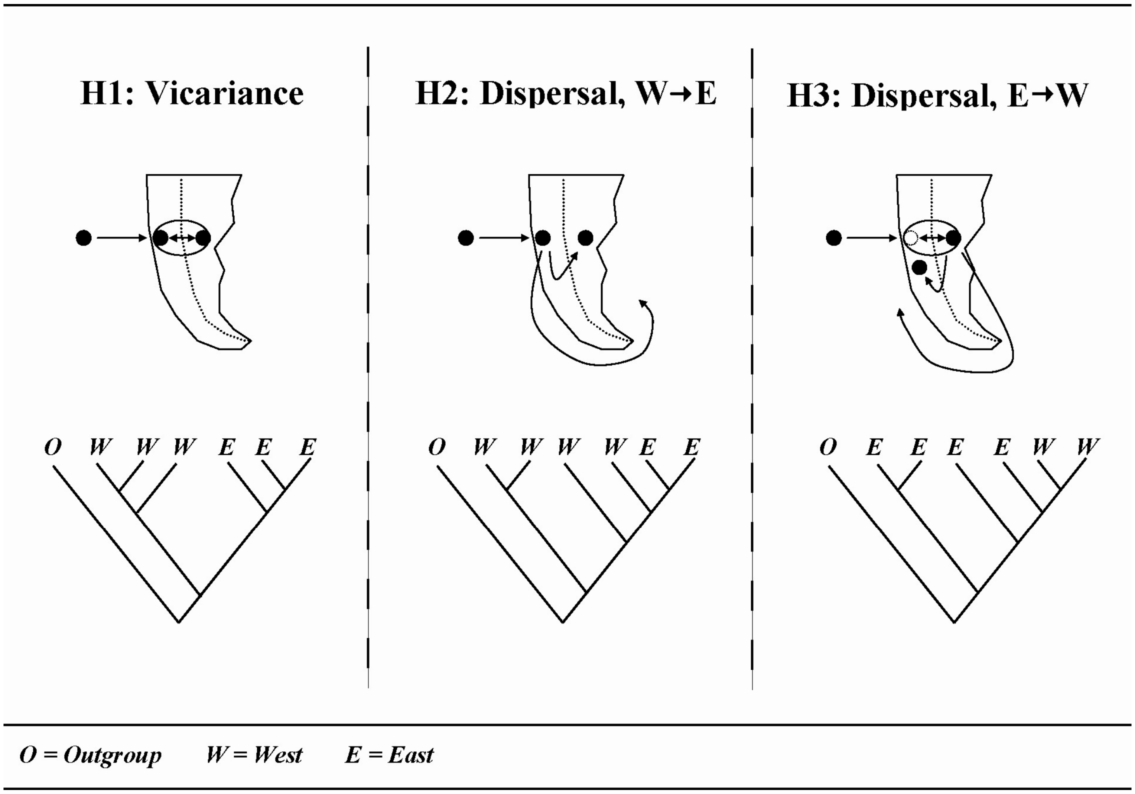


Figure 3.1 Alternative colonization histories for *Galaxias maculatus* in Patagonia, South America

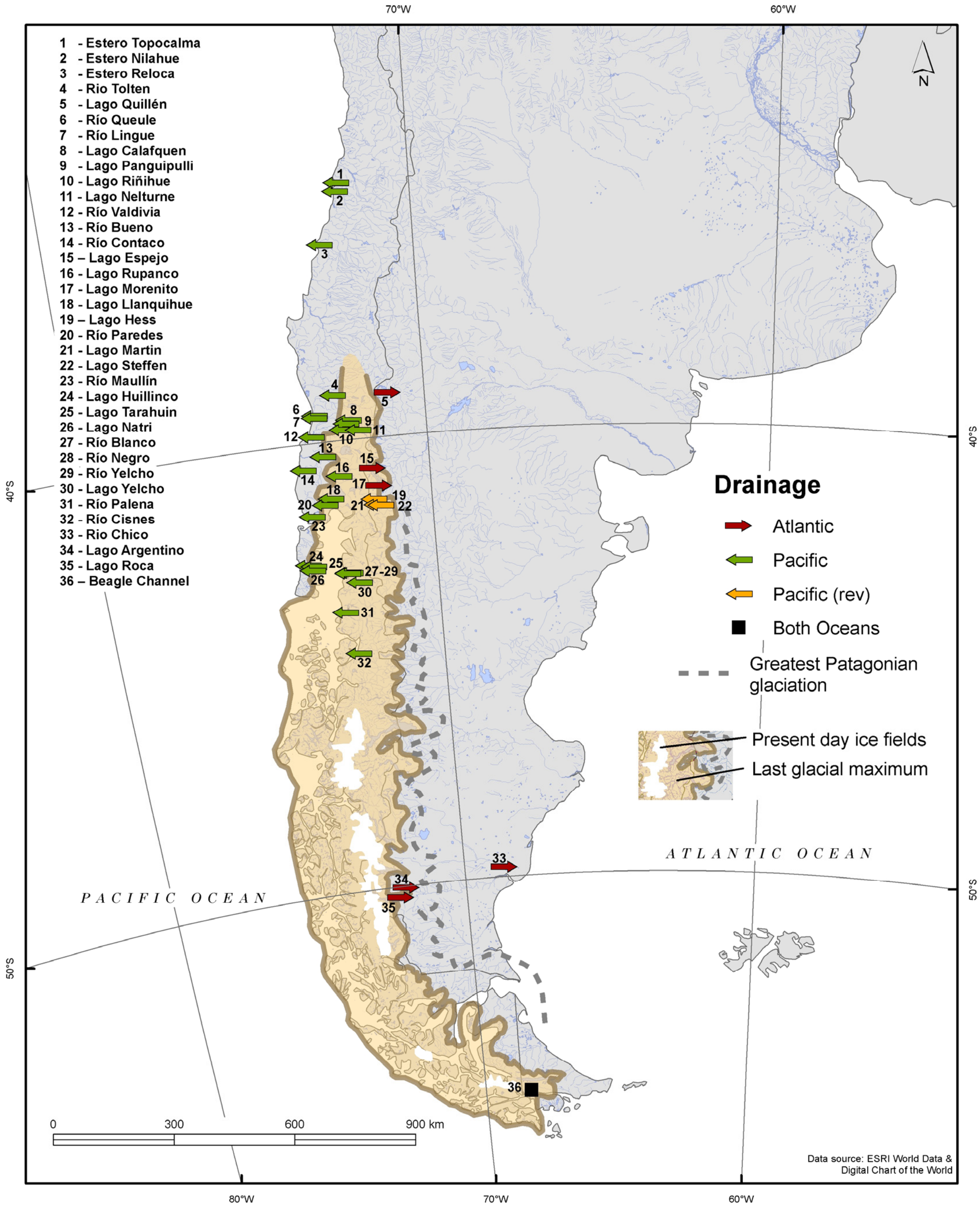


Figure 3.2 Collection locations for *Galaxias maculatus* throughout Patagonia, South America. Sampled locations are represented by arrows that indicate contemporary drainage direction, either Atlantic or Pacific. Pacific (rev) denotes a drainage reversal, from Atlantic to Pacific, which likely occurred following the retreat of Pleistocene glaciers (Turner et al. 2005). The extent of the Last Glacial Maximum and contour of the Great Patagonian Glaciation (GPG) were adapted from Clapperton (1993) and Turner et al. (2005).

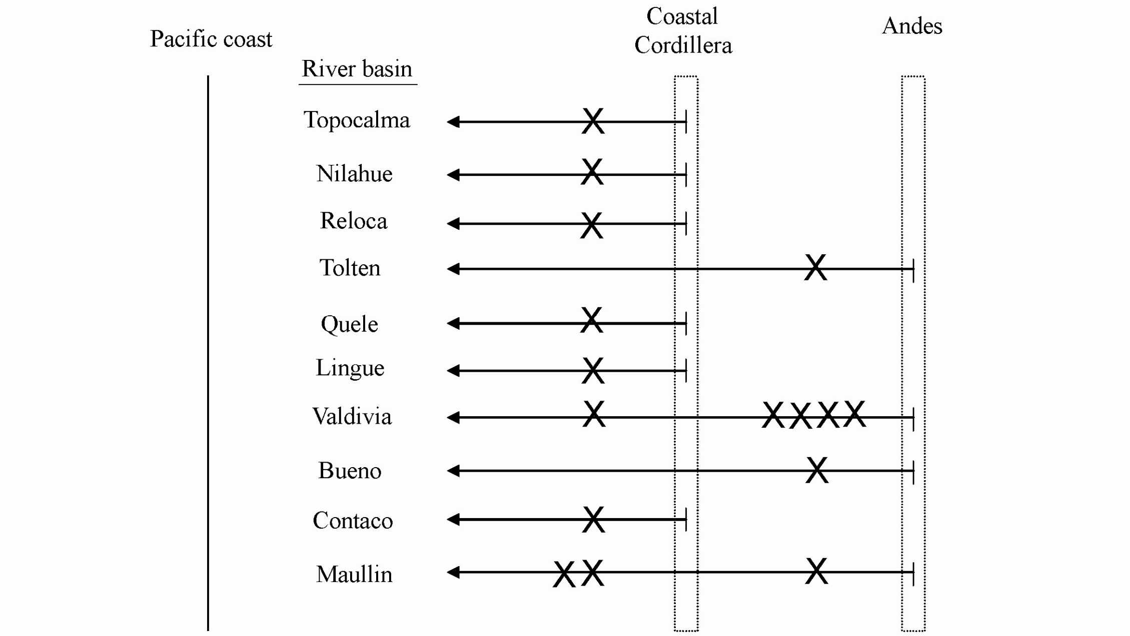


Figure 3.3 Sampling regime for post-hoc Analysis of Molecular Variance (AMOVA) conducted for a subset of collection location in Chile between latitudes 34°S and 40°S. I grouped each population as "coastal" or "Andean" for AMOVA analysis based on two criteria (Table 3.2): (2A) location of headwaters and (2B) location of collection site. The diagram depicts the number of collection locations (X) that are coastal versus Andean.

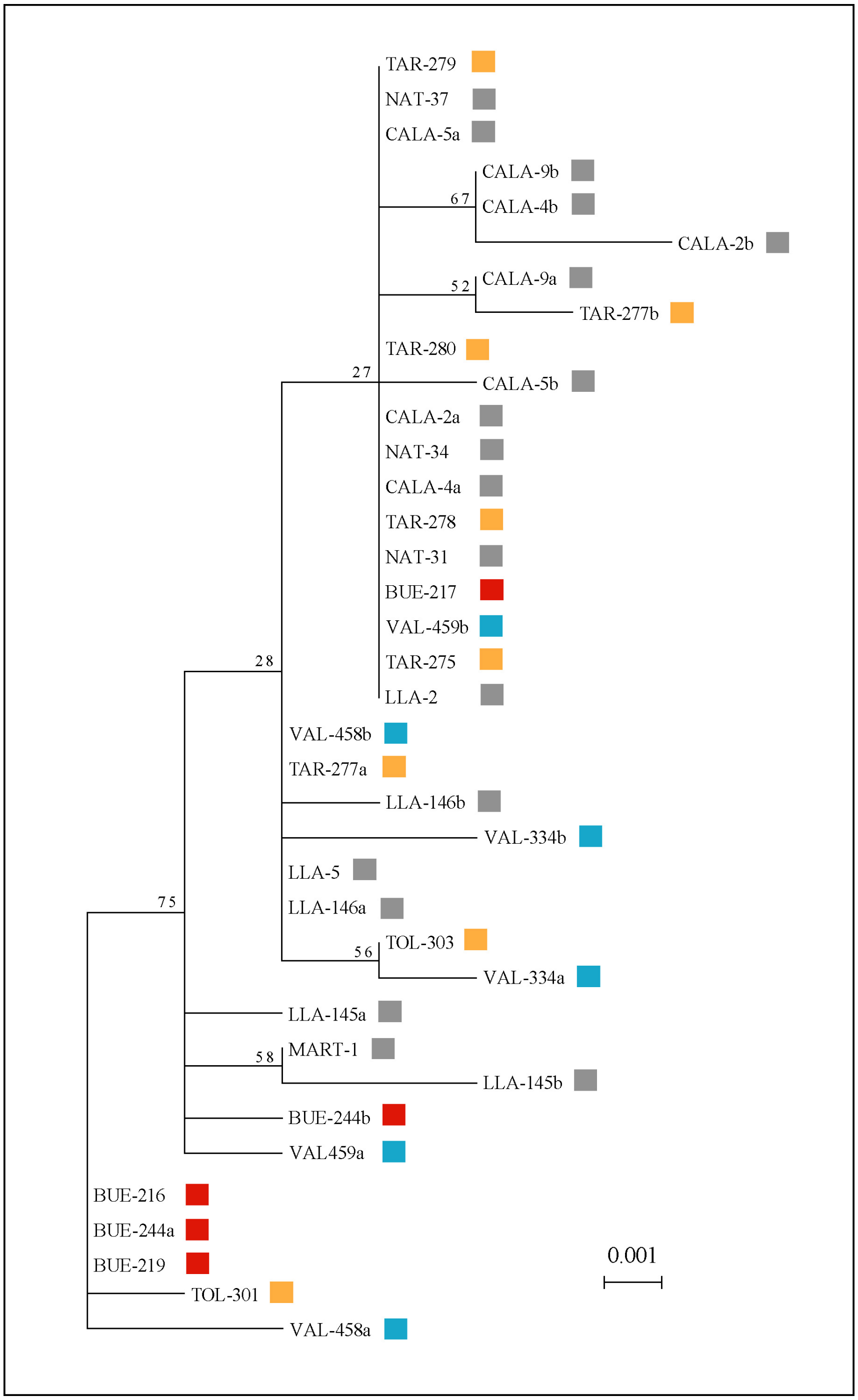


Figure 3.4 Unrooted maximum likelihood tree of nuclear haplotypes from 26 individuals. The subset of individuals screened for nuclear locus ANL22 were selected to represent the full spectrum of mitochondrial diversity (mitochondrial haplogroups 1-8). Capitalized acronyms in the sequence names indicate the collection location of each individual [Tarahuin (TAR), Natri (NAT), Argentino (CALA), Bueno (Bue), Valdivia (VAL), Llanquihue (LLA), Tolten (TOL) Martin (MART)], numbers represent unique individuals and the letters ‘a’ and ‘b’ denote separate alleles of heterozygotes.

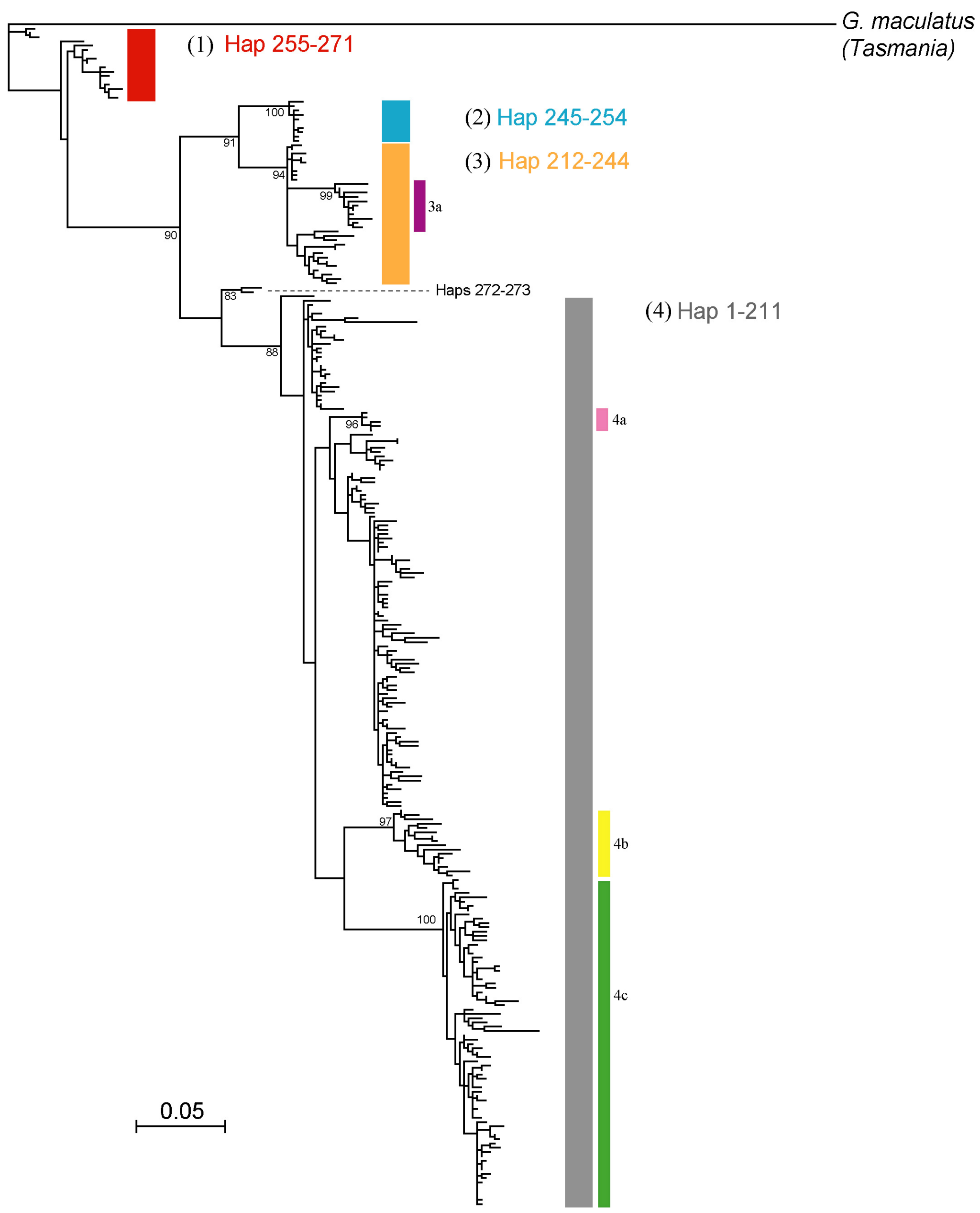


Figure 3.5 Maximum likelihood tree of mitochondrial haplotypes rooted with *Galaxias maculatus* (Tasmania). Strongly supported haplotype clusters are labeled as haplogroups 1-4. Additional strongly supported branches within haplogroups are identified as sub-groups (a-c). Branch support is based on bootstrap resampling.

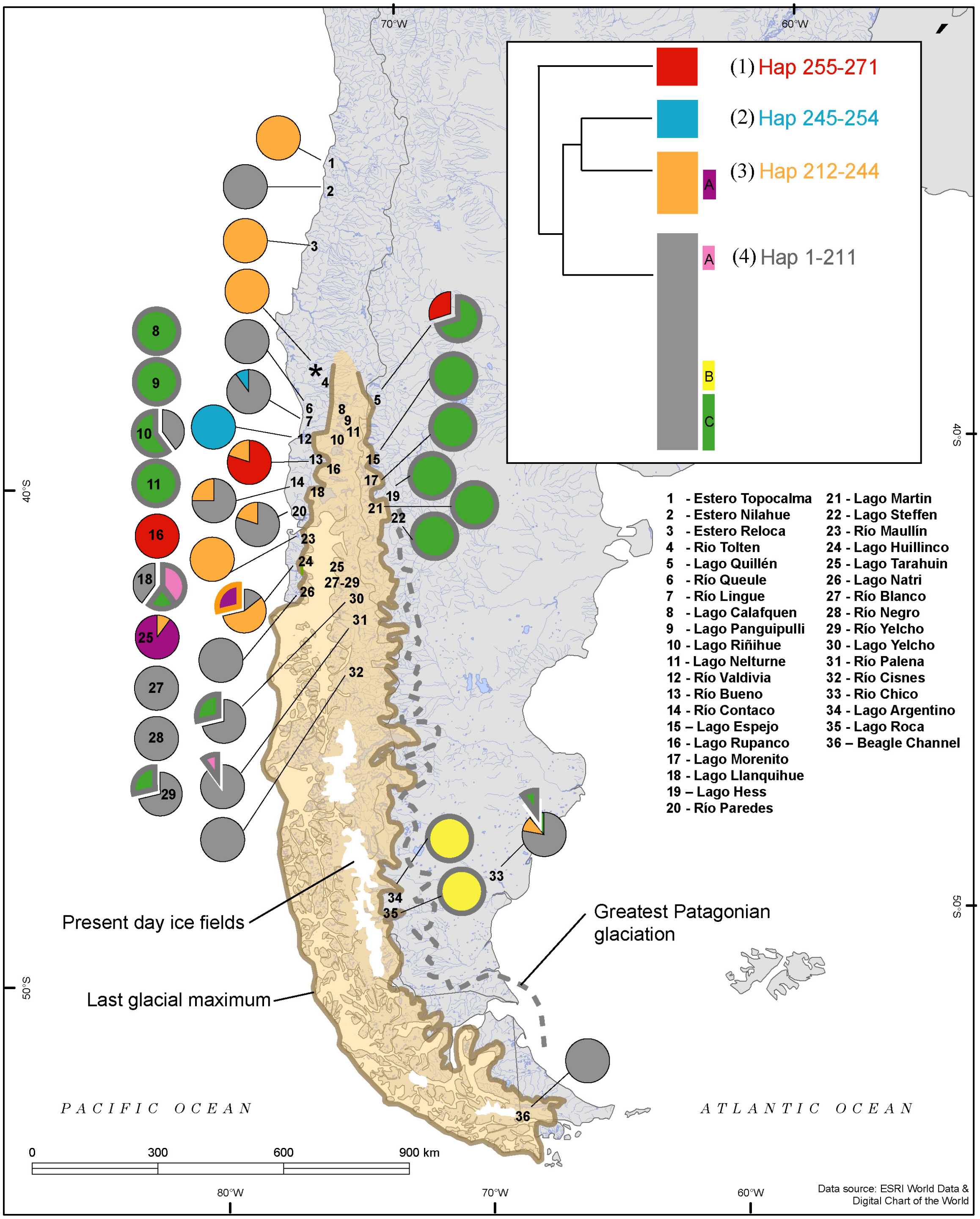


Figure 3.6 Frequency distribution of mitochondrial haplotype groups by sampling location. Haplogroup and sub-group designations follow strongly supported clusters defined in rooted mitchondrial haplotype phylogeny (Figure 3.5). Sub-groups are outlined in colours which correspond to the respective haplogroup within which each is nested.

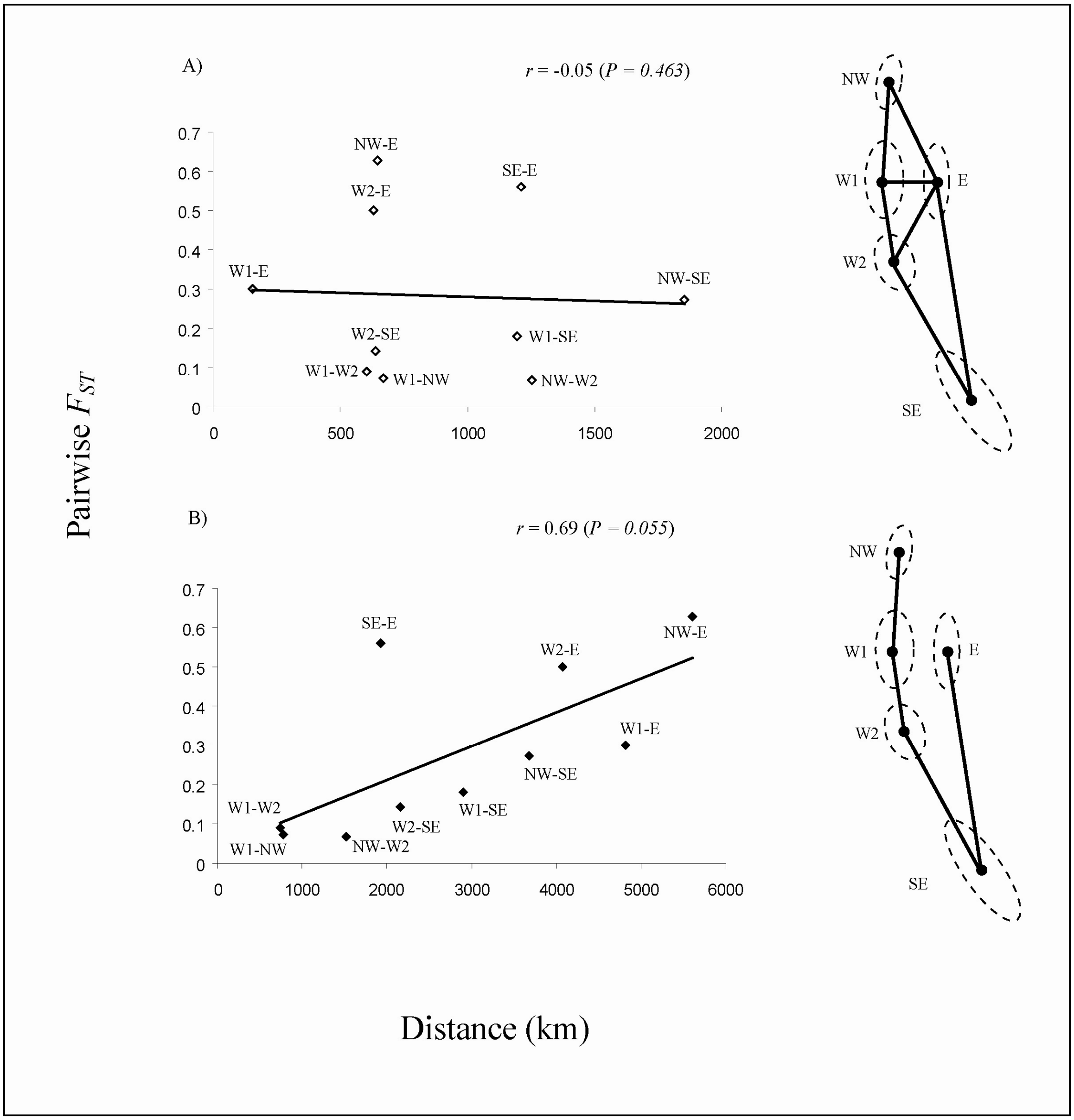


Figure 3.7 Contrasts of pairwise genetic distance versus geographic distance between regional groups assuming marine and trans-Andean dispersal.

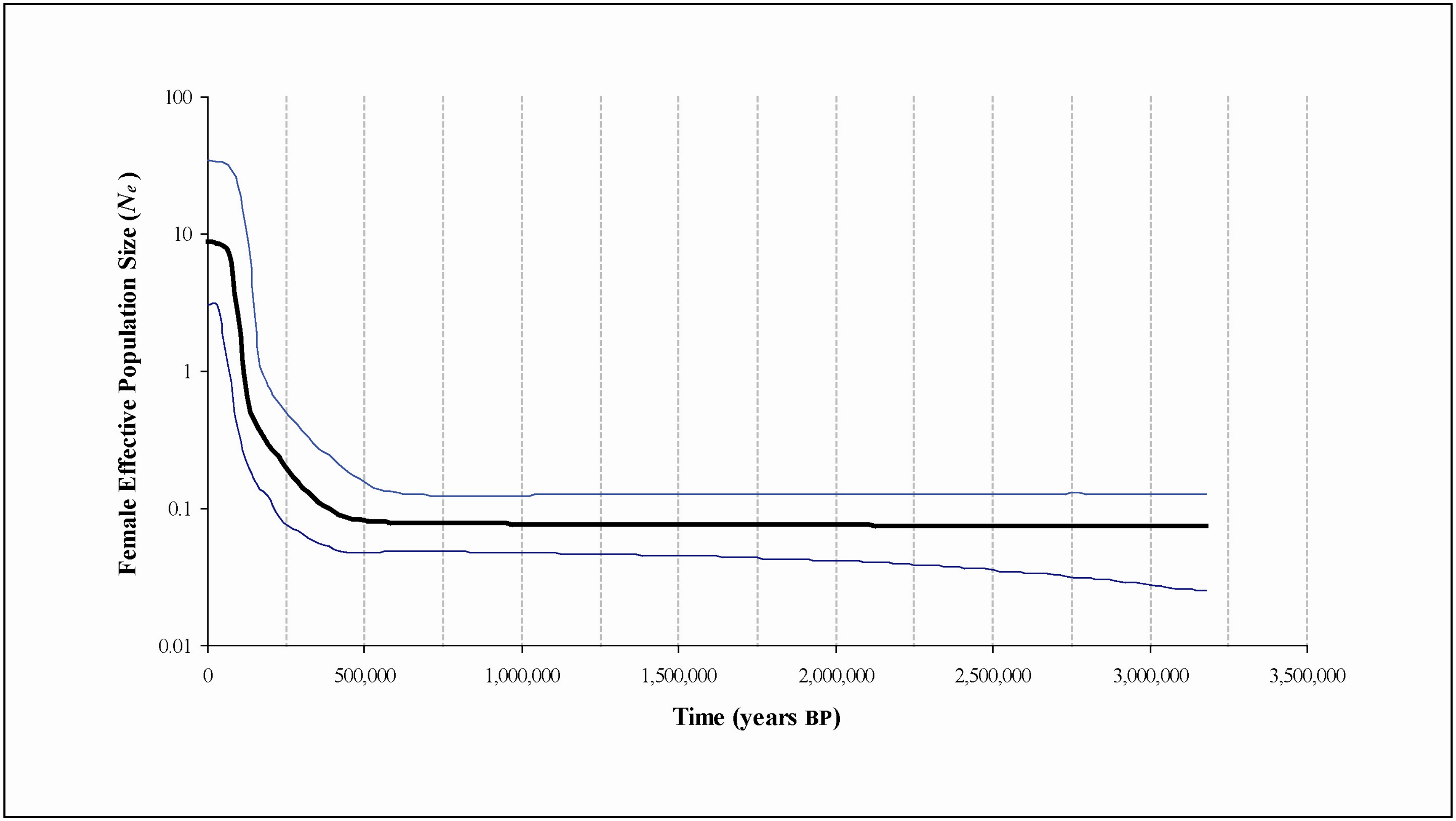


Figure 3.8 Historical estimates of female effective population size through time constructed using the Bayesian Skyline model based on mitochondrial control region haplotypes.

CHAPTER 4

DRAINAGE CAPTURE ACROSS THE PATAGONIAN ANDES: A MULTILOCUS PHYLOGEOGRAPHIC PERSPECTIVE FROM THE FRESHWATER FISH *GALAXIAS PLATEI*

This chapter is currently in review for publication as a *Research Article* in the journal of *Proceedings of the Royal Society of London B: Biologial Sciences*. The primary author of the article is Tyler S. Zemlak. The article is co-authored by Sandra J. Walde, Evelyn M. Habit and Daniel E. Ruzzante. All authors contributed to the conception and design of the study, and all participated in sample collection. TSZ generated the molecular data, and TSZ and DER were responsible for data analysis. TSZ drafted the initial version of the manuscript, and DER and SJW were involved in subsequent versions of the manuscript. All authors reviewed it critically. In the event that the article is accepted for publication, the appropriate copyright permission will be obtained from the publisher *Royal Society Publishing*.

4.1 Abstract

The drainage redirection of some Patagonian river headwaters during the late-Pleistocene is thought to have served as an important mode of connection across an ancient central divide that defines Atlantic and Pacific drainage networks in southernmost South America. Using widespread sampling and multiple loci (1 mtDNA, 7 nuclear) I investigated the demographic history of the freshwater-limited fish species *Galaxias platei* to better understand the potential influences of drainage reversal(s) on Patagonian aquatic biogeography. An ancestral lineage of *G. platei* appears to have been separated into eastern (Atlantic) and western (Pacific) lineages approximately 360 KY BP. Since splitting, descendent lineages maintained small amounts of gene flow in both directions, but approximately 2X greater flow in the direction of E → W. Effective population size was likely greatest in the ancestral lineage, and western descendants have maintained an overall higher effective size than the eastern descendants. It is likely that drainage reversals occurred multiple times and were an important mechanism of gene flow between Atlantic and Pacific catchments for aquatic organisms in Patagonia throughout the Pleistocene glacial cycles.

4.2 Introduction

A classic debate in historical biogeography concerns the relative contributions of vicariance versus dispersal to the disjunct distributions of closely related species across strong physical barriers. A primary example is the existence of closely related species on separate continents. Originally, dispersal was believed to be the principal mechanism connecting closely allied biotas over large oceanic distances via marine tolerant life history stages (e.g. diadromous juvenile fish, seeds), highly vagile adults (e.g. birds) and/or rafting upon drifting debris (Fraser et al. 2010). However, with the rediscovery of plate tectonics in the 1960's, continental drift became a strong alternative that quickly gained the support of the biogeography community. More recently, DNA technology has added insight and the result has been the increased recognition of the importance of both vicariance and dispersal for explaining global biogeographic patterns.

On a finer scale, phylogeographic studies of aquatic species are increasingly recognizing the importance of dispersal and challenging the notion that mountains pose strong barriers (vicariance) to the distribution of freshwater species (Waters and Wallis 2000; Slechtova et al. 2004; Zemlak et al. 2008; Chiang et al. 2010; Zemlak et al. 2010). Collectively these studies suggest that freshwater drainage pathways and their boundaries are dynamic when considered over the appropriate timescales. Aquatic landscapes are continually evolving networks whose structure and connectivity can be influenced by a variety of geological processes. Some of these changes can result in intermittent connections over otherwise strong geographic barriers and can facilitate opportunities for dispersal. For example, drainage catchments defined by tectonically active mountain ranges are continually being uplifted and eroded and changes in the balance between the two processes can alter dispersal avenues. This has been well illustrated in New Zealand where differences in the rates of erosion of the leeward and windward sides of the Alps have resulted in river capture events across mountain boundaries (Craw et al. 2008) which have resulted in important shuffles in the distributions of fishes across otherwise strong geographic barriers (e.g. mountain ridges: Waters and Wallis (2000), Waters et al.

(2001), Burridge et al. (2007), Craw et al. (2007a), Craw et al. (2007b), Craw et al. (2008).

In the present study I assess the strength of the Andean range as a barrier to east ↔ west dispersal in freshwater species and the potential role that Quaternary glacial forcing and associated drainage alterations could have played in facilitating dispersal across the southern Andes. Our focus is the gene flow within an obligate freshwater fish species *Galaxias platei* between Atlantic (eastern) and Pacific (western) drainage networks in Patagonia during the mid- to late-Quaternary. Using a multilocus sequence dataset (8 loci) with individuals of *G. platei* collected from 15 locations in eastern and western Patagonia I asked the questions: (i) Is there evidence for significant gene flow across the Andes post-uplift? If so, (ii) when did the gene flow occur, (iii) which was the predominant direction of movement, and (iii) what physical mechanisms likely facilitated cross-Andean dispersal?

4.2.1 Background

The hydrological landscape of Patagonia is primarily influenced by the Andean orogeny, an uplift which began in the Miocene [*ca.* 23 MY BP (Ramos 1989)]. The north-south orientation of the Andes separates Patagonian drainages into Atlantic and Pacific drainages. All rivers with headwaters west of the Andes drain into the Pacific (herein Chilean). Most headwaters located east of the Andes drain into the Atlantic (herein Argentinean). However, there are a few river systems with headwaters east of the Andes that drain across the Andes into the Pacific (herein trans-Andean). Because of these trans-Andean systems, the actual continental drainage divide does not exactly correspond with the position of the Andean range. Therefore, for the purpose of aquatic biogeography, it is more useful to use the actual drainage boundary (herein Central Drainage Boundary: CDB) when defining eastern and western Patagonia.

Currently, all Patagonian freshwater fish species diversity can be found in Pacific draining systems (i.e. Chilean and trans-Andean), whereas only a subset of that diversity inhabits Atlantic draining systems (i.e. Argentinean) (Dyer 2000; Baigún and Ferriz 2003). Almost all species found in Atlantic draining systems have large range sizes that extend throughout eastern and western Patagonia. The result is an east/west diversity contrast that is created by the restricted ranges of several species found only within Pacific draining systems.

All three widespread Patagonian fishes examined to date (*Galaxias maculatus*, *G. platei*, *Percichthys trucha*) exhibit shallow structure among the populations located east of the Andes, suggesting recent connections among east-flowing river systems. However, haplotypes of the freshwater creole perch (*P. trucha*) collected from eastern Patagonia and some locations west of the Andes differed by an average of 12.3% at mtDNA control region sequences (Ruzzante et al. 2006), which corresponds to a separation of 1.23 MY BP (using a justified range of mutational tempo for this locus: Appendix 4.1). A study of the freshwater puyen (*Galaxias platei*) reached similar conclusions, putting the east-west subdivision within the last 1.5 MY (Ruzzante et al. 2008; Zemlak et al. 2008). *Galaxias maculatus*, a diadromous congener whose genetic structure follows a pattern of isolation by distance around the Andean mountain range, shows an average genetic distance between the most genetically disparate eastern and western haplotype groups of approximately 1.75 MY BP (Zemlak et al. 2010) using an appropriate molecular clock of 2%/MY (Appendix 4.1).

Past work has identified two mechanisms by which fishes and other aquatic organisms likely bridged the *CDB*. The freshwater-limited species, *G. platei*, likely dispersed directly across the boundary during redirections of entire drainage networks probably at the end of the last glacial period. Drainage patterns during the last glaciation differed from contemporary patterns in that current trans-Andean Pacific-draining river systems drained to the Atlantic. Because the headwaters of these systems are located on the eastern slopes of the Andes, a large barrier of ice to the west of the headwater lakes forced drainage into the Atlantic during the LGM. Following the LGM, and the melting of a western barrier of ice, trans-Andean headwaters assumed their contemporary Pacific orientation. The Atlantic → Pacific drainage reversals are thought to have provided an important mechanism of trans-Andean dispersal for *G. platei* from east → west (Zemlak et al. 2008). The phylogeographic structure of the diadromous *G. maculatus* throughout the southern part of its range is best explained by marine dispersal, but drainage reversals appear to have exerted some influence in northern drainages (Zemlak et al. 2010).

Abandoned Atlantic drainage routes were first recognized during early efforts to map the glacial history of the region (Caldenius 1932; Mercer 1976). Strong physical evidence for east → west drainage reversal of trans-Andean rivers exists for some systems (e.g. the Baker river system which currently drains the centrally located and cross border General Carrera/Pueyrredón lake, 46-48°S) and modern dating techniques indicate that the switch from Atlantic to Pacific drainage occurred in this lake when Andean glacial dams collapsed approximately 12 KY BP (Kaplan et al. 2004; Singer et al. 2004; Turner et al. 2005). Evidence of a similar reversal has also been documented for the Manso river drainage situated at 41°S (Tatur et al. 2002).

Trans-Andean systems may have also facilitated dispersal across the Andes in the opposite direction, west → east. A phylogeographic study on the diadromous *G. maculatus* revealed secondary contact zones between divergent eastern and western lineages on both sides of the Andes and in close proximity to the trans-Andean Hua Hum River (Zemlak et al. 2010). In addition, the distribution of haplotypes for the creole perch, *P. trucha*, is most easily explained by assuming bidirectional gene flow, an initial dispersal from west → east followed by population expansion before a subsequent return of some individuals back into the west (Ruzzante et al. 2006).

4.3 Methods and Materials

4.3.1 Sample Collection and Preservation

This study moves up the so-called "evolutionary continuum" that exists between phylogeography and phylogenetics (Avise et al. 1987) to investigate the evolutionary relationship between two sister-clades of the species *G. platei* distributed across the main geographic barrier in Patagonia: the southern Andean mountain range which separates eastern and western Patagonia. My approach targets 30 individuals using sequence variation at eight independent loci (also see 4.4 Discussion for explanation). I based the selection of these 30 individuals on the results of a range-wide single-locus (mtDNA) phylogeographic study of *G. platei* to ensure representative sampling of neutral genetic variation across the species range (Zemlak et al. 2008). The results of (Zemlak et al. 2008) indicated the importance of latitude and river type (Argentinean, Chilean, trans-Andean) in determining the distribution of neutral diversity of *G. platei* in Patagonia. Therefore, the locations were selected so as to represent the contemporary range of *G. platei* and to equally represent each type of river classification: Argentinean (10, of which 1 did not amplify, leaving N= 9), Chilean (10) and trans-Andean (10) (Figure 4.1, Appendix 4.2). My approach uses the Central Drainage Boundary (CDB) in Patagonia to recognize two separate "populations", current Atlantic drainages (Argentinean rivers) and current Pacific drainages (Chilean and trans-Andean). All samples of *G. platei* were collected using gill nets and electrofishing between 1998-2007 from lakes and rivers throughout Patagonia. Samples (gill, muscle, fin, blood) for molecular analyses were fixed in 95% ethanol for storage at -20^{o}C.

4.3.2 Sequence Data

Prior to DNA extraction, ethanol was removed from all tissue samples by exposure to ambient temperature for approximately 120 min. Total genomic DNA was isolated from sub-samples (10 μL of blood or 2x2 mm^2 tissue) using the glassmilk procedure

(Elphinstone et al. 2003) with slight modifications for execution using a MultiPROBE® II HT PLUS EX robotic liquid handling system (PerkinElmer).

Eight independent loci (1 mitochondrial and 7 nuclear) were PCR amplified and sequenced (Appendix 4.3). From the mitochondria, 822 bp of the D-loop of the control region was amplified using the universal primer combination *S-phe* 5'-GCT TTA GTT AAG CTA CG-3' (Nielsen et al. 1994) and *P3* 5'-AAC TTC CAT CCT CAA CTC CCA AAG-3' (Sang et al. 1994). PCRs were each 25 μL: 2.5 μL 10X Reaction Buffer [100mM KCl, 100mM $(NH_4)_2SO_4$, 200mM Tris HCl (pH 8.75, 22°C), 1% Triton X-100, 1mg/ml BSA], 2.5 μL dNTPs (2 μM each), 3.75 μL $MgSO_4$ (20 mM), 13 μL ddH_2O, 0.25 μL of each 10 μM primer, 1 U of Tsg DNA Polymerase (Bio Basic Inc.) and 2.0-2.5 μL of DNA template. A Mastercycler® EP Gradient (Eppendorf) thermal cycler was used to conduct all reactions under the following thermal regime: an initial denaturing temperature of 94ºC for 5 min, followed by 35 cycles of 94ºC for 1 min, 50ºC for 1 min 30, and 72ºC for 1 min 30, and a final extension at 72ºC for 5 min. Nuclear DNA was obtained using newly developed primer sequences listed in Appendix 4.3. Aside from substituting a universally optimal 63ºC annealing temperature for all nuclear loci, the thermal regimes and PCR cocktail compositions used to amplify mtDNA were also used to collect nuclear data.

PCR amplicons for mitochondrial and nuclear loci were visualized using 1.0% agarose gels and sent off site for bidirectional DNA sequencing at Macrogen Inc., Maryland, USA. Each forward and reverse sequence was edited using Sequencher and combined to form bidirectional contigs. Contigs for each locus were aligned in ClustalX2 (Larkin et al. 2007) using default parameters and subsequently checked by eye. Ambiguous nuclear alleles, represented by sequences containing two or more heterozygous base calls, were separated with 100% probability for ANL9, ANL18 and ANL23 using the program PHASE v2.1.1 (Stephens et al. 2001). Alleles were found to be free of recombination using both RDP and MaxChi algorithms as executed in the program RDP3 (Martin and Rybicki 2000). The edited bidirectional contigs will be available in September 2011 from GenBank under the following accession numbers: *HQ169130-HQ169541*.

4.3.3 Development of Anonymous Nuclear Loci

Each nuclear marker represents an Anonymous Nuclear Locus (ANL) designed from a random genomic library of DNA fragments generated for *G. platei* using modified versions of protocols developed for birds and grasshoppers (Jennings and Edwards 2005; Carstens and Knowles 2006). DNA was first isolated from an entire pectoral fin 0.65g of *G. platei* using a standard phenol/chloroform protocol (Sambrook and Russel 2001), concentrated using ethanol precipitation, and resuspended in 30 µL of low TE. Genomic DNA was then fragmented into desirable fragment sizes (i.e. 500-1000 bp) by subjecting the concentrated template to 5s of sonication (Branson Sonifer 150). The resulting fragments were visualized alongside Quickload 100bp DNA Ladder (New England Biolabs) using a 1.0% agarose gel stained with GelGreen (Biotium). The desired range of fragments (i.e. 500-1000 bp range) was excised by razor and gel purified using the Qiaquick Gel Extraction Kit (Qiagen). Any potential unpaired overhangs of DNA strands (usually a consequence of sonication) were "blunted" and prepared for cloning using a Single dA Tailing Kit (Novagen). The prepared sample was then ligated into vectors using a Qiagen PCR Cloning Kit, transformed into alpha-5 competent *Escherichia coli* cells (New England Biolabs) and plated on Invitrogen *im*Media. Positive clones were PCR amplified using M13 primers under standard PCR conditions, visualized using 1.0% agarose gel and sent off site for bidirectional DNA sequencing at Macrogen Inc., Maryland, USA. Each forward and reverse sequence was edited using Sequencher and combined to form bidirectional contigs. Each contig was then blasted in GenBank to ensure no matches with mitochondrial DNA before using Primer3 to design forward and reverse primer sequences. Suitable annealing temperatures for each locus were evaluated using an annealing gradient range of 45°C to 65°C with a Mastercycler® EP Gradient (Eppendorf) thermal cycler using the same PCR recipes used to collect sequence data.

4.3.4 Data Analysis

Locus-specific statistics were generated for all loci using the program Modeltest 3.7 (Posada and Crandall 1998). Calculations included the percentage G+C content, nucleotide (π) and haplotype (h) diversity, average pairwise distance between alleles (k), and Tajima's D (Tajima 1989). Tajima's D was included to ensure that loci were not significantly departing from neutral modes of molecular substitution; analysis using *IMa* software assumes that markers are evolving neutrally.

Pairwise estimates of population divergence, effective size and migration were estimated between Atlantic and Pacific "populations" for *G. platei* using multi-locus sequence data and the analysis software IMa (Hey and Nielsen 2007). IMa is a program for fitting an isolation model with migration to genetic data drawn from two closely allied populations by estimating the posterior probability density of six model parameters, including the following proxies: contemporary and ancestral effective population sizes (θ_1, θ_2, θ_A), relative gene-flow rates per generation between populations (m_1, m_2), and the time of population splitting at time t generations in the past. In the current analysis, population 1 was defined as "Atlantic" (Argentinean) and population 2 was defined as "Pacific" (Chilean, trans-Andean).

The M-mode of the program IMa was used to generate posterior probability densities for each of the parameters in the isolation-with-migration model. Several trial runs were used to establish optimal searching parameters for producing reliable results as per recommendations by Jody Hey in the IMa user manual. The final search parameters used to collect the data employed an MCMC sampling procedure over 10 M generations to generate 100,000 genealogies using 70 heated chains and a geometric heating scheme (g1=0.9, g2=0.7). Prior values for theta, migration and divergence time parameters included: θ_1=10, θ_2=10, θ_A=50, m_1=20, m_2=20, t=10. Differences in the genetic effective sizes between mitochondrial and nuclear markers were controlled by assigning the control region using an inheritance scalar of 0.25. After discarding the first 1.25 M steps as burnin, each model parameter was then estimated independently by recording the peaks of marginal density histograms (HiPt) (Table 4.1A). Credibility intervals were determined for the marginal density histograms based on the 90% highest posterior density (HPD) interval of each marginal distribution. Two independent runs (analyses) using different seed numbers, but identical search parameters, were conducted to ensure that the true stationary distribution was reached; assessed by examining average standard deviations between split frequencies and comparing parameter estimates by eye.

The very similar results produced by the independent analyses conducted under M-mode allowed the pooling of posterior distributions from each of the independent runs (10M steps) for a combined analysis (20M steps) in L-mode. In L-mode the parameter estimates [HiPt, HPD(90Lo), HPD(90Hi)] from the combined distributions were converted into demographically relevant timescales using an appropriate mutation rate and generation time for *G. platei* using the following formulae (Table 4.1B): (1) $N_e=\theta/(4V)$, (2) $M=2\mathrm{N}m$ and (3) $T=tu$, where N_e is the effective population size, M is the migration rate, T is the estimated time since lineage divergence, u is the mutation rate and V is the generation time (Hey and Nielsen 2004). The mutation rates of the loci were scaled by the "asymptotic" galaxiid mtDNA mutation rate for the control region [0.01876 changes/site/MY (Burridge et al. 2008)] as calculated by the program *IMa*, which represents the product of the geometric mean of all locus-specific mutation scalars with the galaxiid mtDNA mutation rate. The generation time used for *G. platei* was 3 years (as recommended by Evelyn Habit, Daniel Ruzzante, Cecilia Carrea and Juan Barriga).

Also conducted in L-mode was an evaluation of the fit of the data to several competing pre-defined models (Table 4.2). The models represent several additional nested variations (16) of the full isolation with migration model that differ in complexity. The most complex (i.e. most heavily parameterized) model is the FULL isolation with migration model (i.e. ABCDE) which allows every parameter to vary independently. All others represent less complex forms and restrict the number of independent variables by making specific variables co-vary or eliminating variables by setting them to zero. The goodness of fit of each model was evaluated using Akaike Information Criterion (AIC) (Akaike 1973) and information theory as recommended by Carstens et al. (2009).

An additional round of analysis using IMa was conducted for the same data set but with the trans-Andean samples removed. The purpose of this analysis was to qualitatively assess the influence of trans-Andean samples on parameter estimates and model selection. Analyses were conducted using exactly the same conditions used to analyze the full data set.

4.4 Results

A total of 412 contigs were recovered from *G. platei* for multilocus phylogeographic analysis (Appendix 4.4). This represents an overall sequencing success of 95% when considering all possible combinations of loci and individuals [(29 individuals x 1 locus) + (29 individuals x 7 loci x 2 because diploid loci) = 435]. The lowest success concerned loci ANL8 (86%) and ANL23 (90%). The remaining loci reach 97% (control region, ANL9, ANL18, ANL21, ANL22) and 100% (ANL20). Full alignments of each locus revealed a high degree of overlap among sequenced fragments, each overlapping for at least 90% of the total size of the targeted region (Appendix 4.4). G+C content varied between 37% (ANL22) and 48% (ANL9) and had an average of 42%. Control region was highly variable and was only matched in haplotype diversity by ANL23 (*ca.* 0.90) (Appendix 4.4). Measures of nucleotide diversity and average pairwise divergence were at least 5- and 7-fold lower for anonymous nuclear loci than for the control region. Tajima's *D* did not reveal deviations from neutral expectations and all loci were free of recombination; two important assumptions necessary for analysis using the IMa model.

Several diagnostic measures available from the output of IMa suggest that sampling by the Markov chains was sufficient to produce reliable estimates of the model parameters using the full dataset (i.e. including trans-Andean systems). Proper mixing of the Markov chains was indicated by high acceptance of swap rates, high ESS values and the absence of trendlines in plots of the parameter estimates over time. Furthermore, independent runs (based on different seed numbers) for analyses involving the full data set showed evidence of convergence on a similar area of stationarity in tree space according to strong similarities between plots and summary statistics derived from the posterior distributions (Table 4.1A, Figure 4.2). The strong similarities in results allowed me to combine the posterior distributions of each run (total of 20 million steps) for the combined estimation of demographic parameters and model optimization using likelihood ratio tests and the AIC.

The same runtime, precautions and diagnostic evaluations were conducted for two separate analyses involving the reduced data set (i.e. trans-Andean samples removed) (Figure 4.3). All criteria necessary for arriving at a single stationary distribution were also satisfied (data not shown). However, an examination of each marginal probability distribution revealed a problem. In both runs, marginal probability distributions obtained for the ancestral population size assumed broad shapes without discernable highpoints. This finding suggests that the reduced dataset is inadequate for estimating the ancestral effective population size. As a consequence, I was unable to further analyze the reduced dataset in L-mode since all parameters are necessary to obtain a joint probability distribution. However, accurate estimations of the marginal probabilities for 5 of the 6 parameters were possible and compared to values obtained using the full dataset to assess the importance of trans-Andean systems for gene flow between eastern and western Patagonia.

4.4.1 Lineage Divergence

The estimated time that elapsed since Atlantic and Pacific populations last shared a common ancester is approximately 360,000 [99,000-15,265,000] yr BP (Table 4.1B). The removal of trans-Andean samples had negligible effects on the estimation of lineage divergence; the estimate for the timing of the east-west split of the *G. platei* population split is slightly earlier (i.e. 0.45 MY BP), but this is a small change relative to the error associated with the estimate (Table 4.1B; Figure 4.3).

4.4.2 Migration

Estimates of migration rates across the CDB are non-zero in both directions (Figure 4.2). This finding suggests that bidirectional gene flow occurred across the CDB in addition, and subsequent, to the initial event that separated *G. platei* into separate ocean catchments *ca.* 360 KY BP. The very low estimated rates of migration suggest that trans-Andean migration events were very infrequent and/or involved low numbers of individuals relative to total population sizes. For example, over a 100 KY period, our estimates suggest that only a fraction of a genetically effective individual was exchanged in either direction: 0.13 [0-0.88] from Pacific to Atlantic and 0.21 [0.08-0.67] from Atlantic to Pacific (Table 4.1B). However, gene flow from Atlantic → Pacific was higher, nearly twice (1.7X) that of the estimated gene flow from Pacific → Atlantic.

The analysis of the data without trans-Andean systems had important implications for migration rate from Pacific → Atlantic; the posterior distribution shifted strongly to the left to assume a high point of zero (Figure 4.3). In other words, the exclusion of trans-Andean samples from the analysis eliminates any evidence of gene flow in the direction of Pacific to Atlantic and suggests that dispersal via trans-Andean routes accounts for all dispersal in the direction of west to east for *G. platei* in this study. Interestingly, the removal of trans-Andean samples had no effect on the migration rate in the Atlantic → Pacific direction.

4.4.3 Effective Population Size

The estimates of effective population size obtained from the peaks of the marginal distributions suggest that the ancestral lineage of *G. platei* was approximately 38- and 4-

fold larger than the descendent lineages occupying Atlantic and Pacific catchments, respectively (Table 4.1A). In demographically meaningful terms, and under a joint probability distribution, these estimates translated to approximately 767,000 [182,000-11,202,000] breeding individuals in the ancestral lineage, while modern populations host roughly 207,000 [96,500-362,000] breeding individuals in Pacific rivers and 17,300 [6,670-60,031] in Atlantic rivers (Table 4.1B).

The removal of trans-Andean systems had most influence on the effective population size of Pacific-draining systems (Figure 4.3). Effective size was estimated to be approximately 1/3rd that of its original size if trans-Andean systems are excluded (1.63 → 0.53).The estimation of Atlantic effective population size was, instead, unaffected by the removal trans-Andean samples.

4.4.4 Model Selection

The model ABBDD (i.e. $\theta_{Atl} \neq \theta_{Pac} = \theta_{A,}$ and $m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$) was ranked as the most likely model to fit the data according to the AIC (Tables 4.2, 4.3). As it concerns effective population size, this model suggests that the effective population size of the ancestral lineage (B) is similar to that of the western lineage (B), but both can be differentiated from the eastern lineage (A). This model also suggests that trans-Andean migration was bidirectional and equal in magnitude in either direction (DD).

However, several other models ranked very high and were only differentiated among other top ranking models by fractional increments according to the AIC. A total of 8 models had values that ranged from 17.33-20.53 with each successively ranked model in this grouping differing by less than a value of one. A strong similarity among these models is that a difference can be recognized between descendant populations (east vs. west) and trans-Andean dispersal was likely, at least in one direction. All models that forced *zero migration* were clumped at the bottom of the ranking with an AIC value range of 602-854, which corresponds to approximately 35- and 50-fold greater than the highest ranked model.

4.5 Discussion

My findings suggest that climate-induced changes in river drainages during one or more glaciations of the Quaternary played a crucial role in producing the contemporary distribution and genetic diversity of *G. platei* throughout its range in Patagonia. It appears that the current range of *G. platei* is, in part, the result of a relatively recent colonization of Atlantic Patagonian river networks. Range expansion across the Andes (west → east) was most likely facilitated by trans-Andean rivers during the onset of the OIS 10 glacial period (341-376 KY BP; Figure 4.4) when the headwaters of Pacific draining systems were deflected towards the Atlantic by a growing barrier of ice across the Andes. The reversal of trans-Andean headwaters during one or more subsequent glacial termination periods is expected to have returned some newly established Atlantic haplotypes into Pacific rivers to form a zone of secondary contact. It is likely that dispersal across the Andes via trans-Andean headwater reversals occurred more than once since the establishment of eastern *G. platei* populations.

The optimal sampling strategy for this level of investigation at the species/population boundary is unclear. The difficulty involves the merger of two fields which traditionally ask different questions and therefore use different methodologies, particularly with respect to the number of individuals and loci sampled: at the population level (phylogeography) extensive population sampling has always been advocated; at the species-level (phylogeny) multiple sampling within a species is generally considered less relevant and instead resources are forcused on increasing the number of loci (Maddison and Knowles 2006; Brito and Edwards 2009). Several theoretical investigations considered the best way to allocate resources when faced with such decisions [e.g. (Pluzhnikov and Donnelly 1996; Felsenstein 2006; Carling and Brumfield 2007)]. One consistent conclusion from this literature is that increasing the number of loci has a critical effect on the accuracy of the parameter estimates [see Maddison and Knowles (2006) and references therein].

In this study I follow the advice of (Felsenstein 2006) and focus on sampling fewer individuals per population and instead devote our resources to increasing the number of loci sequenced. Specifically, I sampled 8 independent loci from each of 29 individuals collected throughout the range of *G. platei*. Within terms of sampling effort, the amount of information collected is equivalent to sequencing 232 individuals for a single locus. I opted for the use of more loci in light of the shallow evolutionary history of *G. platei* across the Andean divide [maximum divergence time of 1.5 MY BP (Zemlak et al. 2008)] which suggests an inherently greater chance of gene tree discordance among loci. By integrating genetic signal over multiple loci I am much more likely to approximate the true population history of this species and thereby more accurately evaluate those demographic parameters pertinent to our questions.

Overall, my approach did produce results that were sufficient to inform our questions of interest. However, future efforts can be taken to tighten confidence intervals associated with parameter estimates and refine the species history of *G. platei* (or other species with similar histories). The consideration of the source of error again returns us to the importance of sampling individuals versus loci. I encourage future efforts concerning Patagonian phylogeography to consider the differences that samples and loci have on parameter estimations and associated confidence intervals.

4.5.1 Lineage Divergence

The results of this study, using seven nuclear loci in addition to an mtDNA locus, indicate that populations of *G. platei* on both sides of the Andes shared a common ancestor as recently as 360 KY BP. This estimate substantially postdates earlier estimates, based on the mtDNA control region only, that the E ↔ W split was as old as 1.5 MY BP (Zemlak et al. 2008). Single-locus estimates using mitochondrial markers are often sensitive to large demographic changes (i.e. ¼ effective size), and this may have contributed to the former exaggerated divergence estimate, as *G. platei* is thought to have experienced one or more severe population bottlenecks in recent history (Ruzzante et al. 2008; Zemlak et al. 2008). Both estimates are much more recent than the start of the Andean uplift at 23 MY BP, however, supporting a role for dispersal as opposed to vicariance mechanisms in explaining genetic differences between eastern and western populations of *G. platei*. Despite the large margin of error associated with the estimate of 360 KY BP (because of a right-skewed posterior distribution for the time parameter), its upper confidence limit (15 MY BP) still significantly postdates the onset of Andean uplift, thus supporting the hypothesis that the Andean divide has been, or is permeable to the dispersal of aquatic organisms.

The best estimate for time of lineage divergence across the *CDB* coincides with the beginning of OIS 10 glacial period [341-376 KY BP, Figure 4.4 (Lisiecki and Raymo 2005)]. I suggest that the drainage reversals documented for the most recent glacial cycle also occurred during earlier cycles. Thus, as OIS 10 glaciers developed, the headwaters of trans-Andean systems were deflected from the Pacific to the Atlantic by a growing barrier of ice to the west, thereby facilitating the dispersal of individuals from Pacific → Atlantic drainages. It is possible that *G. platei* was exclusive to Pacific systems prior to *ca.* 360 KY BP, and the lineage split began when some ancestral (western) haplotypes were transferred to the east. These haplotypes would have formed the underpinnings of the present-day eastern lineage, whereas the remaining ancestral haplotypes underlie the contemporary western lineage. Therefore, a parsimonious explanation of current patterns of genetic diversity in *G. platei* is that drainage diversion involving trans-Andean headwaters allowed *G. platei* to expand into eastern Patagonia, and that limited gene flow has allowed for divergence of the eastern and western lineages.

4.5.2 Migration

Since splitting from a common ancestor, *G. platei* populations appear to have experienced small amounts of bidirectional gene flow across the Andean divide. The estimated migration rates between Atlantic and Pacific drainages were very low and, if taken literally, suggest that less than one effective individual (0.1, W→E; 0.2 E→W) was exchanged every 100 KY. However, these estimates are likely depressed, as several studies suggest that accurate genetic signals from an immigrating lineage are unlikely to be preserved if the immigrants encounter a large established population, due to hybridization, genetic drift, or the elimination of individuals through competitive exclusion [see (Burridge et al. 2006) and references therein]. Therefore, while migration has been non-zero in both directions, I remain skeptical about their estimated magnitude and suggest that they are probably larger.

Exclusion of the haplotypes from the trans-Andean lakes had no effect on estimates of migration rate from Atlantic → Pacific, indicating either (1) that virtually all gene flow from eastern to western drainages occurs through a mechanism other than the reversing drainages, a scenario that seems unlikely in the light of previous findings (Ruzzante et al. 2008; Zemlak et al. 2008; Zemlak et al. 2010), or (2) that eastern genes transferred to the west subsequently spread beyond the trans-Andean drainages into the Chilean systems. The latter scenario seems plausible. Reversals from the Atlantic to the Pacific occurred as the climate warmed, and involved the catastrophic collapse of Andean ice dams that released enormous volumes of water from eastern proglacial in short periods of time (Tatur et al. 2002; Turner et al. 2005). It is likely that such immense fluxes of water breached historical drainage boundaries and facilitated temporary inter-river connections among western drainages. A previous study involving more drainages and more individuals also detected eastern mitochondrial lineages of *G. platei* in both trans-Andean and Chilean systems (Zemlak et al. 2008), supporting the hypothesis of gene flow among western drainages.

In contrast, evidence of migration from Pacific → Atlantic drainages was eliminated when trans-Andean systems were not included in the analysis, indicating that reversing drainages are the principal, or perhaps only, route for west to east gene flow. In addition, the result indicates that any western haplotypes transferred since the founding of the eastern populations (*ca.* 360 KY BP) have not spread to eastern drainages. Opportunities for transfer of western haplotypes from trans-Andean systems to Argentine systems may be much more limited, as these systems flow east only during the cold and arid glacial periods. Thus a plausible explanation for the patterns is that trans-Andean systems were important avenues of dispersal across the CDB in both directions, and that any western haplotypes transferred into the east during west to east reversals stayed in the trans-Andean systems.

4.5.3 Effective Population Size

G. platei appears to have experienced a net loss in total effective population size since the mid- Pleistocene. Our estimates indicate that the effective size of the ancestral population (*ca.* 767,000) was likely 3-fold greater than the combined sum of the current (descendent) Atlantic and Pacific populations (*ca.* 225,000). Climate change during this period (and the associated shifts in habitat) likely contributed to the overall decline in *G. platei* population numbers. The strong genetic bottleneck associated with the LGM detected by (Ruzzante et al. 2008) indicates a sensitivity of *G. platei* to glacial advance, perhaps because the habitat typically occupied by *G. platei* (higher altitude Patagonian lakes and rivers) places this species directly in the path of advancing glaciers. The OIS record suggests that three glacial periods (including the LGM) have occurred since *G. platei* split into eastern and western populations (Figure 4.4), and each advance may have caused loss of some genetic variability.

In addition, my analyses suggest that the effective population size of the Pacific lineage is significantly larger than that of the Atlantic lineage. Estimations from the marginal posterior distributions indicated that the effective population size of the Pacific lineage is approximately 12-fold greater than the Atlantic lineage. I outline two non-exclusive scenarios which may explain why effective size is higher west of the Andes.

First, the combination of long-term stability and the dissected nature of the western habitat may have better preserved genetic diversity in Pacific populations of *G. platei*. The northern part of Chilean Patagonia was never glaciated, and species diversity as well as intraspecific genetic diversity are both currently high in the region. Many populations appear to have persisted in refugia within this region through repeated periods of global cooling, and resident populations likely experienced only limited population reductions, or genetic bottlenecks (Dyer 2000; Premoli et al. 2000; Muellner et al. 2005; Ruzzante et al. 2006).

In contrast, eastern habitat, especially that near the continental divide, was much more unstable throughout the Quaternary. Glacial advance eliminated most of the current eastern lake habitat as ice sheets advanced down the valleys along the eastern slopes of the Andes (Mercer 1976; Clapperton 1993; McCulloch et al. 2000; Coronato et al. 2004; Singer et al. 2004; Rabassa et al. 2005; Rabassa 2008) and the aridity of the climate during glaciations may have led to reductions in suitable habitat for *G. platei*. Secondly, if current eastern populations are the result of range expansion from the west *ca.* 360 KY ago, founder effects probably also contributed to the relative dearth of genetic diversity found in the eastern populations. Therefore, low initial genetic diversity, a relatively young age and perhaps drift, probably explain the smaller effective population size of the eastern population.

4.6 Conclusion

In this study, I place the most likely timing of the divergence of *G. platei* into separate Pacific (west) and Atlantic (east) drainages at *ca.* 360 KY BP, a time that greatly post-dates the uplift of the Andes and thus makes vicariance an implausible explanation for the presence of *G. platei* on both sides of the mountain barrier. The lineages must be linked through dispersal, and the patterns, including low genetic diversity in eastern populations, are most easily explained as a range expansion from west → east. The timing of the divergence coincides with the onset of the OIS 10 glacial period and is consistent with the transfer of individuals during the diversion of trans-Andean headwaters towards the east (Atlantic) as western ice barriers formed during glacial periods. There is evidence of subsequent genetic exchange across the CDB in both directions and likely involving trans-Andean river systems occurring more than once. I also conclude that the repeated glacial cycles of the Quaternary were likely a negative influence on *G. platei* as evidenced by a net reduction in total genetic diversity over the time course of the present study.

Table 4.1 A) Parameter estimates generated from the posterior probability distributions of two independent runs using the program *IMa* (Hey and Nielsen 2007). Statistics for each marginal parameter distribution includes: the value with the highest residence time (*HiPt*) and the lower [*HPD(90Lo)*] and upper [*HPD(90Hi)*] bounds for the estimated 90% highest posterior density. *HiPt* values are also listed for parameters estimated using a joint probability distribution. B) Historical demography of *G. platei* derived from parameter estimates in Table 4.1A. Lower [*HPD(90Lo)*] and upper [*HPD(90Hi)*] bounds for the estimated 90% highest posterior density of each marginal distribution were also converted into demographic terms for purposes of providing a confidence interval. The value of each population demographic was calculated the formulae listed in (Hey and Nielsen 2004).

A)

Source	**Summary Statistic**	**Analysis**	**θ**			**m**		**t**	***log(P)***
			Ancestral	**Pacific**	**Atlantic**	**Pac. → Atl.**	**Atl. → Pac.**		
Marginal distributions	*HPD(90Lo)*	*Run 1*	1.43	0.76	0.05	0.01	1.15	0.06	-
		Run 2	1.43	0.78	0.05	0.01	1.17	0.07	-
	HiPt	***Run 1***	**6.03**	**1.58**	**0.16**	**1.89**	**3.17**	**0.26**	-
		Run 2	**6.03**	**1.65**	**0.14**	**2.01**	**3.19**	**0.24**	-
	HPD(90Hi)	*Run 1*	84.17	2.82	0.47	12.79	10.61	8.29	-
		Run 2	91.13	2.92	0.47	14.03	9.95	10.00	-
Joint distribution	***HiPt***	***Run 1***	**5.99**	**1.33**	**0.29**	**1.67**	**5.27**	**0.37**	**-4.73**
		Run 2	**5.69**	**1.50**	**0.26**	**2.16**	**4.91**	**0.40**	**-4.88**

B)

Summary statistic	**Effective Population Size (# of *individuals*)**			**Migration Rate (*per 100 KY per gene copy*)**		**Lineage Divergence (*yr BP*)**
	Atlantic	**Pacific**	**Ancestral**	**Pac. → Atl.**	**Atl. → Pac.**	
HPD(90Lo)	*6,670*	*96,446*	*181,825*	*0.00*	*0.08*	*99,268*
HiPt	***17,342***	***207,122***	***766,826***	***0.13***	***0.21***	***358,891***
HPD(90Hi)	*60,031*	*362,068*	*11,201,983*	*0.88*	*0.67*	*15,264,342*

Table 4.2 Optimization of the full multilocus dataset to 16 nested variations of the full isolation-with-migration model. Model parameters include the effective population size of Atlantic (θ_{Atl}), Pacific (θ_{Pac}) and ancestral (θ_A) populations and migration between Atlantic and Pacific populations ($M_{Pac\rightarrow Atl}$, $M_{Atl\rightarrow Pac}$). Listed for each model is the likelihood [log(P)] of the model considering the data. Constrained parameters are bolded.

Model	**Model assumptions**		**Parameter estimates**						**log(P)**
	N_e	m	θ_E	θ_W	θ_A	$m_{W\rightarrow E}$	$m_{E\rightarrow W}$	t	
All lineage θ's differ									
ABCDD	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	0.23	1.69	5.80	3.40	**3.40**	0.77	-5.37
ABCD0	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	$m_{Atl\rightarrow Pac} = 0$	0.24	1.90	0.39	9.04	**0.00**	6.45	-6.26
ABC0D	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} = 0$	0.39	1.19	5.94	**0.00**	5.84	0.31	-4.94
ABC00	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	*no mig*	0.20	1.98	0.85	**0.00**	**0.00**	8.94	-300.41
All lineage θ's equal									
AAADE	$\theta_{Atl} = \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	1.46	**1.46**	**1.46**	0.31	3.66	9.68	-11.81
AAADD	$\theta_{Atl} = \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	1.02	**1.02**	**1.02**	2.13	**2.13**	3.88	-28.95
AAA00	$\theta_{Atl} = \theta_{Pac} = \theta_A$	*no mig*	2.13	**2.13**	**2.13**	**0.00**	**0.00**	0.12	-424.17
Descendent lineage θ's equal									
AACDE	$\theta_{Atl} = \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	0.82	**0.82**	5.34	0.00	8.24	0.49	-7.78
AACDD	$\theta_{Atl} = \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	1.02	**1.02**	2.51	2.13	**2.13**	3.88	-28.42
AAC00	$\theta_{Atl} = \theta_{Pac} \neq \theta_A$	*no mig*	2.09	**2.09**	2.22	**0.00**	**0.00**	0.12	-423.98
Ancestral lineage θ same as eastern lineage θ									
ABADE	$\theta_{Pac} \neq \theta_{Atl} = \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	0.35	1.50	**0.35**	2.05	2.08	2.88	-6.02
ABADD	$\theta_{Pac} \neq \theta_{Atl} = \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	0.34	1.50	**0.34**	2.07	**2.07**	2.88	-6.02
ABA00	$\theta_{Pac} \neq \theta_{Atl} = \theta_A$	*no mig*	0.20	1.98	**0.20**	**0.00**	**0.00**	8.94	-300.41
Ancestral lineage θ same as western lineage θ									
ABBDE	$\theta_{Atl} \neq \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	0.06	2.63	**2.63**	20.00	0.85	0.94	-6.63
ABBDD	$\theta_{Atl} \neq \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	0.31	2.07	**2.07**	2.21	**2.21**	2.80	-6.67
ABB00	$\theta_{Atl} \neq \theta_{Pac} = \theta_A$	*no mig*	0.20	1.98	**1.98**	**0.00**	**0.00**	8.94	-300.42

Table 4.3 Likelihoods of each nested population model from Table 4.2 ranked according to the Akaike Information Criterion (AIC) (Akaike 1973). Listed for each model are the parameter assumptions for effective population sizes (θ) and migration rates (m), the likelihood [log(P)], the number of parameters (k) and the AIC value calculated as per Burnham and Anderson (2002) and recommended by Carstens et al. (2009). The least likely models are highlighted in grey.

Model	**Model assumptions**		**log(P)**	***k***	**AIC**	**Rank**
	N_e	m				
ABBDD	$\theta_{Atl} \neq \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	-6.67	2	17.33	1
ABC0D	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} = 0$	-4.94	4	17.88	2
ABADE	$\theta_{Atl} \neq \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	-6.02	3	18.03	3
ABADD	$\theta_{Atl} \neq \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	-6.02	3	18.04	4
ABCDD	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	-5.37	4	18.75	5
ABBDE	$\theta_{Atl} \neq \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	-6.63	3	19.27	6
ABCDE	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	-4.72	5	19.44	7
ABCD0	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	$m_{Atl\rightarrow Pac} = 0$	-6.26	4	20.53	8
AACDE	$\theta_{Atl} = \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	-7.78	4	23.57	9
AAADE	$\theta_{Atl} = \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} \neq m_{Atl\rightarrow Pac}$	-11.81	4	31.62	10
AAADD	$\theta_{Atl} = \theta_{Pac} = \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	-28.95	2	61.90	11
AACDD	$\theta_{Atl} = \theta_{Pac} \neq \theta_A$	$m_{Pac\rightarrow Atl} = m_{Atl\rightarrow Pac}$	-28.42	3	62.84	12
ABB00	$\theta_{Atl} \neq \theta_{Pac} = \theta_A$	*no mig*	-300.42	1	602.83	13
ABA00	$\theta_{Pac} \neq \theta_{Atl} = \theta_A$	*no mig*	-300.41	2	604.82	14
ABC00	$\theta_{Atl} \neq \theta_{Pac} \neq \theta_A$	*no mig*	-300.41	3	606.83	15
AAC00	$\theta_{Atl} = \theta_{Pac} \neq \theta_A$	*no mig*	-423.98	2	851.97	16
AAA00	$\theta_{Atl} = \theta_{Pac} = \theta_A$	*no mig*	-424.17	3	854.33	17

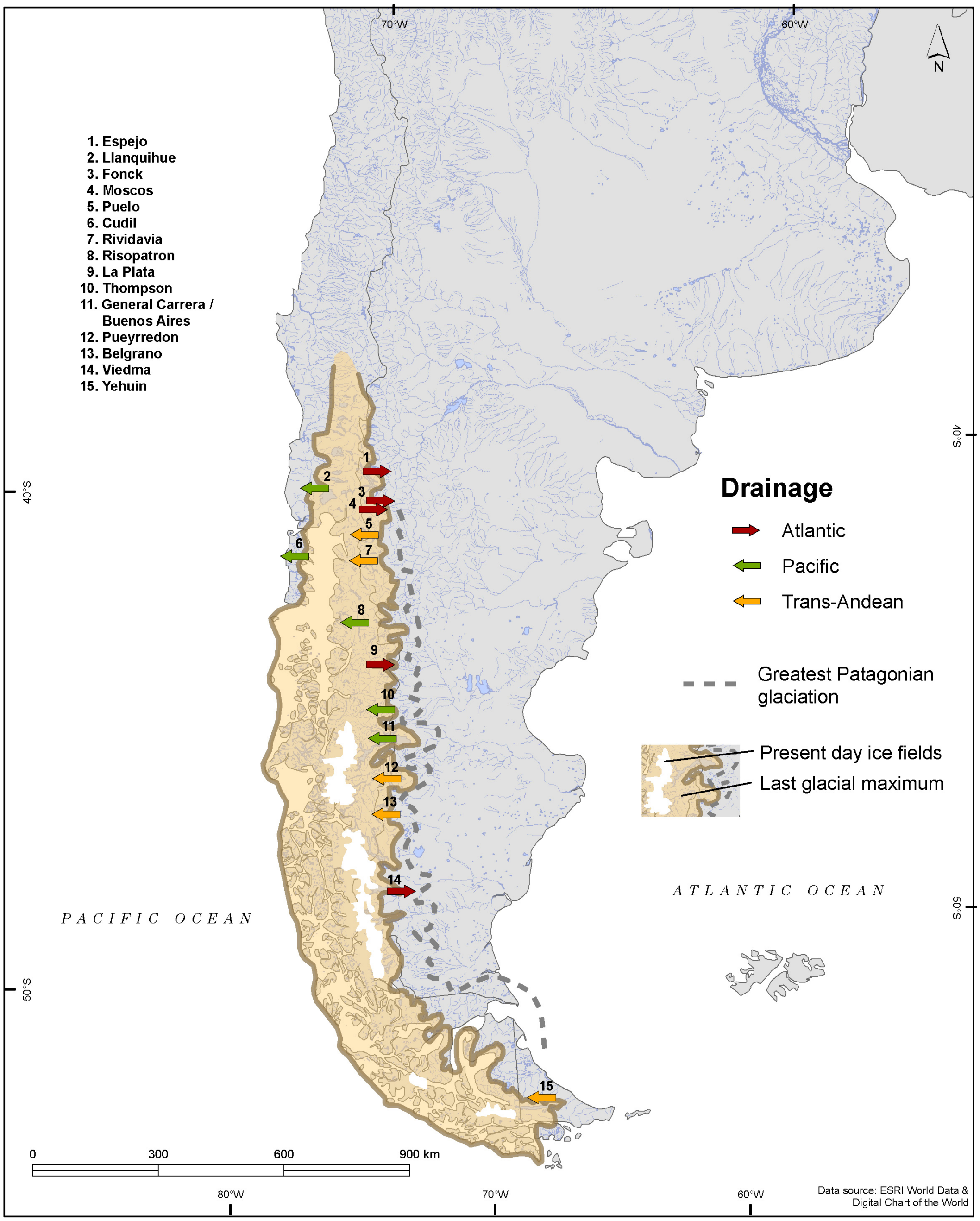


Figure 4.1 Collection locations for *Galaxias platei* throughout Patagonia, South America. Sampled locations are represented by arrows that indicate contemporary drainage direction, either Atlantic or Pacific. Pacific (rev) denotes a drainage reversal, from Atlantic to Pacific, which likely occurred following the retreat of Pleistocene glacier (Turner et al. 2005). The extent of the Last Glacial Maximum (LGM) and contour of the Great Patagonian Glacation (GPG) were adapted from Clapperton (1993) and Turner et al. (2005).

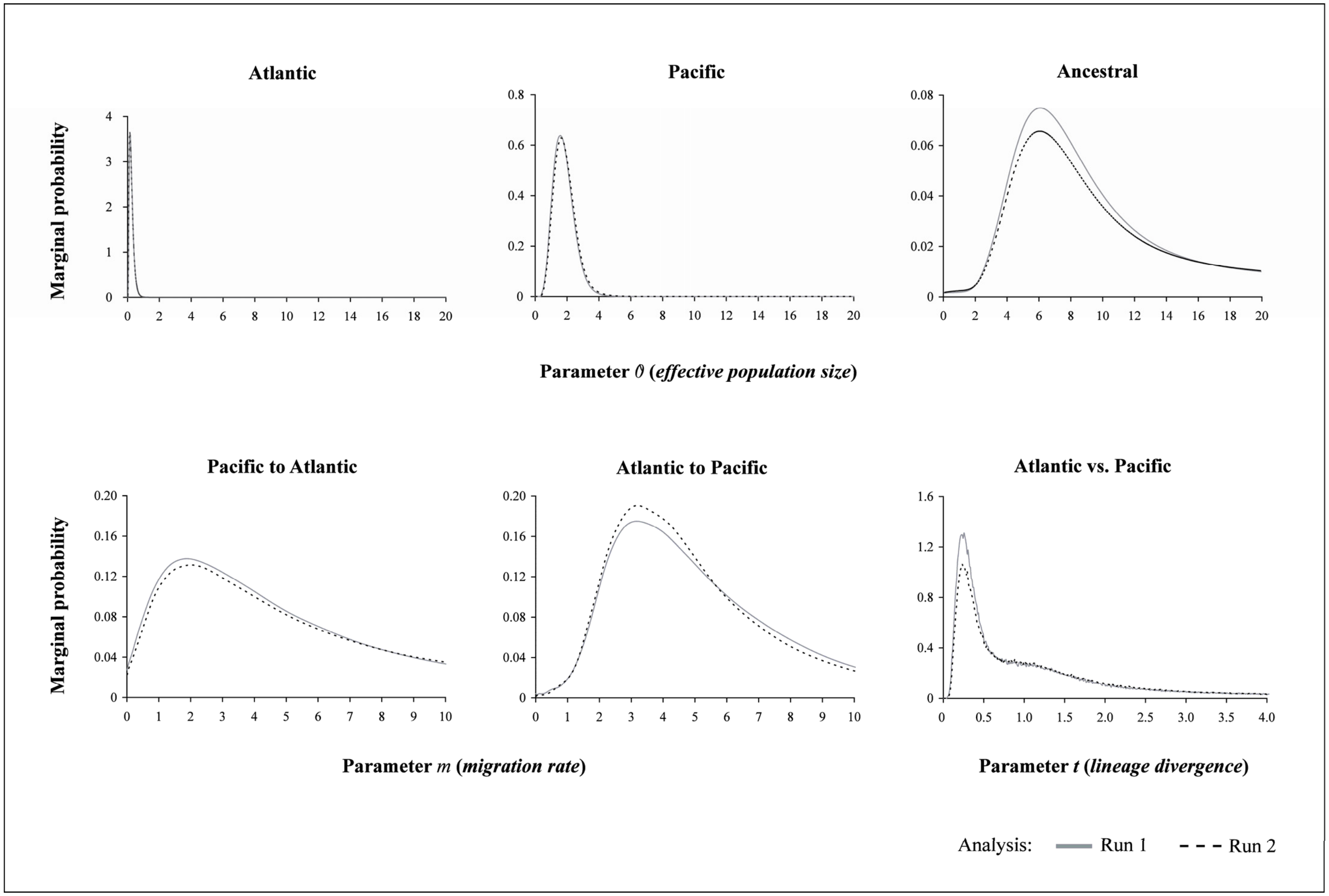


Figure 4.2 Marginal probability distributions for each model parameter generated using 2 independent runs with a full dataset using the program IMa.

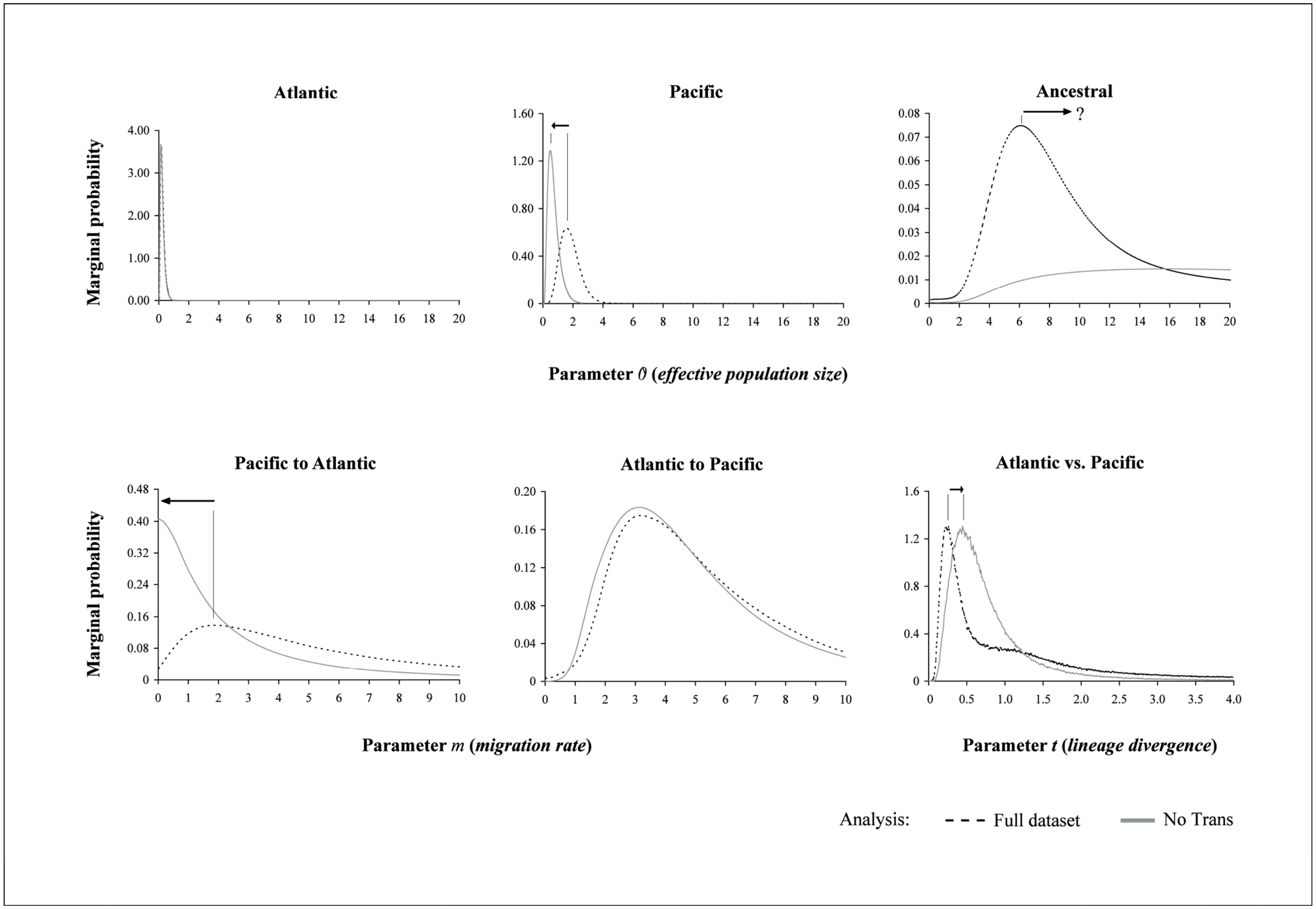


Figure 4.3 Marginal probability distributions for each model parameter generated using a full dataset and reduced dataset in which trans-Andean systems were excluded (No Trans). The effects of trans-Andean exclusion on marginal peaks is indicated by the arrows.

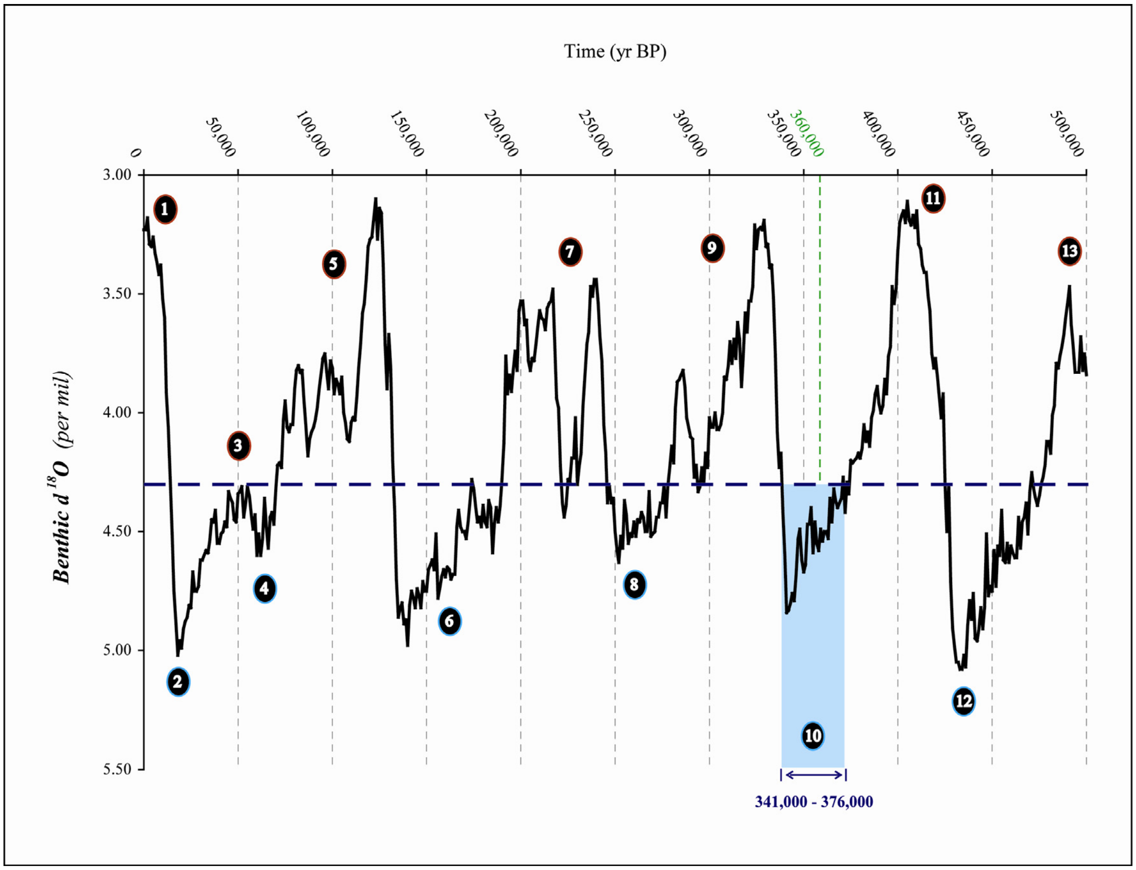


Figure 4.4 Frequency and duration of climatic oscillations over the past 500,000 yr. Oxygen isotope sequences are derived from Lisiecki and Raymo (2005) as a proxy of Quaternary air temperatures for Patagonian South America and are reported in concentrations of oxygen isotope 18 (d18O) per milliliter. Oxygen isotope stage numbering sequence follows that of Lisiecki and Raymo (2005) and are colour-coded to reflect glacial (blue) and inter-glacial (red) periods. Transitions between glacial and inter-glacial conditions follow the recommendations of Ruzzante et al. (2008) of 4.30 d18O per milliliter and are used to estimated the duration of Oxygen Isotope Stage (OIS) 10 in Patagonia (341,000-376,000 yr BP). Also indicated is the estimated time of initial population divergence of *Galaxias platei* (360,000 yr BP) across the Central Drainage Boundary in Patagonia that was estimated in the present study.

CHAPTER 5

CLIMATE INDUCED CHANGES IN THE ANCESTRAL RANGES OF TWO GALAXIID FISHES: THE IMPACT OF QUATERNARY GLACIAL CYCLING ON AQUATIC DIVERSITY IN PATAGONIA

This chapter is currently in being submitted for publication as a *Research Article* in the journal of *Proceedings of the National Academy of Sciences USA*. The primary author of the article is Tyler S. Zemlak. The article is co-authored by Sandra J. Walde, Evelyn M. Habit and Daniel E. Ruzzante. All authors contributed to the conception and design of the study, and all participated in sample collection. TSZ generated the molecular data, and TSZ and DER were responsible for data analysis. TSZ drafted the initial version of the manuscript, and DER and SJW were involved in subsequent versions of the manuscript. All authors reviewed it critically. In the event that the article is accepted for publication, the appropriate copyright permission will be obtained from the publisher *National Academy of Sciences Publishing*.

5.1 Abstract

Patagonia is one of the few areas in the Southern Hemisphere to have been directly influenced by Quaternary glaciers. In this study I evaluate the influence that Quaternary glacial ice had on the genetic diversity of two congeneric fish species, the diadromous *Galaxias maculatus* and the freshwater *G. platei*, using multilocus estimates of effective population size through time. My results indicate that mid-Quaternary glaciations had far-reaching consequences for both species. *Galaxias maculatus* and *G. platei* each experienced severe genetic bottlenecks during the period when Patagonia ice sheet advance reached its maximum positions *ca.* 1.1-0.6 MY BP. Concordant drops in effective size during this time suggests that range sizes were under similar constraints and implies that there were not significant brackish/marine refugia for *G. maculatus*. Declines in effective sizes continued for both species into the late-Pleistocene, but were longer lasting for *G. platei*. The earlier onset of population declines for *G. platei* suggests that this species was vulnerable to modest glacial advances. *Galaxias maculatus* exhibited a strong population recovery during the late-Quaternary (ca. 400 KY-present). Unusually long and warm inter-glacials associated with the late-Quaternary may have helped facilitate a strong population rebound in this primarily coastal species.

5.2 Introduction

Patagonia is one of the few regions in the Southern Hemisphere to have been heavily influenced by Quaternary ice (Ehlers and Gibbard 2007). Ice sheet advances of the past 1 MY were particularly extensive (Singer et al. 2004; Rabassa 2008; Rabassa et al. 2011) and are expected to have played important roles in shaping contemporary landscapes. The most extensive glacial advance took place during the Great Patagonian Glaciation (GPG) of approximately 1.1-1.0 MY BP (Rabassa 2008; Rabassa et al. 2011). Fourteen to 16 glaciations appear to have followed the GPG, each advance slightly less extensive than the previous (Singer et al. 2004). The direct impact of glacial ice was most severe west of the Andes, where it extended from the mountains to the Pacific coast on several occasions, expanding north as far as 35°S (Clapperton 1993; McCulloch et al. 2000; Rabassa 2008). Glacial advances were much less extensive to the east of the Andes, but did descend from the Andean peaks to intrude onto the eastern foothills and extend slightly onto the Patagonian Steppe in southern Patagonia. The indirect effects of these glacial cycles were also widespread. Southern Hemisphere ice sheets are expected to have caused significant changes in the position of the southern westerlies and Patagonian climate (Compagnucci 2011). Substantial drops in sea-level also exposed large parts of the Argentinean continental shelf which held important consequences for climate (Ponce et al. 2011), freshwater drainages (Martinez and Kutschker 2011) and available species habitat (Ruzzante et al. 2011).

Patagonian glaciations undoubtedly affected biodiversity patterns across both terrestrial and aquatic fauna and flora. Indeed, a number of phylogeographic studies exist today for both terrestrial [reviewed in Sersic et al. (2011)] and aquatic taxa (Ruzzante et al. 2006; Ruzzante et al. 2008; Zemlak et al. 2008; Xu et al. 2009; Zemlak et al. 2010). In general, genetic structure within several taxa suggests that species ranges were sundered into one or more glacial refugia in the past. It is likely that these refugia were located east and west of the Andes, both within the glaciated regions as cryptic refugia (Muellner et al. 2005; Zemlak et al. 2008; Xu et al. 2009; Cosacov et al. 2010) and outside glacial limits in mid- to low-latitude refugia (Dyer 2000; Premoli et al. 2000; Ruzzante et al. 2006). For aquatic species, it is also possible that brackish refugia were available for marine-tolerant forms (Zemlak et al. 2010). The present-day distribution of some of these refugial lineages over large geographic distances also suggests many populations/species reclaimed habitat following the melt of the ice sheets [e.g. Ruzzante et al. (2006), Morando et al. (2007), Cosacov et al. (2010)]. The mechanisms underlying enhanced dispersal in less vagile species, such as fishes, has attracted a lot of attention. The transition between glacial and inter-glacial periods appears to have kept aquatic environments unstable and malleable throughout the past 1 MY and created/destroyed many opportunities for inter-drainage dispersal (Zemlak et al. 2008; Zemlak et al. 2010; Ruzzante et al. 2011). Examples include periodic drainage reversals across the Andean divide, the formation of large proglacial lakes and the possibility of inter-river coalescence on an exposed continental shelf in eastern Patagonia.

Sequence-based studies of the phylogeographic history of Patagonian species have primarily relied on the genetic signal available from a single organellar locus (i.e. mitochondrial or chloroplast DNA). In some cases, a second independent nuclear locus has been used for a sub-set (Ruzzante et al. 2006; Zemlak et al. 2010) or all (Xu et al. 2009) of the study taxa. While these studies have been very informative as a first approach to understanding the general influences of historical landscapes on biodiversity in Patagonia, there are important limitations. One problem is the inability of a single-locus to account for the stochastic nature of the lineage sorting process; chance alone can create very different genetic histories at different loci within the same genome. Single gene trees therefore may deviate from the actual species/population tree (Edwards and Beerli 2000). This can lead to inaccurate estimations of evolutionary parameters (e.g. population divergence time) or even lead to a false interpretations in the worst case scenario (e.g. sex-biased dispersal). Another issue is that organellar genes alone are unlikely to preserve genetic signal beyond a recent bottleneck (Heled and Drummond 2008; Ho and Shapiro 2011). Considering the glacial history of Patagonia, it is therefore likely that organellar genes are limited to investigating only recent events. To move beyond these limitations it becomes necessary to integrate the signal from multiple independent loci (Zhang and Hewitt 2003; Brito and Edwards 2009). Currently, there is only one multilocus study concerning Patagonian species (Breitman et al. 2011).

Phylogeographic approaches in Patagonia also generally focus on a single taxon. Multi-species comparisons (comparative phylogeography) are limited to four studies which target xeric plants of the Steppe (Jakob et al. 2009), lizards of central Chile (Victoriano et al. 2008) rodents of southern Patagonia and Tierra del Fuego (Lessa et al. 2010) and freshwater fish (Ruzzante et al. 2008). Contrasting the phylogeographic histories of multiple co-distributed species is a powerful way of understanding regional trends by clarifying the breadth of influence that historical processes had on shaping biogeographical regions (Kholodova 2009). At the same time, differences in phylogeographic histories are also useful for identifying novel responses to shared environmental changes and help identify key biological characteristics that may benefit/disadvantage individuals when facing changing habitats.

In the present study, I move beyond the limitations of single-locus/single-taxon methods and compare the multilocus phylogeographic signal of two congeneric, but ecologically different, species of cold-water fishes in Patagonia. *Galaxias platei* is a freshwater species most abundant in the high altitude headwaters of drainage systems associated with the Andean foothills (Cussac et al. 2004; Habit et al. 2010), and *Galaxias maculatus* is an amphidromous species (Barriga et al. 2002) inhabiting estuarine and freshwater environments of lower to mid reaches of river systems (Cussac et al. 2004; Zattara and Premoli 2005; Habit et al. 2010) as well as higher-elevation and headwater lakes/rivers where the species exhibits a resident and landlocked life history (Barriga et al. 2002; Zattara and Premoli 2005; Barriga et al. 2007). Both species are widespread throughout Patagonia.

The specific objective of this study is to compare the changes in effective ancestral population size throughout the Quaternary for the two species, *G. maculatus* and *G. platei*, in an attempt to clarify the breadth of influence that successive glaciations had on aquatic diversity in Patagonia. I specifically use estimates of range-wide changes in effective population size through time as a proxy for the effects of glaciations on range size in each species. Due to their overlapping contemporary distributions, any shared patterns across species can help us to understand the general effect(s) of past climate on regional aquatic diversity in Patagonia. Contrasting patterns should instead yield important insights regarding the potential roles that species-specific life histories may have played in helping/limiting survival during past shifts in climate and landscapes. The specific questions I address are: (i) How general were the influences of Quaternary glaciations in affecting the distributions of cold-adapted fishes? (ii) Was the magnitude of glacial advance important in determining range sizes? (iii) As a diadromous fish, did access to marine/brackish refugia afford *G. maculatus* any advantage over the freshwater-limited *G. platei*?

5.3 Methods and Materials

The multilocus dataset of *G. platei* (1 mtDNA, 7 nuclear) was collected for a previous phylogeographic study (Chapter 4) and will be available in GenBank in September 2011 under the following accession numbers: HQ169130-HQ169541. The data was collected from 29 individuals from 15 river systems throughout the range of *G. platei* (Table 5.1, Figure 5.1). The multilocus sequence dataset of *G. maculatus* (1 mtDNA, 6 nuclear) is new.

5.3.1 Sample Collection and Preservation (*G. maculatus*)

A total of 94 individuals were selected from 15 lake/river locations chosen to represent the distributional range of *G. maculatus* in Patagonia (Table 5.1, Figure 5.1). All samples used for the present study derive from a tissue bank of Patagonian fishes located in the laboratory of DER. All samples in the tissue bank were collected using gill nets and electrofishing between 1998-2011. Samples were originally fixed in 95% ethanol in the field and transferred to Dalhousie University for storage at -20^{o}C.

5.3.2 Sequence Data (*G. maculatus*)

Total genomic DNA was isolated from *G. maculatus* tissue sub-samples using the glassmilk procedure (Elphinstone et al. 2003) with slight modifications for execution using a MultiPROBE® II HT PLUS EX robotic liquid handling system (PerkinElmer). Subsequently, the mitochondrial control region and the nuclear locus ANL22 were amplified using the primers and procedures described in Zemlak et al. (2010) (Chapter 3). Five additional nuclear markers were amplified using Exon-Primed Intron-Crossing (EPIC) primers published by Li et al. (2010), including: 1777E4, 36298E1, 14867E4, 18049E2, 19231E4. The standard PCR cocktail and thermocycler regime suggested by Li et al. (2010) were used to amplify all EPIC markers. A Mastercycler® EP Gradient (Eppendorf) thermal cycler was used to conduct all reactions.

PCR amplicons for mitochondrial and nuclear loci were visualized using 2.0% agarose gels and sent off site for bidirectional DNA sequencing at Macrogen Inc., Maryland, USA. Each forward and reverse sequence was edited using CondonCode Aligner 3.5 (CodonCode Corporation) and combined to form bidirectional contigs. Contigs for each locus were aligned in ClustalX2 (Larkin et al. 2007) using default parameters and subsequently checked by eye. All nuclear loci contained ambiguous nuclear alleles, represented by sequences containing 2 or more heterozygous base calls, and were separated with 100% probability using the program PHASE v2.1.1 (Stephens et al. 2001). All loci were found to be free of recombination using both RDP and MaxChi algorithms as executed in the program RDP3 (Martin and Rybicki 2000). The edited contigs used for analysis are available from GenBank under the following accession numbers: HQ902942-HQ904063.

5.3.3 Data Analysis

Changes in effective population size over time were compared between *G. maculatus* and *G. platei* using skyline plots generated with Migrate v.3.2.1 (Beerli 2009). The parameter

θ was used as a proxy for effective population size (N_e). I used the Bayesian search strategy in Migrate to analyze the datasets for both species (Beerli and Felsenstein 2001; Beerli 2006). Search parameters were separately optimized for each species using the recommendations in the Migrate Documentation Version 3.2 distributed with the software. Several experimental runs were conducted in which search parameters were altered to ensure an unbiased and widespread search of tree space using Markov Chain Monte Carlo (MCMC) simulation. Each dataset was run with default values (low sampling increments and short runtime) and used rough estimates of F_{ST} to find suitable starting values of θ: 0.001 for *G. maculatus* and 0.0005 for *G. platei*. Subsequently, multiple test runs were conducted for each species in which the sampling frequency, chain length and heating schemes were altered to produce a smooth, unimodal posterior distribution for θ. The final search parameters used for *G. maculatus* consisted of a single long chain of 100 million generations, sampled every 1,000 generations to obtain a sample of 100,000 genealogies. A fixed heating scheme, with swapping among four independent chains at different fixed temperatures (1.0; 1.5; 3.0; 1,000,000), was used to improve MCMC searching. After discarding the first 5,000 trees as burnin, parameters were updated at a frequency of 0.5 using a posterior bin size of 2,000 and a uniform prior (min=0, max=0.2, delta=0.02). For *G. platei*, a single long chain of 10 million generations, sampled every 1,000 generations was used to obtain a sample of 10,000 genealogies using the same 4-chain heating strategy. Again, 5,000 trees were discarded as burnin and parameters were updated at a frequency of 0.5, but with a different uniform prior (min=0, max=1.0, delta=0.1) and using a smaller bin size (1,500). An inheritance scalar of 1 was used for the control region, whereas 4 was used for all nuclear loci. An additional, and independent run using different seed numbers, was conducted for each species data set and compared to the original optimized run to ensure sufficient runtime and convergence on a stationary distribution. Finally, four additional replicate runs (using different seed numbers) were conducted for each species using the web-based computing power available from Computational Biology Service Unit (CBSU) at Cornell University. The final results represent a total of 6 independent runs for each species.

The time scale of the skyline plots (x-axis) generated by Migrate is in units of expected mutations per generation (Figure 5.2). I converted each axis into calendar years using species-specific generation times (G_{Gm} = 1 ± 0.2 yrs and G_{Gp} = 3 ± 0.2 yrs) and substitution rates (μ_{Gm} = 0.026 and μ_{Gp} = 0.011 changes/site/MY). Generation time has been well studied in *G. maculatus*. Studies of New Zealand and Australian populations suggest that *G. maculatus* is an annual species (i.e. generation time = 1 year) (McDowall 1968; Pollard 1971; Chapman et al. 2006). Similar conclusions have also been reported for Patagonian populations (Campos 1973; Barriga et al. 2002). I employed a generation time of 3 years for *G. platei* based on information on age at first reproduction collected from *G. platei* from lakes Moreno and Rivadavia in the provinces of Rio Negro and Neuquén in Argentina. Mitochondrial substitution rate in galaxiids has been well investigated using a number of dated river capture and mountain building events in New Zealand (Burridge et al. 2008). The use of multiple events of varying age (0.007-5.0 MY BP) confirmed that time-dependency is a factor in estimating mtDNA substitution rates in galaxiid fishes, with relatively young geological events/population separations (i.e. <200 KY BP) providing an accelerated estimate of substitution rates when compared to older geological events [see Ho and Larson (2006)]. Two ranges were reported: 0.031-0.125 changes/site/MY from river isolation events younger than 200 KY; 0.011-0.026 changes/site/MY for galaxiid rates derived from older events (>200 KY). A single average substitution rate for the control region/cytochrome *b* (0.01876 changes/site/MY) was obtained using an asymptotic calculation (Burridge et al. 2008). However, the application of a general clock in the present study is not appropriate considering that the two species represent opposite ends of the spectrum in galaxiid fishes with respect to generation times and body sizes; two primary factors influencing mtDNA substitution rates in animals [see Martin and Palumbi (2003)]. *Galaxias platei* can reach standard lengths (SL) as great as 37 cm (Milano et al. 2006), but more commonly averages an SL between 15-25 cm [see Table 2 in Milano et al. (2006); Evelyn Habit personal observations]. Average total length for *G. maculatus* is instead generally around 10 cm or less, although occasionally individuals can be found that measure up to 19 cm in SL (Allen 1989; Allen et al. 2003). Therefore, based on the predictions of Martin and Palumbi (1993), *G. maculatus* is expected to have a faster mtDNA substitution rate (short generation time, small body size) than *G. platei* (long generation time, large body size). Metabolic rate is another important factor (Martin and Palumbi 1993), but is unlikely to be relevant considering that both species are cold-water poikilotherms. Thus, taking into account time-dependency, as well as physiological differences between species, I employed the extremes of the range reported for galaxiid fishes based on older events (i.e. 0.2-5.0 MY) and used substitution rates of 0.011 and 0.026 changes/site/MY for *G. platei* and *G. maculatus*, respectively (Figure 5.3). Furthermore, I address the uncertainty around these estimates by (i) also presenting results assuming ± 0.2 year differences in generation times and (ii) by discussing the implications that more extreme values of generation times would have on the x-axis (timeline) of the skyline plots and the conclusions of this study. I only considered influences of modifying generation time on the time axis since it is strongly correlated with mutation rate and body size. My species specific approach is likely to result in more accurate estimates of the timing of changes in effective population sizes in each species than the alternative of using an average substitution rate and an average generation time estimated for all galaxiids (Burridge et al. 2008).

5.4 Results

The demographic timelines for each species were constructed from six independent runs using Migrate (Figure 5.2). Overall, the replicate runs for each species were strongly concordant and imply an unbiased and adequate search of parameter/tree space. The greatest variation within each species timeline concerns coalescent points and time intervals involving large changes in theta (i.e. rapid growth, genetic bottleneck). The variation among runs was incorporated into each estimate of effective population size (y-axis) by averaging over the 6 runs and including a min/max range. Any variation introduced to the x-axis by replicate runs was incorporated as error additively with the uncertainty associated with generation times.

Each species experienced a strong genetic bottleneck during the middle Pleistocene (0.6-1.1 MY BP; Figure 5.3). The timing and the degree of overlap in this bottleneck between species was strongly dependant on the units used to scale the x-axis. Left untransformed (expected mutations/generation; Figure 5.2), the bottlenecks were synchronous at 0.02 mutations/generation. The transformation of the x-axis into calendar years, however, separated the timing of these events. Using the best estimates of generation times in each species (G_{Gm} = 1 yr, G_{Gp} = 3 yrs) resulted in the placement of bottlenecks between 0.75 – 0.90 MY BP in *G. maculatus* and 0.61 – 0.67 MY BP in *G. platei* (Figure 5.3). These ranges were further extended to 0.63 - 1.13 MY BP in *G. maculatus* and 0.58 - 0.72 MY BP in *G. platei* when a margin of error (± 0.2 yrs) was allowed for estimates of generation time. Over the course of each species bottleneck, population sizes were reduced by an average of 41% in *G. maculatus* and 34% in *G. platei*. The timing of these bottlenecks thus broadly correspond to the middle Pleistocene (0.6-1.1 MY BP), a period in the Quaternary of drastic climate change and marked glacial advances in Patagonia (Rabassa 2008; Rabassa et al. 2011).

Following these middle Pleistocene bottlenecks, each species continued to experience consistent and long-term population declines. *Galaxias maculatus* was the first to show signs of recovery with population losses stabilizing within the period of 240,000 – 360,000 yr BP (OIS 7-11; Figure 5.3). By 135,000 [112,000-168,000] yr BP, *G. maculatus* had transitioned to a period of strong population growth that persisted throughout the remainder of the Pleistocene. This period represented the greatest rate of change, as well as the greatest magnitude of change, in effective population size for *G. maculatus*. Beginning at a historical low of 0.014 (0.013-0.016), θ increased by over 600% to reach a new high of 0.085 (0.075-0.099) by the end of the Pleistocene. *Galaxias platei*, however, did not show signs of stabilization/recovery until 38,000 (36,000-41,000) yr BP, well into the last glacial period (OIS 2). Even then, population gains were more modest at a 200% increase in theta [0.007 to 0.014 (0.013-0.015)]. Therefore, while the estimates from the most recent times suggest that effective size in *G. maculatus* is approximately 6 times larger than that of *G. platei*, this difference represents only a very recent departure from parity that existed as recently as 240-360 KY BP.

Before the genetic bottlenecks, the effective population size trajectories for *G. maculatus* and *G. platei* differed from each other (Figure 5.3). During this time the effective population size for *G. maculatus* increased with the genetic signal coalescing between 2.17 – 2.25 [1.81-2.81] MY BP at a point when *theta* was low [0.030 (0.016-0.036)]. By the early- to mid-Pleistocene [1.14 (0.95-1.41) MY BP], effective population size in *G. maculatus* had peaked at 0.073 (0.071-0.075) to gross a population size increase of approximately 243% since the time of coalescence. The shape of the growth curve during this period was most likely non-linear and separated into two stages at approximately the mid-way point by a brief cessation of growth. Based on the shape of the plot, population growth rate was greatest in the earliest phase. *Galaxias platei* instead, experienced continuous losses throughout an overlapping, but shorter period. The point of coalescence in *G. platei* is between 1.37-1.43 [1.29-1.53] MY BP, at which point theta was at its highest at approximately 0.071 (0.066-0.077). In the period leading to the mid-Pleistocene bottleneck, *Galaxias platei* had lost 41% of its population to end the period with an average theta of 0.042 (0.031-0.044).

5.5 Discussion

In this study I described the long-term effects of glacial ice on the diversity of two aquatic taxa in Patagonia. Early Quaternary glaciations appear to have been most important for headwater species, such as *G. platei*, whose range is predominantly associated with Andean headwaters and in close proximity to mountain glaciers. Stronger advances associated with the mid-Pleistocene, however, appear to have had far-reaching effects on aquatic diversity in Patagonia. Beginning with the peak glacial periods of approximately 1.1-0.6 MY BP, *G. platei* and *G. maculatus* each demonstrated strong and concordant decreases in effective population sizes consistent with range-wide genetic bottlenecks. Subsequent glaciations of diminishing extent were also likely to have placed constraints on range sizes, but were much less intrusive than previous cycles. *Galaxias maculatus* demonstrated the earliest signs of recovery from the effects of Pleistocene ice with evidence of population gains soon after the mid-Brunhes (Tzedakis et al. 2009) event of approximately 400 KY BP. It is possible that the unusually warm and long inter-glacials of the late-Pleistocene could have improved coastal conditions and facilitated a speedier recovery for this species.

5.5.1 The Potential Impacts of Pleistocene Glaciers on Aquatic Diversity

My results indicate that *G. maculatus* and *G. platei* each suffered a strong genetic bottleneck during the mid-Pleistocene (0.6-1.1 MY BP). I suggest that these strong decreases in population size were primarily the result of strong contractions in range sizes imparted by the extreme climate fluctuations that were affecting Patagonia and the rest of the Globe at this time.

Starting with the GPG [1.1 MY BP, (Singer et al. 2004)] and ending with the Coldest Pleistocene Glaciation [CPG, 0.6-0.7 MY BP (Rabassa 2008)], this period witnessed several strong advances of the Patagonian ice sheet. The direct impact of Pleistocene glaciations on aquatic habitat in Patagonia is expected to have been important, with each advance eliminating the majority of temperate lakes/rivers and displacing or eliminating their contents. Atmospheric circulation also changed during glacial periods with the Westerlies either shifting northwards or declining in intensity (Compagnuci 2011). Aridity increased on most of the Patagonian Steppe placing further stress on eastern freshwater habitat located outside of the glacial limits (but see Martinez & Kutschker 2011). These mid-Pleistocene climate events were likely very difficult for aquatic organisms and can most easily explain the ubiquitous loss of genetic diversity in each species throughout their ranges in Patagonia. At this point, however, I am uncertain if the species bottlenecks of *G. maculatus* and *G. platei* occurred simultaneously or were the result of independent climatic events of the mid-Pleistocene (see 5.4.3 Uncertainties in the demographic timelines).

The constraints on range sizes imparted by Quaternary ice do not appear to be limited to peak glacial cycles. Following the mid-Pleistocene bottlenecks, each species continued to experience population declines throughout most the remaining epoch, but at lower rates. Interestingly, the magnitude of glacial advance during this period was also abating. A total of 14-16 ice sheet advances may have occurred in Patagonia following the GPG (Rabassa et al. 2005). Unlike glacial sequences of the Northern Hemisphere, however, each successive advance of the Patagonian ice sheet was of diminishing extent (Singer et al. 2004). The concordant and negative relationship between species abundance and the magnitude of ice sheet advance therefore suggests that glacial dynamics held strong limitations on range sizes for aquatic organisms in Patagonia for most of the Pleistocene. Perhaps glacier size was very important in affecting the size, number and/or connectivity of any aquatic refugia during this time.

Within the period of 240,000-360,000 KY BP population size in *G. maculatus* stabilized and ended a long-term interval of decline that had persisted since as early as the GPG 1.1 MY BP. Subsequently, population size accelerated exponentially throughout the remainder of the Pleistocene and reached an unprecedented level by the Holocene interglacial. Can the unusually warm inter-glacials following the mid-Bruhnes account for population recovery in *G. maculatus*? The mid-Bruhnes event (approximately 430 KY BP) refers to the climatic transition period between the OIS 12 glacial and the OIS 11 interglacial and represents the largest amplitude change in Pleistocene climate *as per* global benthic δ^{18}O ocean sediment proxies (Tzedakis et al. 2009). Furthermore, the mid-Brunhes was a transition point back to warm interglacials ending the period of cool interglacials that lasted approximately 430-1.1 MY BP (Tzedakis et al. 2009). The warm interglacials of the past 430,000 yrs could have benefited *G. maculatus* in any number of ways. For example, sea-levels were higher during late-Quaternary interglacials (Rabineau et al. 2006; Olson and Hearty 2009) and could have dramatically increased habitat size. Local embayments were also much warmer during this time (Ortlieb et al. 1996) and could have encouraged faster growth and shorter generation times.

The strong genetic bottleneck and subsequent long-term population decline in *G. maculatus* throughout the mid- to late-Pleistocene are contrary to the conclusions reached by a previous study (Zemlak et al. 2010). A skyline-plot based on *ca.* 300 control region sequences found evidence for long-established maternal effective population sizes (~3.2 MY) which remained relatively constant until 0.5 MY BP when population sizes rapidly increased several orders of magnitude (Zemlak et al. 2010). This demographic pattern was interpreted as evidence that *G. maculatus* was resilient to past glacials in Patagonia and explained by marine refugia and ice-avoidance. The findings of this study, however, indicated otherwise and instead suggest that effective size was negatively impacted by Pleistocene ice. I suggest that the present multilocus analysis is more representative of the true demographic history of *G. maculatus* throughout the Pleistocene for two reasons. First, single-locus analyses are much less likely to be informative beyond a population bottleneck (Heled and Drummond 2008). Therefore, the long-term period of population size (0.5-3.2 MY BP) illustrated by Zemlak et al. (2010) is more likely to reflect an artifact of genetic purge imparted by reductions in population size throughout the mid- to late-Pleistocene than a real pattern. Second, the mutation rate implemented is general for galaxiid fishes (0.01876 changes/site/MY) and likely underestimates the pace of substitution at the mtDNA control region for this species.

5.5.2 Late Pliocene to Mid-Quaternary Population Dynamics

The coalescent point of the genetic signal of *G. maculatus* falls within the range of 1.81-2.81 MY BP and could represent the timing of the first establishment of the species in Patagonia. *Galaxias maculatus* is the most widespread diadromous fish in the Southern Hemisphere with a distribution that includes Australia, Tasmania, New Zealand and Patagonia (McDowall 1970, 1971). Biogeographic and phylogeographic evidence suggest that Patagonian populations represent the most recent range expansion of this species and arrived via long distance marine dispersal from west-Pacific sources (Berra et al. 1996; Waters and Burridge 1999; McDowall 2000; Waters et al. 2000). Within this context, the initially low and variable demographic history of *G. maculatus* estimated by the present data could be the explained by adjustments to a new location. Examples of the potential pressures facing a range expansion include: (i) selective pressures to new environmental conditions, (ii) losses in genetic diversity due to drift and founder effects and (iii) competition with other established species. Alternatively, it is possible that *G. maculatus* was already established in Patagonia at this time and dwindling population numbers are simply a response to early Patagonian glaciations or other unknown ecological pressures.

For *G. maculatus*, the period leading up to the GPG was characterized by long-term and step-wise gains in population size. I suggest that this period is when *G. maculatus* expanded its range to attain its current widespread distribution throughout Chile and Argentina. The phylogeographic structure of contemporary populations of *G. maculatus* in Patagonia is best explained by a model of isolation-by-distance through the marine realm (Zemlak et al. 2010). Expansion likely initiated from an area within, or near, the Lakes District of Chile and subsequently spread north and south along the coast. After achieving a widespread distribution throughout Chile, southern populations continued southward and invaded Tierra del Fuego and mainland Argentina via the Atlantic/Pacific connection at the southernmost tip of South America (Zemlak et al. 2010). I suggest that the first (and most rapid) growth phase in the skyline-plot is attributable to the initial expansion of *G. maculatus* throughout Chile. The second growth phase most likely reflects the subsequent spread of southern Chilean populations throughout Argentina.

Galaxias platei experienced a different demographic trajectory. This species suffered consistent losses in effective population size leading up to the GPG. This early onset of population decline could be explained by the fact that this species is currently most abundant in headwater lakes along the Andean mountains, potentially making it more vulnerable to the weaker glaciations of the early Quaternary. Substantial ice caps had formed at the peaks of the southern Andes as early as 14 MY BP (Heusser 2003; Ehlers and Gibbard 2007). The oldest known Patagonian glaciation is expected to have occurred between 7 and 5 MY BP and possessed outlet glaciers which extended beyond the mountain front (Rabassa et al. 2005). In the subsequent period leading up to the GPG (*ca.* 1.1-1.0 MY) a minimum of 8 additional glacial advances occurred during which time glacial lobes are expected to have reached several high elevation lakes and rivers (Rabassa et al. 2005). Therefore, it is very likely that at least part of the range of *G. platei* was repeatedly occupied by ice in the period leading up to the GPG and probably contributed to population losses in *G. platei* by forcing contractions in range size.

5.5.3 Uncertainties in the Demographic Timelines

I took several precautionary measures to produce reliable estimates of the timing and magnitude of changes in population sizes of the two widespread *Galaxias* species featured in this study. However, the number of factors involved in constructing a calibrated Skyline plot for two species makes the nature of this study inherently complicated and prone to several sources of uncertainty. Therefore, I identify these uncertainties below and discuss the potential influences that such uncertainties could have on the shape of Skyline plot and any associated conclusions:

(i) *Inadequate sampling* – too few individuals, or unrepresentative geographic sampling, may have led to missed haplotypes and thus an underestimation of population age (Ruzzante et al. 2008; Ho and Shapiro 2011). Inadequate sampling is more likely for *G. platei*, but only with respect to the number of individuals analyzed (n=29); the geographic distribution of samples is representative of the entire range of *G. platei*. Furthermore, it is unlikely that additional sampling will dramatically influence the shape of the skyline plot and the conclusions of this study since the widespread geographic sampling used in this study most certainly encapsulates the main genetic subdivisions that comprise deeper phylogeographic structure at mtDNA loci of this species (Zemlak et al. 2008).

(ii) *Phylogenetic resolution* – the first step to generating Skyline plots is to estimate a genealogy from a group of genetic samples (e.g. sequence data). The shape of skyline plot is then generated from the information contained within a given genealogy, or group of genealogies. Of particular importance to the Skyline plot is the size of each coalescent interval and the number of lineages within each interval [see Drummond et al. (2005)]. Considering that the genealogy itself is an estimation, any error with respect to the topology and/or the branch lengths (phylogenetic error) can substantially influence the accuracy of demographic reconstructions (Ho and Shapiro 2011). In this study I accounted for this source of error by summarizing over several (n=6) independent runs for each species data set. The replicate runs were strongly concordant and suggestive of strong phylogenetic resolve among genealogies.

(iii) *Population structure* – an assumption of the Skyline method is that genetic samples were collected from panmictic populations. The violation of this assumption has not yet been investigated in depth (Ho and Shapiro 2011), however, there is a valid concern that population structure can drive patterns in the skyline-plot (Pannell 2003; Ho and Shapiro 2011). Within the present study, however, population structure in *G. maculatus* is very unlikely considering the high propensity for populations of diadromous fishes to remain connected (Ward et al. 1994; McDowall 1999). This violation is more likely in *G. platei* considering its exclusively freshwater lifestyle and the strong genetic structure detected across the Andes at the mtDNA control region (Zemlak et al. 2008). Still, it is unlikely that trans-Andean structure will effect the main conclusion of this study: that a strong population bottleneck occurred between 0.6-0.7 MY BP. A previous study (Zemlak et al. 2008) showed that the initial separation of *G. platei* across the Andes was likely to have occurred much more recently, approximately 360 KY BP, and with subsequent (but infrequent) bouts of bidirectional gene flow. Furthermore, deep genetic structure is more likely to be reflected in the skyline plot as a positive gain, instead of the bottleneck detected in this study. Population size (N) is estimated for each unit (coalescent interval) in the skyline-plot according to the relationship $N_i = \gamma i(i-1)/2$, where *i* denotes the number of lineages in a given coalescent and γ represents the length of the coalescent interval. Additional structure should therefore increase the number of lineages (*i*) in the coalescent interval and interpreted as a population gain.

(iv) *Selection* – another assumption of skyline-plot methods is that sequences are assumed to be evolving neutrally. However, there is abundant evidence that the mitochondrial genome is under purifying selection (e.g. Bazin et al. 2006, but see McCusker & Bentzen 2010). In the case of mtDNA, (weak) purifying selection would create an excess of mutations near the tips of the genealogy [see Ho & Shapiro (2011) and references therein]. In the present study, the biases associated with purifying selection could help explain why each species plot ends with a phase of positive gains in recent evolutionary history, but is unlikely to influence the positions or severities of population bottlenecks located earlier in the Quaternary.

(v) *Evolutionary variability around the coalescence* – the timing of coalescent points in the genealogy form important components of the skyline estimation. Stochastic variation in a single locus can dramatically alter the timing of important events recovered in the skyline-plot. I controlled for stochastic error in the lineage sorting process by using multiple independent loci for each species.

(vi) *Scaling the time axis* – conversion of the x-axis of skyline plots generated by Migrate require an accurate generation time and mutation rate for each species. I provided reasonable estimates of generation time and mutation rate in each species. However, there are a number of uncertainties regarding these estimations, which when combined, could result in substantial changes in the timing of important demographic events. For example, generation time is expected to vary geographically, as well as temporally. Increasing or decreasing the generation times of each species can strongly influence the degree of overlap between species bottlenecks (Figure 5.3). Changing the mutation rate would have similar effects (data not shown). I attempted to accommodate for geographic and temporal variation in generation time and mutation rate in these species by introducing confidence intervals of ± 0.2 yrs (see section 5.2 Materials and Methods).

5.6 Conclusion

My results suggest that climate cycling during the Quaternary held important consequences for the distributions and genetic diversity of the cold-adapted Patagonian fishes *G. maculatus* and *G. platei*. Both galaxiid species demonstrated declines in effective population size in response to the growth of the Patagonian ice throughout the mid- to late-Pleistocene. Of particular importance was the magnitude of glacial advance. I detected strong and concordant declines in effective population sizes (genetic bottlenecks) for both galaxiid species during the mid-Pleistocene (0.6-1.1 MY BP) at a time when glacial advances reached their maximum positions in Patagonia. Following these bottlenecks, contractions in population size continued in each species into the late-Pleistocene, albeit at diminishing rates. The similar fate of these two widespread species, despite very different ecologies, carries important implications for the phylogeographic history of other high latitude aquatic species. I suggest that strong glacial advances of the past 1 MY were very likely to have eliminated a large proportion of the neutral genetic diversity in cold-adapted aquatic species of Patagonia by constraining range sizes and exposing populations to the eroding effects of drift.

Table 5.1 Sampling locations (Lake/River) for specimens of *Galaxias maculatus* and *G. platei* used for genetic analysis. Locations are numbered according to latitudinal position and correspond to labels in Figure 5.1. Listed for each sampling location is its catchment membership (river basin), current ocean drainage, geospatial coordinates and the number of individuals collected for each species.

Lake/River	**Latitude (dd mm ss)**	**Longitude (dd mm ss)**	**River Basin**	**Ocean Drainage**	**# Individuals**	
					G.m.	***G.p.***
Topocalma River	34 17 51 S	71 57 46 W	Topocalma	Pacific	1	
Nilahue River	34 29 01 S	72 00 58 W	Nilahue	Pacific	5	
Reloca River	35 37 49 S	72 33 45 W	Reloca	Pacific	3	
Lake Quillen	39 25 15 S	71 18 11 W	Limay	Atlantic	6	
Lake Calafquen	39 34 16 S	72 14 27 W	Valdivia	Pacific	5	
Valdivia River	39 51 46 S	73 21 12 W	Valdivia	Pacific	5	
Bueno River	40 19 27 S	73 05 38 W	Bueno	Pacific	5	
Lake Espejo	40 37 42 S	71 45 16 W	Limay	Atlantic	6	2
Lake Llanquihue	41 15 44 S	72 59 40 W	Maullin	Pacific		2
Lake Fonck	41 19 09 S	71 46 30 W	Manso	Atlantic		2
Lake Moscos	41 20 47 S	71 37 05 W	Manso	Atlantic		2
Lake Martin	41 30 42 S	71 40 09 W	Manso	Atlantic	5	
Lake Steffen	41 30 53 S	71 34 57 W	Manso	Atlantic	7	
Lake Puelo	42 08 06 S	71 38 06 W	Puelo	Pacific		2
Cudil River	42 22 29 S	73 48 22 W	Chiloe	Pacific		2
Lake Rivadavia	42 39 04 S	71 40 48 W	Futalaufquen	Pacific		2
Lake Risopatron	44 15 51 S	72 31 20 W	Palena	Pacific		2
Lake La Plata	44 52 58 S	71 50 55 W	Senguerr	Atlantic		2
Lake Thompson	45 38 18 S	71 47 15 W	Aysen	Pacific		2
Lake General Carrera	46 17 52 S	71 56 14 W	Aysen	Pacific		2
Lake Pueyrredon	47 25 34 S	71 54 00 W	Baker	Pacific		2
Lake BrazoChico	47 47 24 S	73 30 10 W	Baker	Pacific	12	
Lake Belgrano	47 50 28 S	72 07 46 W	Nansen/Bravo	Pacific		2
Lake Viedma	49 42 16 S	72 03 28 W	Santa Cruz	Atlantic		1
Lake Argentino	50 13 50 S	72 31 08 W	Santa Cruz	Atlantic	6	
Lake Roca	50 31 51 S	72 48 40 W	Santa Cruz	Atlantic	6	
Lake Duyuo	50 33 31 S	75 19 17 W	Duque de York	Pacific	10	
Lake Yehuin	54 24 00 S	67 44 00 W	Azopardo	Pacific		2
Ushuaia Coast	54 48 15 S	68 14 17 W	Beagle Channel	Atlantic/Pacific	12	
					94	**29**

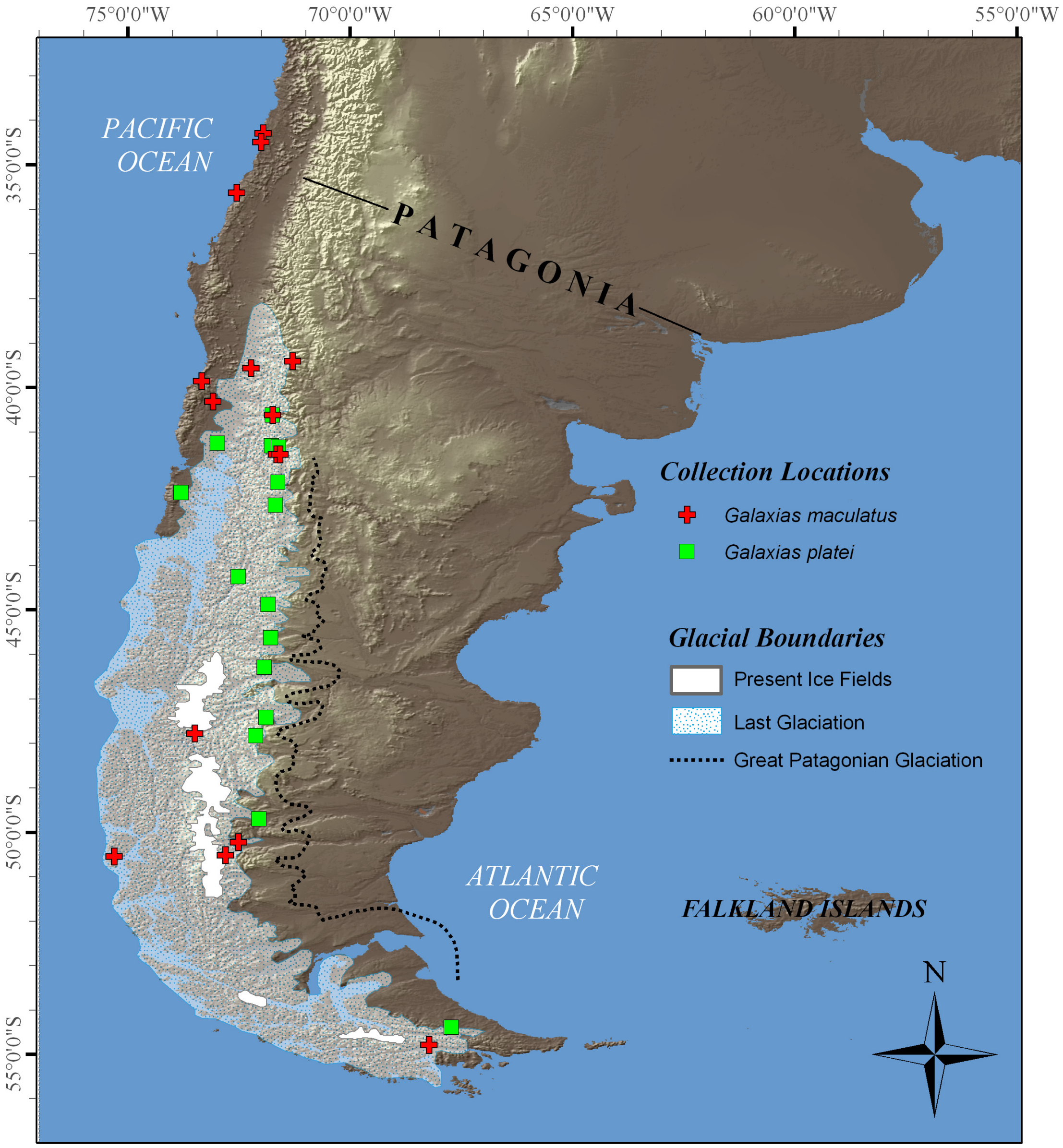


Figure 5.1 Collection locations for *Galaxias maculatus* (+) and *G. platei* (□) throughout Patagonia, South America. The extent of the Last Glacial Maximum (LGM) and contour of the Great Patagonian Glaciation (GPG) were adapted from Clapperton (1993) and Turner et al. (2005).

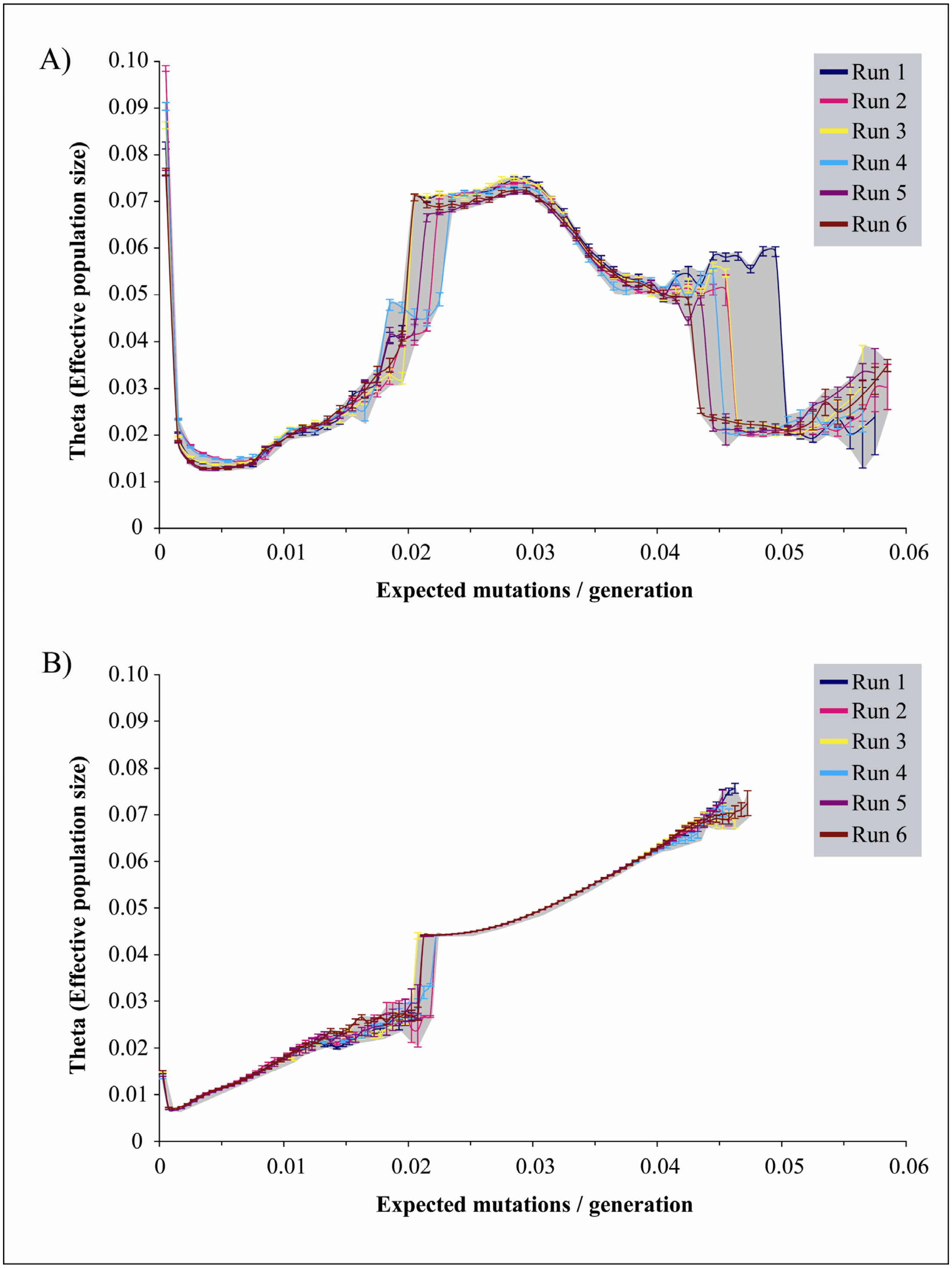
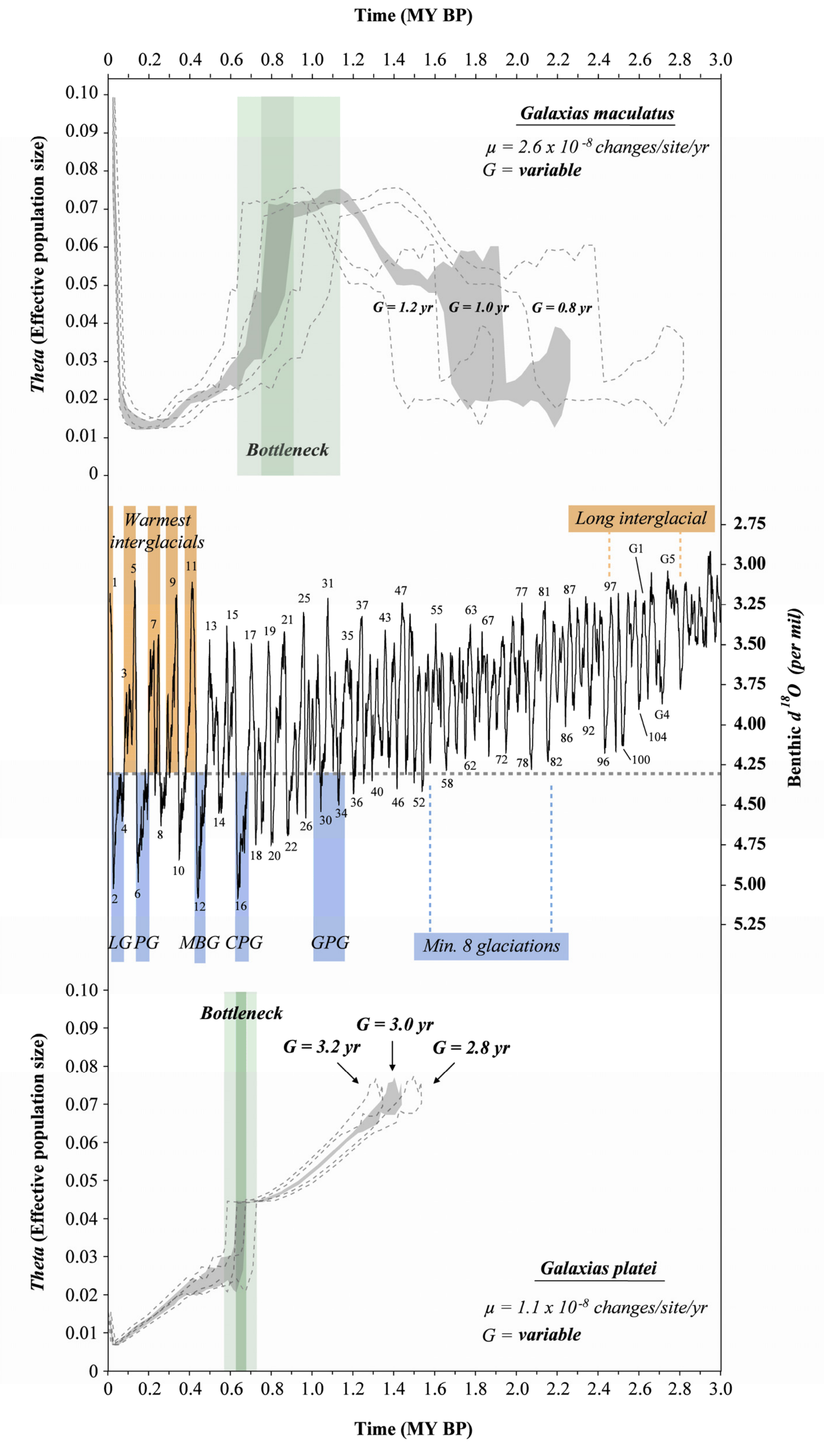


Figure 5.2 Uncalibrated Skyline plots of A) *Galaxias maculatus* and B) *G. platei* based on the sequence variation at multiple loci. The plots represent the relative changes in effective population size (θ) through time (expected mutations per generation) for each species. Shown for each species are 6 independent Skyline analyses (Runs 1-6) used to assess the consistency of demographic reconstructions (or phylogenetic error).

[See subsequent page for figure]

Figure 5.3 Skyline plots from Figure 5.1 with the x-axes transformed into calendar years using species-specific mutation rates and generation times. Multiple generation times are used to depict the magnitude of influence that calibration uncertainties have on the time axis. Also shown is the frequency and duration of climatic oscillations over the past 1.2 MY according to oxygen isotope 18 ($d^{18}O$) sequences derived from Lisiecki and Raymo (2005). Transitions between glacial and inter-glacial conditions follow the recommendations of Ruzzante et al. (2008) of 4.30 $d^{18}O$ per milliliter. Superimposed on oxygen isotope sequences are important historical climate events in Patagonia (Rabassa et al. 2005; Rabassa et al. 2008; Tzedakis et al. 2009; Rabassa et al. 2011). Abbreviated climate events include: Last Glaciation (LG), Penultimate Glaciation (PG), Mid-Brunhes Glaciation (MBG), Coldest Pleistocene Glaciation (CPG), Great Patagonian Glaciation (GPG).



Time (MY BP)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
Theta (Effective population size)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Galaxias maculatus
μ = 2.6 x 10 -8 changes/site/yr
G = variable
G = 1.2 yr
G = 1.0 yr
G = 0.8 yr
Bottleneck
Warmest interglacials
Long interglacial
Benthic d18O (per mil)
2.75
3.00
3.25
3.50
3.75
4.00
4.25
4.50
4.75
5.00
5.25
1
3
5
7
9
11
13
15
17
19
21
25
31
35
37
43
47
55
63
67
77
81
87
97
G1
G5
G4
104
100
96
92
86
82
78
72
62
58
52
46
40
36
34
30
26
22
20
18
16
14
12
10
8
6
4
2
LG
PG
MBG
CPG
GPG
Min. 8 glaciations
Bottleneck
G = 3.0 yr
G = 3.2 yr
G = 2.8 yr
Galaxias platei
μ = 1.1 x 10-8 changes/site/yr
G = variable

CHAPTER 6: CONCLUSION

A modified version of this chapter will be integrated with Chapter 1 and submitted for consideration as an *Invited Review* in the journal of *Molecular Ecology* with co-authors Daniel E. Ruzzante and Sandra J. Walde.

6.1 **Introduction**

My thesis focused on the roles in which historical changes in the physical and climatic environments of Patagonia influenced the abundance and distribution of two widespread fishes, *Galaxias maculatus* and *G. platei*. My results indicated that processes which started as early as the Miocene (southern Andean uplift) and operated throughout the Quaternary (Andean orogeny and glacial cycles) played important roles in shaping the contemporary distributions and genetic diversity of the widespread galaxiid fishes. In this final chapter I first briefly summarize the major findings of my thesis and then explore useful directions for future studies.

6.2 Thesis summary

6.2.1 Dispersal in widespread fishes

My work highlighted the importance of the Andes mountain range in creating a strong, but penetrable barrier to dispersal between eastern and western Patagonian for widespread galaxiid fishes. The primary mechanism of dispersal between the east and west differs between *G. platei* and *G. maculatus*. The freshwater-limited species, *G. platei*, utilized a direct and previously overlooked route of trans-Andean dispersal which involved the redirection of entire drainage basins. As a diadromous fish, *G. maculatus* instead gained access to eastern Patagonia using its tolerance to marine environments and spread into adjacent drainages via ocean connections. Eventually, this resulted in a stepping-stone dispersal pattern around the southernmost tip of South America and into Atlantic draining basins of eastern Patagonia. However, some populations in the northernmost extent of its range were also influenced by drainage reversals and two of the most divergent lineages were swapped to either side of the Andes mountain range to complete what appears to be a full ring around the Andes. Multilocus estimates of cross-Andean gene flow in *G. platei* reaffirmed that drainage reversals were likely a bidirectional means of dispersal between eastern and western Patagonia aquatic species and was probably available on multiple occasions as climate conditions transitioned between glacial and inter-glacial conditions.

6.2.2 Glacial refugia

My work has also yielded several important insights into the likely locations of Quaternary aquatic glacial refugia both east and west of the Andes. Several authors have recognized the importance of the northern parts of Chilean Patagonia and south-central Chile as physically and climatically stable over long evolutionary time based on reports of high population- and species-level biodiversity (Dyer 2000; Premoli et al. 2000; Muellner et al. 2005; Ruzzante et al. 2006). My work suggests that this temperate area, located immediately north of the maximum positions of the Patagonian ice sheet, was also likely occupied by *G. platei* and *G. maculatus* during one or more glacial periods. Combined with phylogeographic evidence from another fish species [*Percichthys trucha* (Ruzzante et al. 2006)] and a crab [*Aegla alacalufi* (Xu et al. 2009)], this region appears to have served as a general refuge for several aquatic species in the past.

Perhaps most important, however, was the finding that *G. platei* likely survived one or more glacial advance at higher latitudes, or within “cryptic” refugia [as defined by Stewart et al. (2010)]. Genetic signal from *G. platei* suggests that a small ice-free refuge existed in the glaciated southwest in the area surrounding Lake Riesco. Corroborative genetic signal from the freshwater crab (*Aegla alacalufi*) and fossil pollen records from *Nothofagus* trees (pollen records) also suggests that Lake Riesco remained ice-free in the past as glacial refuge (Markgraf et al. 1996; Xu et al. 2009). Phylogeographic signal in *G. platei* also revealed further structure in eastern Patagonia that suggests additional glacial refugia. The eastern lineage of *G. platei* shows a strong sub-division in which separate southern lineage forms a zone of secondary contact at 47°S (Lake Pueyrredon/Cochrane) with a centrally located lineage. Both eastern lineages of *G. platei* are likely to have been separate cryptic refugia because of their south and central locations with respect to a northern refuge originally proposed by Ruzzante et al. (2006) to explain phylogeographic patterns in *P. trucha*.The presence of any eastern refugia in the past was unexpected because of the enhanced aridity of eastern Patagonia during glacial periods. However, recent work on Patagonian shingle formation (Martinez and Kutschker 2011), climate simulations (Compagnucci 2011) and sea-level variation (Ponce et al. 2011) suggest that the east was more hospitable to aquatic environments than previously thought.

The genetic structure of *G. maculatus* can also be explained by populations surviving in more than one refuge in eastern Patagonia. Control region sequence data revealed two strongly supported haplotype groups located in northern (Limay and Manso river systems) and southern (Santa Cruz river system) Argentine Patagonia. However, at this point, I am uncertain if this structure was created by the effects of isolation-by-distance, the repeated isolation into separate glacial refugia, or by a combined effect. More work is required to estimate the timing of range expansion of *G. maculatus* from Chile to Argentina and better establish the positions of this species throughout Patagonia in the past. But, considering the very similar demographic histories of *G. maculatus* and *G. platei* throughout the Pleistocene (Chapter 5), it is does seem likely that both species were likely to have shared similar glacial refugia.

6.3 Future work

Overall, my thesis provides a solid foundation concerning the degree to which Quaternary landscapes and climate have shaped the abundance, distribution and connectivity of two widespread fish species through time. This work has set the benchmark for phylogeographic studies in Patagonia and provides a very good starting-point from which we can continue to grow knowledge about the diversification of populations and species in Patagonia. In the next section I discuss some future directions and ideas worth pursuing to bring us closer to this goal.

6.3.1 Post-glacial dispersal in eastern Patagonian fish populations

One important uncertainty which remains in Patagonian fish phylogeography is the process(es) responsible for creating, or maintaining, genetic homogeneity among fish populations that are currently distributed throughout several isolated drainage basins. For example, the creole perch *P. trucha* reveals minimal structure over its entire distribution throughout Argentinean Patagonia (33-47^{o}S) according to control region mtDNA sequences (Ruzzante et al. 2006). One explanation is that large moraine-dammed proglacial lakes formed at the base of the eastern foothills as glaciers receded and joined otherwise isolated river headwaters under a common body of water (Ruzzante et al. 2006). For example, Lakes General Carrera/Buenos Aires and Cochrane represent "successor lakes" of a much larger proglacial lake that formed alongside the ice field following the Last Glacial (Bell 2008). Such a scenario would have allowed uninhibited mixing among drainages homogenization of genetic signal. Presumably, such conditions would also been available on multiple occasions with each glacial cycle. Rabassa et al. (2011), however, points out that this scenario is very unlikely considering that most eastern glaciers were of cirque formations and thus confined by deep valleys associated with the surrounding foothills. Therefore, any associated moraine-dammed proglacial lakes would have been under similar constraints and unable to facilitate dispersal on the scale implied by genetics. However, I argue that such constraints could have varied by latitude. More specifically, the southeastern fringes of the ice sheet (high latitudes) routinely extended outside of the influences of the foothills and terminated on the flat Steppe. It is possible, therefore, that proglacial lake influence could have been more important at higher latitudes. Although this point is moot for explaining the genetic patterns of the warm-adapted and northerly distributed species *P. trucha* (earlier example), other high latitude proglacial lakes could still have been important for cold-adapted fishes with more southerly distributions such as *G. platei* which also showed minimal structure over several degrees of latitude (i.e. 47-54^{o}S).

An alternative explanation that is gaining support is that fish dispersed via inter-drainage stream coalescence on an exposed continental shelf during lowered sea levels of glacial periods. This hypothesis gained support soon after Martinez and Kutschker (2011) suggested that river outflow in eastern Patagonia was likely to have remained high during multiple glacial advances. More specifically, work on the origin, distribution and formation of Patagonian shingles (gravels) throughout eastern Patagonia indicates that the fluvial energy responsible for depositing these gravels remained high for long periods and was asynchronous with the timing of glacial cycles. Stream outflow was instead determined by a host of factors (e.g. tectonics, sea-level, seasonality) that made it possible to maintain strong outflow throughout peak glacial advances. When combined with the idea that the surface area of the Patagonian Steppe roughly doubled during glacial periods (Ponce et al. 2011), it becomes very likely that adjacent drainages were connected during one or more glacial periods and may have facilitated north-south movements in aquatic organisms (Ruzzante et al. 2011).

Phylogeographic signal, using primarily mtDNA sequence data, has been useful thus far for investigating the relationships among independent glacial lineages. However, to investigate patterns within glacial lineages, it becomes necessary to consider faster evolving genomic regions of sufficient genetic resolution. A useful step would be to employ microsatellites or Single Nucleotide Polymorphisms (SNPs) to test between alternative hypotheses of post-glacial dispersal in freshwater fishes, i.e. proglacial lake versus stream coalescence, within the context of landscape genetics. Specifically, I would use Mantel correlograms to compare two models of dispersal between headwater locations occupied by a single glacial lineage (as defined by phylogenetics). To represent river coalescence points on an exposed shelf, I would reconstruct drainage patterns using RiverTools and apply a least-cost distance through these hypothesized river networks. The alternative, representing proglacial lakes, could be calculated by straight-line Euclidean distance between headwater locations. It would also be interesting to take this analysis a step further and consider if there are any latitudinal breaks in the support for each alternative, which may suggest that each mechanism was working in concert. Those interested should also consider consulting the following studies to help plan sample design and analyses: Amaud (2003) (original research article), Storfer et al. (2007) (review).

6.3.2 Biogeographic implications of drainage reversals

Another important unknown is the collection of factors that interact to make certain aquatic species more prone to cross-mountain dispersal than others. Many of the species ranges of freshwater fishes in Patagonia are restricted to only Pacific drainages (Dyer 2000; Baigun and Ferriz 2003). This pattern suggests that cross-Andean dispersal has not occurred in all species. What contributes to these idiosyncratic patterns of dispersal when similar mechanisms should be available to all aquatic organisms? Several authors suggest an ecological component whereby those species occupying high elevation streams and headwaters of tributaries should be more prone to river capture and other mechanisms of inter-drainage dispersal over mountain ridges (Slechtova et al. 2004; Burridge et al. 2006; Chiang et al. 2010). For Patagonian species, however, such a prediction does not always hold. *Galaxias platei* is consistently found in the high elevation headwaters of Patagonian tributaries and its phylogeographic structure is well explained by drainage reversals. However, *Aplochiton zebra*, another galaxiid species, is also found in the headwaters of some trans-Andean headwaters (Cussac et al. 2004) but is absent from Atlantic-draining systems. Undoubtedly, habitat preference will certainly play an important role in determining the availability of species to the influences of stream capture events, however, it does not appear to be the only factor and more work is needed to understand what other contributing factors are working to filter some species over others.

Several trans-Andean river systems in Patagonia are expected to have undergone drainage reversal(s) in the past. Physical evidence is available for the Baker system regarding its most recent diversion from Atlantic to Pacific during the melting of the Patagonian Ice Sheet following the Last Glacial Maximum (LGM) (Turner et al. 2005; Bell 2008). In Chapter 1 I also identified several systems based on the criterion of headwater position with respect to the Andes, i.e. located on opposite side of Andes with respect to the ocean in which they drain, and included: the Hua Hum, Puelo, Futaleufu, Palena, Baker, Pascua, Chico and Grande rivers. Furthermore, the phylogeographic signal from *G. platei* and *G. maculatus* (Chapters 2-4) indicated that the headwaters of the Hua Hum, Palena and Baker recently underwent oceanic reversals (<300 KY), and perhaps on more than one occasion. Future studies should concentrate on one or more of the aforementioned river basins to compare the genetic structure of several aquatic taxa from diverse backgrounds and ecologies (e.g. freshwater and diadromous fishes, crabs, insects, frogs, plants). Broader perspectives will be difficult because sample overlap is important; two (or more) geographic regions are the common units from which comparisons of genetic structure and gene flow among species are based. Therefore, within the context of studying drainage reversals, studies should aim to compare the biological contents of the headwaters of adjacent trans-Andean and Atlantic river drainages. Contrasting the phylogeographic histories of multiple co-distributed populations/species is a powerful way of precipitating regional trends to clarify the breadth of influence that historical processes had on shaping biogeographical regions (Kholodova 2009). At the same time, differences in phylogeographic histories is also useful for identifying novel responses to shared environmental changes and helps identify key biological characteristics that may benefit/disadvantage individuals when facing changing habitats (e.g. Ruzzante et al. 2008). Researchers are again advised to consider faster evolving regions considering how recent drainage reversals are expected to have occurred.

Finally, and in conjunction with genetic-based studies, future efforts should also consider collaborating with the appropriate geological expert to help characterize the physical history of any drainage system(s) of interest. Using independently dated river capture events Burridge et al. (2008) presents a very useful method for calibrating molecular clocks in New Zealand fishes. An accurately characterized and dated history of river capture events involving trans-Andean headwaters would make a similar approach feasible for Patagonian species. Some work has already been done concerning the most recent drainage reversal event involving the Baker system (Turner et al. 2005; Bell 2008). However, more work is required to elaborate on these, and other, events both spatially and temporally. This information would not only improve our understanding of the evolutionary history of species associated with specific drainages, but it would also provide a very useful molecular tool for reconstructing the evolutionary histories of similar species in other geographic regions and at other temporal scales.

6.4 Conclusion

My thesis represents a cohesive collection of phylogeographic studies that answers several questions concerning the diversification of aquatic species in Patagonia. The investigations of genetic structure and diversity of *G. maculatus* and *G. platei* were particularly useful for identifying the likely locations of Quaternary glacial refugia and mechanisms of inter-drainage dispersal used by the *Galaxias spp.* and perhaps other aquatic species. However, like any good scientific endeavor, my thesis raised more questions than I could answer. To help guide the efforts of future studies, I outlined two interesting examples, specifically: post-glacial dispersal in fishes and the biogeographic implications of drainage reversals. By no means is this list comprehensive. The examples are only provided to compliment the work of my thesis and provide further inspiration for developing a more cohesive picture of the forces shaping the beautiful and exciting biome of temperate South America.

REFERENCES

Aigo, J., V. Cussac, S. Peris, S. Ortubay, S. Gomez, H. Lopez, M. Gross, J. Barriga, and M. Battini. 2008. Distribution of introduced and native fish in Patagonia (Argentina): patterns and changes in fish assemblages. Rev. Fish. Biol. Fish. 18:387-408.

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle *in* B. N. Petrov, and F. Csaki, eds. Second International Symposium on Information Theory, Akademiai Kiado, Budapest.

Allen, G. R. 1989. Freshwater Fishes of Australia. T.H.F. Publications, Neptune City, NJ.

Allibone, R. M., and G. P. Wallis. 1993. Genetic variation and diadromy in some native New Zealand galaxiids (Teleostei: Galaxiidae). Biological Journal of the Linnean Society 50:19 - 33.

Allnutt, T. R., A. C. Newton, A. Lara, A. Premoli, J. J. Armesto, R. Vergara, and M. Gardner. 1999. Genetic variation in *Fitzroya cupressoides* (alerce), a threatened South American conifer. Molecular Ecology 8:975-987.

Allnutt, T. R., A. C. Newton, A. Premoli, and A. Lara. 2003. Genetic variation in the threatened South American conifer *Pilgerodendron uviferum* (Cupressaceae), detected using RAPD markers. Biological Conservation 114:245-253.

Amante, C., and B. W. Eakins. 2008. ETOPO1 1 Arc-Minute Global Relief Model: Procedures, Data Sources and Analysis. National Geophysical Data Center, NESDIS, NOAA, U.S. Department of Commerce, Boulder, CO.

Amaud, J. F. 2003. Metapopulation genetic structure and migration pathways in the land snail Helix aspersa: influence of landscape heterogeneity. Springer, Dordrecht, PAYS-BAS.

Arana, M. V., L. A. Gallo, G. G. Vendramin, M. J. Pastorino, F. Sebastiani, and P. Marchelli. 2010. High genetic variation in marginal fragmented populations at extreme climatic conditions of the Patagonian Cypress *Austrocedrus chilensis*. Molecular Phylogenetics and Evolution 54:941-949.

Avila, L. J., M. Morando, and J. W. Sites. 2006. Congeneric phylogeography: hypothesizing species limits and evolutionary processes in Patagonian lizards of the *Liolaemus boulengeri* group (Squamata : Liolaemini). Biological Journal of the Linnean Society 89:241-275.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489-522.

Azpilicueta, M. M., P. Marchelli, and L. A. Gallo. 2009. The effects of Quaternary glaciations in Patagonia as evidenced by chloroplast DNA phylogeography of Southern beech *Nothofagus obliqua*. Tree Genetics & Genomes 5:561-571.

Baigun, C., and R. Ferriz. 2003. Distribution patterns of native freshwater fishes in Patagonia (Argentina). Organisms Diversity & Evolution 3:151-159.

Bard, E., and R. E. M. Rickaby. 2009. Migration of the subtropical front as a modulator of glacial climate. Nature 460:380-U393.

Barker, J. R., and D. M. Lambert. 1988. A genetic analysis of populations of *Galaxias maculatus* from the Bay of Plenty: implications for natal river return. New Zealand Journal of Marine and Freshwater Research 22:321 - 326.

Barriga, P. Juan, Battini, A. Miguel, Macchi, J. Patricio, Milano, Daniela, Cussac, and E. Victor. 2002. Spatial and temporal distribution of landlocked Galaxias maculatus and Galaxias platei (Pisces: Galaxiidae) in a lake in the South American Andes. Scientific and Industrial Research Publishing, Wellington, NOUVELLE-ZELANDE.

Barriga, J. P., M. A. Battini, and V. E. Cussac. 2007. Annual dynamics variation of a landlocked Galaxias maculatus (Jenyns 1842) population in a Northern Patagonian river: occurrence of juvenile upstream migration. Journal of Applied Ichthyology 23:128-135.

Bazin, E., S. Glemin, and N. Galtier. 2006. Population size does not influence mitochondrial genetic diversity in animals. Science 312:570-572.

Becker, L. A., M. A. Pascual, and N. G. Basso. 2007. Colonization of the southern Patagonia Ocean by exotic chinook salmon. Conserv Biol 21:1347 - 1352.

Beerli, P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics 22:341-345.

Beerli, P. 2009. How to use migrate or why are markov chain monte carlo programs difficult to use? Pp. 42-79 *in* G. Bertorelle, M. W. Bruford, H. C. Hauffe, A. Rizzoli, and C. Vernesi, eds. Population Genetics for Animal Conservation. Cambridge University Press, Cambridge, UK.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences of the United States of America 98:4563-4568.

Beheregaray, L. B. 2008. Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. Molecular Ecology 17:3754-3774.

Bekessy, S. A., T. R. Allnutt, A. C. Premoli, A. Lara, R. A. Ennos, M. A. Burgman, M. Cortes, and A. C. Newton. 2002. Genetic variation in the vulnerable and endemic Monkey Puzzle tree, detected using RAPDs. Heredity 88:243-249.

Bekkevold, D., M. M. Hansen, and K. L. Mensberg. 2004. Genetic detection of sex-specific dispersal in historical and contemporary populations of anadromous brown trout *Salmo trutta*. Mol Ecol 13:1707 - 1712.

Bell, C. M. 2008. Punctuate drainage of an ice-dammed Quaternary lake in southern South America. Geografiska Annaler: Series A, Physical Geography 90:1-17.

Benn, D. I., and D. J. A. Evans. 1998. Glaciers & Glaciation. Oxford University Press, New York.

Berger, A. 1988. Milankovitch Theory and climate. Reviews of Geophysics 26:624-657.

Bernatchez, L., and C. C. Wilson. 1998. Comparative phylogeography of nearctic and palearctic fishes. Molecular Ecology 7:431-452.

Bernatchez, L., and C. C. Wilson. 1999. Diadromy and genetic diversity in Nearctic and Palearctic fishes: A reply. Molecular Ecology 8:529 - 530.

Berra, T. M., L. Crowley, W. Ivantsoff, and P. A. Fuerst. 1996. *Galaxias maculatus*: An explanation of its biogeography. Marine and Freshwater Research 47:845 - 849.

Bowen, B. W., A. Muss, L. A. Rocha, and W. S. Grant. 2006. Shallow mtDNA coalescence in Atlantic pygmy angelfishes (genus *Centropyge*) indicates a recent invasion from the Indian Ocean. Journal of Heredity 97:1-12.

Breitman, M. F., L. J. Avila, J. W. Sites Jr, and M. Morando. 2011. Lizards from the end of the world: Phylogenetic relationships of the *Liolaemus lineomaculatus* section (Squamata: Iguania: Liolaemini). Molecular Phylogenetics and Evolution In Press, Corrected Proof.

Brito, P., and S. Edwards. 2009. Multilocus phylogeography and phylogenetics using sequence-based markers. Genetica 135:439-455.

Brumfield, R. T., and S. V. Edwards. 2007. Evolution into and out of the Andes: A Bayesian analysis of historical diversification in *Thamnophilus* antshrikes. Evolution 61:346-367.

Burridge, C. P., D. Craw, D. Fletcher, and J. M. Waters. 2008. Geological dates and molecular rates: Fish DNA sheds light on time dependency. Molecular Biology and Evolution 25:624-633.

Burridge, C. P., D. Craw, and J. M. Waters. 2006. River capture, range expansion, and cladogenesis: The genetic signature of freshwater vicariance. Evolution 60:1038-1049.

Burridge, C. P., D. Craw, and J. M. Waters. 2007. An empirical test of freshwater vicariance via river capture. Molecular Ecology 16:1883-1895.

Caldenius, C. C. 1932. Las Glaciaciones cuaternarias en la Patagonia y Tierra del Fuego. Geografiska Annaler: Series A, Physical Geography 14:1-164.

Campos, H. 1973. Migration of galaxias maculatus (Jenyns) (galaxiidae, pisces) in Valdivia Estuary, Chile. Hydrobiologia 43:301-312.

Cano, J. M., H. S. Makinen, and J. Merila. 2008. Genetic evidence for male-biased dispersal in the three-spined stickleback (*Gasterosteus aculeatus*). Molecular Ecology 17:3234 - 3242.

Carling, M. D., and R. T. Brumfield. 2007. Gene sampling strategies for multi-locus population estimates of genetic diversity (*theta*). PLoS ONE 2:e160.

Carnaval, A. C., M. J. Hickerson, C. F. B. Haddad, M. T. Rodrigues, and C. Moritz. 2009. Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science 323:785 - 789.

Carstens, B. C., and L. L. Knowles. 2006. Variable nuclear markers for *Melanoplus oregonensis* identified from the screening of a genomic library. Molecular Ecology Notes 6:683 - 685.

Carstens, B. C., and L. L. Knowles. 2007. Shifting distributions and speciation: species divergence during rapid climate change. Molecular Ecology 16:619-627.

Carstens, B. C., H. N. Stoute, and N. M. Reid. 2009. An information-theoretical approach to phylogeography. Molecular Ecology 18:4270-4282.

Chapman, A., D. Morgan, S. Beatty, and H. Gill. 2006. Variation in Life History of Land-Locked Lacustrine and Riverine Populations of <i>Galaxias maculatus</i> (Jenyns 1842) in Western Australia. Environmental Biology of Fishes 77:21-37.

Chiang, T. Y., H. D. Lin, K. T. Shao, and K. C. Hsu. 2010. Multiple factors have shaped the phylogeography of Chinese spiny loach *Cobitis sinensis* in Taiwan as inferred from mitochondrial DNA variation. J. Fish Biol. 76:1173-1189.

Clapperton, C. M. 1993. The Glaciers and Glacial Landforms of South America. Quaternary Geology and Geomorphology of South America. Elsevier, Amsterdam, The Netherlands.

Compagnucci, R. 2011. Atmospheric circulation over Patagonia since the Jurassic to present: a review through proxy data and climatic modeling scenarios. Biological Journal of the Linnean Society Accepted.

Coronato, A., O. Martínez, J. Rabassa, J. Ehlers, and P. L. Gibbard. 2004. Glaciations in Argentine Patagonia, southern South America. Pp. 49-67. Developments in Quaternary Science. Elsevier.

Coronato, A. M. J., F. Coronato, E. Mazzoni, M. Vázquez, and J. Rabassa. 2008. The Physical Geography of Patagonia and Tierra del Fuego. Pp. 13-55. Developments in Quaternary Science. Elsevier.

Cosacov, A., A. N. Sersic, V. Sosa, L. A. Johnson, and A. A. Cocucci. 2010. Multiple periglacial refugia in the Patagonian steppe and post-glacial colonization of the Andes: the phylogeography of *Calceolaria polyrhiza*. Journal of Biogeography 37:1463-1477.

Craw, D., C. Burridge, L. Anderson, and J. M. Waters. 2007a. Late Quaternary river drainage and fish evolution, Southland, New Zealand. Geomorphology 84:98 - 110.

Craw, D., C. Burridge, and J. Waters. 2007b. Geological and biological evidence for drainage reorientation during uplift of alluvial basins, central Otago, New Zealand. N. Z. J. Geol. Geophys. 50:367-376.

Craw, D., C. P. Burridge, P. Upton, D. L. Rowe, and J. M. Waters. 2008. Evolution of biological dispersal corridors through a tectonically active mountain range in New Zealand. Journal of Biogeography 35:1790-1802.

Craw, D., and J. Waters. 2007. Geological and biological evidence for regional drainage reversal during lateral tectonic transport, Marlborough, New Zealand. J. Geol. Soc. 164:785-793.

Croizat, L., G. Nelson, and D. E. Rosen. 1974. Centers of origin and related concepts. Systematic Zoology 23:265-287.

Crowell, J. C., and L. A. Frakes. 1970. Phanerozoic glaciation and the causes of ice ages. Am J Sci 268:193-224.

Cussac, V., S. Ortubay, G. Iglesias, D. Milano, M. E. Lattuca, J. P. Barriga, M. Battini, and M. Gross. 2004. The distribution of South American galaxiid fishes: the role of biological traits and post-glacial history. Journal of Biogeography 31:103-121.

Dick, C. W., D. W. Roubik, K. F. Gruber, and E. Bermingham. 2004. Long-distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae : *Euglossini*) revealed by comparative mitochondrial DNA phylogeography. Molecular Ecology 13:3775-3785.

Diraison, M., P. R. Cobbold, D. Gapais, E. A. Rossello, and C. Le Corre. 2000. Cenozoic crustal thickening, wrenching and rifting in the foothills of the southernmost Andes. Tectonophysics 316:91-119.

Droxler, A. W., R. B. Alley, W. R. Howard, R. Z. Poore, and L. H. Burckle. 2003. Unique and Exceptionally Long interglacial marine isotope stage 11.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. Bmc Evolutionary Biology 7.

Drummond, A. J., A. Rambaut, B. Shapiro, and O. G. Pybus. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution 22:1185 - 1192.

Dyer, B. 2000. Systematic review and biogeography of the freshwater fishes of Chile. Estudios Oceanologicos 19:77-98.

Edwards, S., and P. Beerli. 2000. Perspective: Gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution 54:1839-1854.

Ehlers, J., and P. L. Gibbard. 2007. The extent and chronology of cenozoic global glaciation. Quaternary International 164-65:6-20.

Elphinstone, M. S., G. N. Hinten, M. J. Anderson, and C. J. Nock. 2003. An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. Mol. Ecol. Notes 3:317-320.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol. Bioinform. 1:47-50.

Felsenstein, J. 2006. Accuracy of coalescent likelihood estimates: Do we need more sites, more sequences, or more loci? Molecular Biology and Evolution 23:691-700.

Fraser, C. I., R. Nikula, and J. M. Waters. 2010. Oceanic rafting by a coastal community. Proceedings of the Royal Society B: Biological Sciences:*In Press*.

Glasser, N. F., K. N. Jansson, S. Harrison, and J. Kleman. 2008. The glacial geomorphology and Pleistocene history of South America between 38^{o}S and 56^{o}S. Quaternary Science Reviews 27:365-390.

Grant, W. S., and B. W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. Journal of Heredity 89:415-426.

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52:696 - 704.

Guindon, S., F. Lethiec, P. Duroux, and O. Gascuel. 2005. PHYML Online - a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res. 33:W557 - W559.

Habit, E., P. Piedra, D. E. Ruzzante, S. J. Walde, M. C. Belk, V. E. Cussac, J. Gonzalez, and N. Colin. 2010. Changes in the distribution of native fishes in response to introduced species and other anthropogenic effects. Glob. Ecol. Biogeogr. 19:697-710.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis software program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Hare, M. P. 2001. Prospects for nuclear gene phylogeography. Trends in Ecology & Evolution 16:700-706.

Harrison, S. P., and J. Dondson. 1993. Climates of Australia and New Guinea Since 18,000 yr B.P. Pp. 265-293 *in* H. E. Wright, J. E. Kutzback, T. Webb III, W. F. Ruddiman, F. A. Street-Perrott, and P. J. Bartlein, eds. Global Climates Since the Last Glacial Maximum. University of Minnesota Press, Minneapolis.

Heled, J., and A. Drummond. 2008. Bayesian inference of population size history from multiple loci. BMC Evolutionary Biology 8:289.

Heusser, C. J., T. V. Lowell, L. E. Heusser, A. Moreira, and S. Moreira. 2000. Pollen sequence from the Chilean Lake District during the Llanquihue glaciation in marine Oxygen isotope Stages 4-2. Journal of Quaternary Science 15:115-125.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907-913.

Hey, J., and R. Nielsen. 2004. Multilocus Methods for Estimating Population Sizes, Migration Rates and Divergence Time, With Applications to the Divergence of Drosophila pseudoobscura and D. persimilis. Genetics 167:747-760.

Hey, J., and R. Nielsen. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proceedings of the National Academy of Sciences of the United States of America 104:2785-2790.

Hilton, N., D. Sugden, A. Payne, and C. Clapperton. 1994. Glacier modeling and the climate of Patagonia during the last glacial maximum. Quaternary Research 42:1-19.

Himes, C. M. T., M. H. Gallardo, and G. J. Kenagy. 2008. Historical biogeography and post-glacial recolonization of South American temperate rain forest by the relictual marsupial *Dromiciops gliroides*. Journal of Biogeography 35:1415-1424.

Ho, S. Y. W., and G. Larson. 2006. Molecular clocks: when timesare a-changin'. Trends in Genetics 22:79-83.

Ho, S. Y. W., M. J. Phillips, A. Cooper, and A. J. Drummond. 2005. Time Dependency of Molecular Rate Estimates and Systematic Overestimation of Recent Divergence Times. Molecular Biology and Evolution 22:1561-1568.

Ho, S. Y. W., and B. Shapiro. 2011. Skyline-plot methods for estimating demographic history from nucleotide sequences. Molecular Ecology Resources In press.

Howard, W. R. 1997. Palaeoclimatology: A warm future in the past. Nature 388:418-419.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.

Hulton, N. R. J., R. S. Purves, R. D. McCulloch, D. E. Sugden, and M. J. Bentley. 2002. The Last Glacial Maximum and deglaciation in southern South America. Quaternary Science Reviews 21:233-241.

Hutchings, J. A., and L. Gerber. 2002. Sex-biased dispersal in a salmonid fish. Proc Biol Sci 269:2487 - 2493.

Iriondo, M. 2000. Patagonian dust in Antarctica. Quaternary International 68:83-86.

Jakob, S. S., E. Martinez-Meyer, and F. R. Blattner. 2009. Phylogeographic analyses and paleodistribution modeling indicate Pleistocene *in situ* survival of *Hordeum* species (Poaceae) in southern Patagonia without genetic or spatial restriction. Molecular Biology and Evolution 26:907-923.

Kaplan, M. R., C. J. Fogwill, D. E. Sugden, N. Hulton, P. W. Kubik, and S. Freeman. 2008. Southern Patagonian glacial chronology for the Last Glacial period and implications for Southern Ocean climate. Quaternary Science Reviews 27:284-294.

Kholodova, M. 2009. Comparative phylogeography: Molecular methods, ecological interpretation. Molecular Biology 43:847-854.

Kidd, D. M., and X. H. Liu. 2008. GEOPHYLOBUILDER 1.0: an ARCGIS extension for creating 'geophylogenies'. Molecular Ecology Resources 8:88-91.

Kim, I., C. J. Phillips, J. A. Monjeau, E. C. Birney, K. Noack, D. E. Pumo, R. S. Sikes, and J. A. Dole. 1998. Habitat islands, genetic diversity, and gene flow in a Patagonian rodent. Molecular Ecology 7:667-678.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120.

Kohfeld, K. E., and S. P. Harrison. 2001. DIRTMAP: the geological record of dust. Earth-Science Reviews 54:81-114.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, and R. Lopez. 2007. Clustal W and clustal X version 2.0. Bioinformatics 23:2947 - 2948.

Ledru, M. P., D. D. Rousseau, F. W. Cruz, C. Riccomini, I. Karmann, and L. Martin. 2005. Paleoclimate changes during the last 100,000 yr from a record in the Brazilian Atlantic rainforest region and interhemispheric comparison. Quaternary Research 64:444-450.

Lee, J. Y., and S. V. Edwards. 2008. Divergence across Australia's Carpentarian Barrier: Statistical phylogeography of the red-backed fairy wren (*Malurus melanocephalus*). Evolution 62:3117-3134.

Lemmen, D. S., A. Dukrodkin, and J. M. Bednarski. 1994. Late-glacial drainage systems along the northwestern margin of the Laurentide Ice Sheet. Quaternary Science Reviews 13:805-828.

Lessa, E. P., J. A. Cook, and J. L. Patton. 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. Proceedings of the National Academy of Sciences of the United States of America 100:10331 - 10334.

Lessa, E. P., G. D’ElÍA, and U. F. J. PardiÑAs. 2010. Genetic footprints of late Quaternary climate change in the diversity of Patagonian-Fueguian rodents. Molecular Ecology 19:3031-3037.

Li, C., J.-J. Riethoven, and L. Ma. 2010. Exon-primed intron-crossing (EPIC) markers for non-model teleost fishes. BMC Evolutionary Biology 10:90.

Lisiecki, L. E., and M. E. Raymo. 2005. A Pliocene-Pleistocene stack of 57 globally distributed benthic delta O-18 records. Paleoceanography 20.

Lisiecki, L. E., and M. E. Raymo. 2007. Plio-Pleistocene climate evolution: trends and transitions in glacial cycle dynamics. Quaternary Science Reviews 26:56-69.

Lopez-Gamundi, O. R., and L. A. Buatois. 2010. Introduction: Late Paleozoic glacial events and postglacial transgressions in Gondwana. Geological Society of America Special Papers 468:v-viii.

Maddison, W. P., and L. L. Knowles. 2006. Inferring phylogeny despite incomplete lineage sorting. Syst. Biol. 55:21-30.

Mancini, M. V., A. R. Prieto, M. M. Paez, F. Schäbitz, and J. Rabassa. 2008. Late Quaternary Vegetation and Climate of Patagonia. Pp. 351-367. Developments in Quaternary Science. Elsevier.

Mangerud, J., M. Jakobsson, H. Alexanderson, V. Astakhov, G. K. C. Clarke, M. Henriksen, C. Hjort, G. Krinner, J. P. Lunkka, P. Moller, A. Murray, O. Nikolskaya, M. Saarnisto, and J. I. Svendsen. 2004. Ice-dammed lakes and rerouting of the drainage of northern Eurasia during the Last Glaciation. Quaternary Science Reviews 23:1313-1332.

Marchelli, P., and L. A. Gallo. 2004. The combined role of glaciation and hybridization in shaping the distribution of genetic variation in a Patagonian southern beech. Journal of Biogeography 31:451-460.

Marchelli, P., and G. Leonardo. 2006. Multiple ice-age refugia in a southern beech of South America as evidenced by chloroplast DNA markers. Conservation Genetics 7:591-603.

Markgraf, V. 1987. Paleoenvironmental changes at the northern limit of the sub-antarctic *Nothofagus* forest, Lat. 37°S, Argentina. Quaternary Research 28:119-129.

Markgraf, V. 1989. Southern Westerlies during the Last Glacial Maximum - reply. Quaternary Research 31:426-432.

Markgraf, V. 1993. Climatic History of Central and South America since 18,000 yr B.P.: Comparison of Pollen Records and Model Simulations. Pp. 357-385 *in* H. E. Wright, J. E. Kutzback, T. Webb III, W. F. Ruddiman, F. A. Street-Perrott, and P. J. Bartlein, eds. Global climates since the last glacial maximum. University of Minnesota Press, Minneapolis.

Markgraf, V., M. McGlone, and G. Hope. 1995. Neogene paleoenvironmental and paleoclimatic change in southern temperate ecosystems: a southern perspective. Trends in Ecology & Evolution 10:143-147.

Markgraf, V., E. Romero, and C. Villagran. 1996. History and Paleoecology of South American *Nothofagus* Forests. Pp. 354-386 *in* T. T. Veblen, R. S. Hill, and J. Reed, eds. The Ecology and Biogeography of *Nothofagus* Forests. Yale University Press, New Haven.

Martin, A. P., and S. R. Palumbi. 1993. Body size, metabolic rate, generation time, and the molecular clock. Proceedings of the National Academy of Sciences 90:4087-4091.

Martin, D., and E. Rybicki. 2000. RDP: Detection of recombination amongst aligned sequences. Bioinformatics (Oxford) 16:562 - 563.

Martinez, O., and A. Kutschker. 2011. The "rodados Patagonicos" (Patagonian shingle formation) of eastern Patagonia: environmental conditions of gravel sedimentation. Biological Journal of the Linnean Society Accepted.

Mathiasen, P., and A. C. Premoli. 2010. Out in the cold: genetic variation of *Nothofagus pumilio* (Nothofagaceae) provides evidence for latitudinally distinct evolutionary histories in austral South America. Molecular Ecology 19:371-385.

McCulloch, R. D., M. J. Bentley, R. S. Purves, N. R. J. Hulton, D. E. Sugden, and C. M. Clapperton. 2000. Climatic inferences from glacial and palaeoecological evidence at the last glacial termination, southern South America. Journal of Quaternary Science 15:409-417.

McCusker, M. R., and P. Bentzen. 2010. Positive relationships between genetic diversity and abundance in fishes. Molecular Ecology 19:4852-4862.

McDowall, R. M. 1968. Galaxias maculatus (Jenyns) the New Zealand Whitebait. Fisheries Research Division, Marine Department, Wellington, NZ.

McDowall, R. M. 1969. Relationships of galaxioid fishes with a further discussion of salmoniform classification. Copeia:796 - &.

McDowall, R. M. 1970. The galaxiid fishes of New Zealand. Bulletin of the Museum of Comparative Zoology 139:341 - 432.

McDowall, R. M. 1971a. Galaxiid fishes of South-America. Zool. J. Linn. Soc. 50:33-73.

McDowall, R. M. 1971b. The galaxiid fishes of South America. Zool. J. Linn. Soc. 50:33 - 73.

McDowall, R. M. 1978. Generalized tracks and dispersal in biogeography. Systematic Zoology 27:88 - 104.

McDowall, R. M. 1999. Diadromy and genetic diversity in Nearctic and Palearctic fishes. Molecular Ecology 8:527 - 528.

McDowall, R. M. 2000. Biogeography of the southern cool-temperate galaxioid fishes: evidence from metazoan macroparasite faunas. Journal of Biogeography 27:1221 - 1229.

McDowall, R. M. 2006. Crying wolf, crying foul, or crying shame: alien salmonids and a biodiversity crisis in the southern cool-temperate galaxioid fishes? Rev. Fish. Biol. Fish. 16:233-422.

McDowall, R. M. 2007. On amphidromy, a distinct form of diadromy in aquatic organisms. Fish and Fisheries 8:1 - 13.

Mercer, J. H. 1976. Glacial history of southernmost South America. Quaternary Research 6:125-166.

Mercer, J. H. 1983. Cenozoic Glaciation in the Southern Hemisphere. Annual Review of Earth and Planetary Sciences 11:99-132.

Meserve, P. L., and D. A. Kelt. 1990. The role of aridity and isolation on central Chilean small mammals: a reply. Journal of Biogeography 17:681-684.

Milano, D., V. E. Cussac, P. J. Macchi, D. E. Ruzzante, M. F. Alonso, P. H. Vigliano, and M. A. Denegri. 2002. Predator associated morphology in *Galaxias platei* in Patagonian lakes. J. Fish Biol. 61:138-156.

Milano, D., D. E. Ruzzante, V. E. Cussac, P. J. Macchi, R. A. Ferriz, J. P. Barriga, J. C. Aigo, M. E. Lattuca, and S. J. Walde. 2006. Latitudinal and ecological correlates of morphological variation in *Galaxias platei* (Pisces, Galaxiidae) in Patagonia. Biological Journal of the Linnean Society 87:69-82.

Morando, M., L. J. Avila, J. Baker, and J. W. Sites. 2004. Phylogeny and phylogeography of the *Liolaemus darwinii* complex (Squamata : Liolaemidae): Evidence for introgression and incomplete lineage sorting. Evolution 58:842-861.

Morando, M., L. J. Avila, C. R. Turner, and J. W. Sites. 2007. Molecular evidence for a species complex in the patagonian lizard *Liolaemus bibronii* and phylogeography of the closely related *Liolaemus gracilis* (Squamata : Liolaemini). Molecular Phylogenetics and Evolution 43:952-973.

Muellner, A. N., K. Tremetsberger, T. Stuessy, and C. M. Baeza. 2005. Pleistocene refugia and recolonization routes in the southern Andes: insights from *Hypochaeris palustris* (Asteraceae, Lactuceae). Molecular Ecology 14:203-212.

Nichols, R. 2001. Gene trees and species trees are not the same. Trends in Ecology & Evolution 16:358-364.

Nielsen, J. L., D. Tupper, and W. K. Thomas. 1994. Mitochondrial DNA polymorhpism in unique runs of chinook salmon (*Oncorhynchus tshawytscha*) from the Sacramento San Joaquin river basin. Conserv. Biol. 8:882-884.

Noonan, B. P., and K. P. Wray. 2006. Neotropical diversification: the effects of a complex history on diversity within the poison frog genus *Dendrobates*. Journal of Biogeography 33:1007-1020.

Olson, S. L., and P. J. Hearty. 2009. A sustained +21 m sea-level highstand during MIS 11 (400 ka): direct fossil and sedimentary evidence from Bermuda. Quaternary Science Reviews 28:271-285.

Ortlieb, L., A. Diaz, and N. Guzman. 1996. A warm interglacial episode during oxygen isotope stage 11 in northern Chile. Quaternary Science Reviews 15:857-871.

Palma, R. E., E. Rivera-Milla, J. Salazar-Bravo, F. Torres-Perez, U. F. J. Pardinas, P. A. Marquet, A. E. Spotorno, A. P. Meynard, and T. L. Yates. 2005. Phylogeography of *Oligoryzomys longicaudatus* (Rodentia : Sigmodontinae) in temperate South America. Journal of Mammalogy 86:191-200.

Palumbi, S. R., F. Cipriano, and M. P. Hare. 2001. Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. Evolution; international journal of organic evolution 55:859-868.

Pannell, J. R. 2003. Coalescence in a metapopulation with recurrent local extinction and recolonization. Evolution 57:949-961.

Pascual, M. A., V. Cussac, B. Dyer, D. Soto, P. Vigliano, S. Ortubay, and P. Macchi. 2007. Freshwater fishes of Patagonia in the 21st Century after a hundred years of human settlement, species introductions, and environmental change. Aquatic Ecosystem Health & Management 10:212-227.

Pastorino, M. J., and L. A. Gallo. 2002. Quaternary evolutionary history of *Austrocedrus chilensis*, a cypress native to the Andean-Patagonian forest. Journal of Biogeography 29:1167-1178.

Pastorino, M. J., and L. A. Gallo. 2009. Preliminary operational genetic management units of a highly fragmented forest tree species of southern South America. Forest Ecology and Management 257:2350-2358.

Pastorino, M. J., L. A. Gallo, and H. H. Hattemer. 2004. Genetic variation in natural populations of *Austrocedrus chilensis*, a cypress of the Andean-Patagonian Forest. Biochemical Systematics and Ecology 32:993-1008.

Pastorino, M. J., P. Marchelli, M. Milleron, C. Soliani, and L. A. Gallo. 2009. The effect of different glaciation patterns over the current genetic structure of the southern beech *Nothofagus antarctica*. Genetica 136:79-88.

Pearson, O. P., and M. F. Smith. 1999. Genetic similarity between *Akodon olivaceus* and *Akodon xanthorhinus* (Rodentia : Muridae) in Argentina. Journal of Zoology 247:43-52.

Perez-Losada, M., G. Bond-Buckup, C. G. Jara, and K. A. Crandall. 2004. Molecular systematics and biogeography of the southern South American freshwater "crabs" *Aegla* (Decapoda : Anomura : Aeglidae) using multiple heuristic tree search approaches. Syst. Biol. 53:767-780.

Perrin, N., and V. Mazalov. 2000. Local competition, inbreeding, and the evolution of sex-biased dispersal. American Naturalist 155:116 - 127.

Pluzhnikov, A., and P. Donnelly. 1996. Optimal sequencing strategies for surveying molecular genetic diversity. Genetics 144:1247-1262.

Pollard, D. A. 1971. The biology of a landlocked form of the normally catadromous salmoniform fish Galaxias maculatus (Jenyns). I. Life cycle and origin. Marine and Freshwater Research 22:91-124.

Ponce, J., J. Rabassa, A. Coronato, and A. Borromei. 2011. Paleogeographic evolution of the Atlantic coast of Pama and Patagonia since the Last Glacial maximum to the Middle Holocene. Biological Journal of the Linnean Society Accepted.

Posada, D., and K. A. Crandall. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817-818.

Premoli, A. C., T. Kitzberger, and T. T. Veblen. 2000. Isozyme variation and recent biogeographical history of the long-lived conifer *Fitzroya cupressoides*. Journal of Biogeography 27:251-260.

Quiroga, M. P., and A. C. Premoli. 2010. Genetic structure of *Podocarpus nubigena* (Podocarpaceae) provides evidence of Quaternary and ancient historical events. Palaeogeography Palaeoclimatology Palaeoecology 285:186-193.

Rabassa, J. 2008. Late Cenozoic Glaciations in Patagonia and Tierra del Fuego. Pp. 151-204 *in* J. Rabassa, ed. Developments in Quaternary Science. Elsevier.

Rabassa, J., A. Coronato, and O. Martinez. 2011. Late Cenozoic glaciations in Patagonia and Tierra del Fuego: an updated review. Biological Journal of the Linnean Society Accepted.

Rabassa, J., A. M. Coronato, and M. Salemme. 2005. Chronology of the Late Cenozoic Patagonian and their correlation with biostratigraphic glaciations units of the Pampean region (Argentina). J. South Am. Earth Sci. 20:81-103.

Rabineau, M., S. Berné, J.-L. Olivet, D. Aslanian, F. Guillocheau, and P. Joseph. 2006. Paleo sea levels reconsidered from direct observation of paleoshoreline position during Glacial Maxima (for the last 500,000 yr). Earth and Planetary Science Letters 252:119-137.

Ramos, V. A. 1989. The birth of South America. Am. Scientist 77:444-450.

Ringuelet, R. A. 1955. Vinculaciones faunísticas de la zona boscosa del Nahuel Huapi y el dominio zoogeográfico austral cordillerano. Ministerio de Educación de la Nación, Universidad Nacional de Eva Perón (Universidad Nacional de La Plata) Facultad de Ciencias NAturales y Museo Tomo XVIII.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Rosen, D. E. 1974. Phylogeny and zoogeography of salmoniform fishes and relationships of *Lepidogalaxias salmandroides*. Bulletin of the American Museum of Natural History 153:265 - 326.

Rosen, D. E. 1978. Vicariant patterns and historical explanations of biogeography. Systematic Zoology 27:159-188.

Rozas, J., and R. Rozas. 1999. DnaSP version 3: An integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174 - 175.

Ruzzante, D., S. Walde, P. Macchi, M. Alonso, and J. Barriga. 2011. Phylogeography and phenotypic diversification in the Patagonian fish *Percichthys trucha*: the roles of Quaternary glacial cycles and natural selection. Biological Journal of the Linnean Society Accepted.

Ruzzante, D. E., S. J. Walde, V. E. Cussac, M. L. Dalebout, J. Seibert, S. Ortubay, and E. Habit. 2006. Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism. Molecular Ecology 15:2949-2968.

Ruzzante, D. E., S. J. Walde, J. C. Gosse, V. E. Cussac, E. Habit, T. S. Zemlak, and E. D. M. Adams. 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. Molecular Ecology 17:2234-2244.

Sambrook, J., and D. W. Russel. 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

Sang, T. K., H. Y. Chang, C. T. Chen, and C. F. Hui. 1994. Population structure of the Japanese eel, *Anguilla japonica*. Molecular Biology and Evolution 11:250-260.

Sersic, A., A. Cosacov, A. A. Cocucci, L. A. Johnson, R. Pozner, L. J. Avila, and J. W. Sites Jr. 2011. Emerging phylogeographic patterns of plants and terrestrial vertebrates from Patagonia. Biological Journal of the Linnean Society Accepted.

Sidlauskas, B., G. Ganapathy, E. Hazkani-Covo, K. P. Jenkins, H. Lapp, L. W. McCall, S. Price, R. Scherle, P. A. Spaeth, and D. M. Kidd. 2010. Linking big: the continuing promise of evolutionary synthesis. Evolution 64:871-880.

Singer, B. S., R. P. Ackert, and H. Guillou. 2004. Ar-40/Ar-19 and K-Ar chronology of Pleistocene glaciations in Patagonia. Geological Society of America Bulletin 116:434-450.

Slechtova, V., J. Bohlen, J. Freyhof, H. Persat, and G. B. Delmastro. 2004. The Alps as a barrier to dispersal in cold-adapted freshwater fishes? Phylogeographic history and taxonomic status of the bullhead in the Adriatic freshwater drainage. Molecular Phylogenetics and Evolution 33:225-239.

Smith, M. F., D. A. Kelt, and J. L. Patton. 2001. Testing models of diversification in mice in the *Abrothrix olivaceus/xanthorhinus* complex in Chile and Argentina. Molecular Ecology 10:397-405.

Smith, S. A., and E. Bermingham. 2005. The biogeography of lower Mesoamerican freshwater fishes. Journal of Biogeography 32:1835-1854.

Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos, and P. S. Soltis. 2006. Comparative phylogeography of unglaciated eastern North America. Molecular Ecology 15:4261-4293.

Stephens, M., N. J. Smith, and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. American Journal of Human Genetics 68:978 - 989.

Stewart, J. R., A. M. Lister, I. Barnes, and L. Dalen. 2010. Refugia revisited: individualistic responses of species in space and time. Proceedings of the Royal Society B-Biological Sciences 277:661-671.

Storfer, A., M. A. Murphy, J. S. Evans, C. S. Goldberg, S. Robinson, S. F. Spear, R. Dezzani, E. Delmelle, L. Vierling, and L. P. Waits. 2006. Putting the `landscape' in landscape genetics. Heredity 98:128-142.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599.

Tatur, A., R. del Valle, M. M. Bianchi, V. Outes, G. Villarosa, J. Niegodzisz, and G. Debaene. 2002. Late Pleistocene palaeolakes in the Andean and Extra-Andean Patagonia at mid-latitudes of South America. Quaternary International 89:135-150.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Tremetsberger, K., E. Urtubey, A. Terrab, C. M. Baeza, M. A. Ortiz, M. Talavera, C. Konig, E. M. Temsch, G. Kohl, S. Talavera, and T. F. Stuessy. 2009. Pleistocene refugia and polytopic replacement of diploids by tetraploids in the Patagonian and Subantarctic plant *Hypochaeris incana* (Asteraceae, Cichorieae). Molecular Ecology 18:3668-3682.

Turner, K. J., C. J. Fogwill, R. D. McCulloch, and D. E. Sugden. 2005. Deglaciation of the eastern flank of the North Patagonian Icefield and associated continental-scale lake diversions. Geogr. Ann. Ser. A-Phys. Geogr. 87A:363-374.

Tzedakis, P. C., D. Raynaud, J. F. McManus, A. Berger, V. Brovkin, and T. Kiefer. 2009. Interglacial diversity. Nature Geosci 2:751-755.

Victoriano, P. F., J. C. Ortiz, E. Benavides, B. J. Adams, and J. W. Sites. 2008. Comparative phylogeography of codistributed species of *Chilean Liolaemus* (Squamata : Tropiduridae) from the central-southern Andean range. Molecular Ecology 17:2397-2416.

Wainer, I., G. Clauzet, M. P. Ledru, E. Brady, and B. Otto-Bliesner. 2005. Last Glacial Maximum in South America: Paleoclimate proxies and model results. Geophysical Research Letters 32.

Wakeley, J., and J. Hey. 1997. Estimating ancestral population parameters. Genetics 145:847-855.

Wang, J. P., H. D. Lin, S. Huang, C. H. Pan, X. L. Chen, and T. Y. Chiang. 2004. Phylogeography of *Varicorhinus barbatulus* (Cyprinidae) in Taiwan based on nucleotide variation of mtDNA and allozymes. Molecular Phylogenetics and Evolution 31:1143-1156.

Ward, R. D., M. Woodwark, and D. O. F. Skibinski. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J. Fish Biol. 44:213-232.

Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last, and P. D. N. Hebert. 2005. DNA barcoding Australia's fish species. Philos. Trans. R. Soc. B-Biol. Sci. 360:1847-1857.

Waters, J. M., R. M. Allibone, and G. P. Wallis. 2006. Geological subsidence, river capture, and cladogenesis of galaxiid fish lineages in central New Zealand. Biological Journal of the Linnean Society 88:367-376.

Waters, J. M., and C. P. Burridge. 1999. Extreme intraspecific mitochondrial DNA sequence divergence in *Galaxias maculatus* (Osteichthys : Galaxiidae). One of the world's most widespread freshwater fish. Molecular Phylogenetics and Evolution 11:1 - 12.

Waters, J. M., D. Craw, J. H. Youngson, and G. P. Wallis. 2001. Genes meet geology: Fish phylogeographic pattern reflects ancient, rather than modern, drainage connections. Evolution 55:1844-1851.

Waters, J. M., L. H. Dijkstra, and G. P. Wallis. 2000a. Biogeography of a southern hemisphere freshwater fish: how important is marine dispersal? Molecular Ecology 9:1815 - 1821.

Waters, J. M., J. A. Lopez, and G. P. Wallis. 2000b. Molecular phylogenetics and biogeography of galaxiid fishes (Osteichthyes : Galaxiidae): Dispersal, vicariance, and the position of *Lepidogalaxias salamandroides*. Syst. Biol. 49:777 - 795.

Waters, J. M., D. L. Rowe, S. Apte, T. M. King, G. P. Wallis, L. Anderson, R. J. Norris, D. Craw, and C. P. Burridge. 2007. Geological dates and molecular rates: Rapid divergence of rivers and their biotas. Syst. Biol. 56:271 - 282.

Waters, J. M., and G. P. Wallis. 2000. Across the Southern Alps by river capture? Freshwater fish phylogeography in South Island, New Zealand. Molecular Ecology 9:1577-1582.

Waters, J. M., and G. P. Wallis. 2001. Cladogenesis and loss of the marine life-history phase in freshwater galaxiid fishes (Osmeriformes : Galaxiidae). Evolution 55:587 - 597.

Xu, J. W., M. Perez-Losada, C. G. Jara, and K. A. Crandall. 2009. Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufi* in Chilean Patagonia. Molecular Ecology 18:904-918.

Zattara, E. E., and A. C. Premoli. 2005. Genetic structuring in Andean landlocked populations of *Galaxias maculatus*: effects of biogeographic history. Journal of Biogeography 32:5 - 14.

Zemlak, T. S., E. M. Habit, S. J. Walde, M. A. Battini, E. D. M. Adams, and D. E. Ruzzante. 2008. Across the southern Andes on fin: glacial refugia, drainage reversals and a secondary contact zone revealed by the phylogeographical signal of *Galaxias platei* in Patagonia. Molecular Ecology 17:5049-5061.

Zemlak, T. S., E. M. Habit, S. J. Walde, C. Carrea, and D. E. Ruzzante. 2010. Surviving historical Patagonian landscapes and climate: molecular insights from *Galaxias maculatus*. BMC Evolutionary Biology 10.

Zhan, A. B., C. Li, and J. Z. Fu. 2009. Big mountains but small barriers: Population genetic structure of the Chinese wood frog (*Rana chensinensis*) in the Tsinling and Daba Mountain region of northern China. Bmc Genetics 10.

Zhang, D.-X., and G. M. Hewitt. 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Molecular Ecology 12:563-584.

APPENDIX

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Appendix 2.1 Frequency of *Galaxias platei* mitochondrial control haplotypes by collection location.

Location	L Rinihue	L Llanquihue	R Cudil	L Risopatron	L Las Torres	L Los Palos	L Escondida	L Riesco	L Alta	L Thompson	L Azul	L Jeinimeni	L Pollux	L G Carrera / B Aires	L Espejo	L Puelo	L Rivadavia	L La Plata	L Cochrane / Pueyrredon	L Belgrano	L O'Higgins / S Martin	L Viedma	L Yehuin	L Escondido	L Margarita	**Total**
hap-1											4				8	9	4									25
hap-2							1																			1
hap-3					1																					1
hap-4					5	1	8			10	5	1	10	2			4	7	2							55
hap-5																	1		1							2
hap-6														1												1
hap-7															2											2
hap-8					2																					2
hap-9																1										1
hap-10																				7						7
hap-11																				1						1
hap-12																				2						2
hap-13																								1		1
hap-14														1												1
hap-15																					1	1	6	6	2	16
hap-16														6					2							8
hap-17																							1	2		3
hap-18														1												1
hap-19														1												1
hap-20				15				2	1																	18
hap-21				1																						1
hap-22		6				2		6																		14
hap-23						1																				1
hap-31					1																					1
hap-32	1	4																								5
hap-33		1																								1
hap-34		1																								1
hap-36			1																							1
hap-37			1																							1
hap-42			12																							12
hap-43											1								1							2
hap-44																			5							5
hap-45																			1							1
hap-46																	1									1
hap-47																						1				1
hap-48																						1				1
hap-49												1														1
hap-50												1														1
hap-51		1																								1
hap-52				1																						1
hap-53						1																				1
hap-54		1																								1
hap-55														1												1
hap-56														1												1
hap-57														1												1
hap-58														1												1
hap-59														1												1
hap-60																						1				1
hap-61														1												1
hap-62	1																									1
Total	2	14	14	17	9	5	9	8	1	10	10	3	10	18	10	10	10	7	12	10	1	4	7	9	2	212

Appendix 3.1 Maximum likelihood analysis of all mtDNA control region haplotypes from the present study and a subset of homologous haplotypes derived from New Zealand and Tasmanian populations of *Galaxias maculatus*.

Appendix 3.2 Maximum likelihood analysis of ~150 haplotypes derived from Tasmanian and New Zealand origins using partial isolates of control region sequences.

Phylogenetic analyses presented in Appendix 3.1 and 3.2 were conducted using partial isolates of control region sequences from Waters et al. (2000), GenBank accession numbers AF240184-AF240339, to justify the use of a single sequence as an outgroup. The files are in Newick format and can be obtained from the following link:

http://www.biomedcentral.com/1471-2148/10/67/additional/

Appendix 3.3. Geographic distances (km) between regional collection locations used in Mantel tests (see Table 3.1). Distances above diagonal are direct distances between regions and represent a trans-Andean dispersal model. Below the diagonal are distances measured assuming a marine-mediate route of dispersal (Figure 3.7).

[redacted]	**NW**	**W1**	**E**	**W2**	**SE**
NW	-	669	647	1253	1852
W1	782	-	155	602	1194
E	5600	4818	-	630	1212
W2	1523	741	4077	-	640
SE	3679	2897	1921	2156	-

Appendix 3.4 Frequency of *Galaxias maculatus* mitochondrial control region haplotypes by collection location.

	Estero Topocalma	Estero Nilahue	Estero Reloca	Rio Tolten	Rio Queule	Rio Lingue	Lago Calafquen	Lago Panguipulli	Lago Rinihue	Lago Neltume	Rio Valdivia	Rio Bueno	Rio Contaco	Lago Rupanco	Lago Llanquihue	Rio Paredes	Rio Maullin	Lago Huillinco	Lago Tarahuin	Lago Natri	Rio Blanco	Rio Negro	Rio Yelcho	Lago Yelcho	Rio Palena	Rio Cisnes	Lago Quillen	Lago Espejo	Lago Morenito	Lago Hess	Lago Martin	Lago Steffen	Rio Chico	Lago Argentino	Lago Roca	Beagle Channel	**Total**
Hap-1																																				1	**1**
Hap-2					1																																**1**
Hap-3															1										1												**2**
Hap-4																					1																**1**
Hap-5																																				1	**1**
Hap-6					1																																**1**
Hap-7																																				1	**1**
Hap-8													1																								**1**
Hap-9																					1																**1**
Hap-10																					1																**1**
Hap-11					1																																**1**
Hap-12																						1															**1**
Hap-13						1																															**1**
Hap-14																						1															**1**
Hap-15																						1															**1**
Hap-16																		1																			**1**
Hap-17																					1																**1**
Hap-18																										1											**1**
Hap-19																										1											**1**
Hap-20																						1															**1**
Hap-21						1																															**1**
Hap-22																1																					**1**
Hap-23															1																						**1**
Hap-24													1																								**1**
Hap-25																															1						**1**
Hap-26																																1					**1**
Hap-27																																1					**1**
Hap-28																														1							**1**
Hap-29																															1						**1**
Hap-30																																1					**1**
Hap-31																													1								**1**
Hap-32																																1					**1**
Hap-33																														1							**1**
Hap-34																														1		1					**2**
Hap-35																																1					**1**
Hap-36																														1							**1**
Hap-37																														1							**1**
Hap-38																													1								**1**
Hap-39																											2										**2**
Hap-40																											1										**1**
Hap-41																											1										**1**
Hap-42																											1										**1**
Hap-43																											1										**1**
Hap-44																											1										**1**
Hap-45																															2						**2**
Hap-46																														1							**1**
Hap-47																												1									**1**
Hap-48																												1									**1**
Hap-49																															1						**1**
Hap-50																												1									**1**
Hap-51																												1		1	3	2					**7**
Hap-52																																1					**1**
Hap-53																												1									**1**
Hap-54																														1							**1**
Hap-55																																1					**1**
Hap-56																																1					**1**
Hap-57																												1									**1**
Hap-58																												1									**1**
Hap-59																												1									**1**
Hap-60																														1							**1**
Hap-61																															1						**1**
Hap-62																																1					**1**
Hap-63																																1					**1**
Hap-64																												1									**1**
Hap-65															1																						**1**
Hap-66																								1													**1**
Hap-67																																	1				**1**
Hap-68																							1														**1**
Hap-69																							1														**1**
Hap-70															1																						**1**

Appendix 3.4 continued

	Estero Topocalma	Estero Nilahue	Estero Reloca	Rio Tolten	Rio Queule	Rio Lingue	Lago Calafquen	Lago Panguipulli	Lago Rinihue	Lago Neltume	Rio Valdivia	Rio Bueno	Rio Contaco	Lago Rupanco	Lago Llanquihue	Rio Paredes	Rio Maullin	Lago Huillinco	Lago Tarahuin	Lago Natri	Rio Blanco	Rio Negro	Rio Yelcho	Lago Yelcho	Rio Palena	Rio Cisnes	Lago Quillen	Lago Espejo	Lago Morenito	Lago Hess	Lago Martin	Lago Steffen	Rio Chico	Lago Argentino	Lago Roca	Beagle Channel	**Total**
Hap-71							1																														**1**
Hap-72							1																														**1**
Hap-73							1																														**1**
Hap-74									1																												**1**
Hap-75									1																												**1**
Hap-76							1																														**1**
Hap-77								1																													**1**
Hap-78									1																												**1**
Hap-79										1																											**1**
Hap-80								1																													**1**
Hap-81							1																														**1**
Hap-82							1																														**1**
Hap-83								1																													**1**
Hap-84								1																													**1**
Hap-85									1																												**1**
Hap-86							1																														**1**
Hap-87							1																														**1**
Hap-88									1																												**1**
Hap-89										1																											**1**
Hap-90								1																													**1**
Hap-91									1																												**1**
Hap-92										1																											**1**
Hap-93							1																														**1**
Hap-94										2																											**2**
Hap-95										1																											**1**
Hap-96										1																											**1**
Hap-97							1																														**1**
Hap-98										1																											**1**
Hap-99										1																											**1**
Hap-100										1																											**1**
Hap-101						1																															**1**
Hap-102																					1																**1**
Hap-103																																			1		**1**
Hap-104																																			1		**1**
Hap-105																																		1			**1**
Hap-106																																		4			**4**
Hap-107																																		1			**1**
Hap-108																																			1		**1**
Hap-109																																		1			**1**
Hap-110																																			1		**1**
Hap-111																																			1		**1**
Hap-112																																		1			**1**
Hap-113																																		1			**1**
Hap-114																																		1			**1**
Hap-115																																			1		**1**
Hap-116																																			1		**1**
Hap-117																																			1		**1**
Hap-118																																			1		**1**
Hap-119													1																								**1**
Hap-120																																	1				**1**
Hap-121																																	1				**1**
Hap-122																					1																**1**
Hap-123																																				1	**1**
Hap-124																																				1	**1**
Hap-125																									1												**1**
Hap-126						1																															**1**
Hap-127																										1											**1**
Hap-128																									1												**1**
Hap-129																							1														**1**
Hap-130																										1											**1**
Hap-131																									1												**1**
Hap-132																1																					**1**
Hap-133																					1																**1**
Hap-134																							1														**1**
Hap-135					1																																**1**
Hap-136																									1												**1**
Hap-137																																	1				**1**
Hap-138																						1															**1**
Hap-139						1																															**1**
Hap-140																										1											**1**
Hap-141																										1											**1**
Hap-142																1																					**1**
Hap-143					1																																**1**
Hap-144						1																															**1**
Hap-145																							1														**1**
Hap-146																																				1	**1**

Appendix 3.4 continued

	Estero Topocalma	Estero Nilahue	Estero Reloca	Rio Tolten	Rio Queule	Rio Lingue	Lago Calafquen	Lago Panguipulli	Lago Rinihue	Lago Neltume	Rio Valdivia	Rio Bueno	Rio Contaco	Lago Rupanco	Lago Llanquihue	Rio Paredes	Rio Maullin	Lago Huillinco	Lago Tarahuin	Lago Natri	Rio Blanco	Rio Negro	Rio Yelcho	Lago Yelcho	Rio Palena	Rio Cisnes	Lago Quillen	Lago Espejo	Lago Morenito	Lago Hess	Lago Martin	Lago Steffen	Rio Chico	Lago Argentino	Lago Roca	Beagle Channel	**Total**
Hap-147																									1												**1**
Hap-148																									1												**1**
Hap-149																					1																**1**
Hap-150															1																						**1**
Hap-151																									1												**1**
Hap-152		1																																			**1**
Hap-153		1																																			**1**
Hap-154																							1														**1**
Hap-155		1																																			**1**
Hap-156					1																																**1**
Hap-157																																	1				**1**
Hap-158																																				1	**1**
Hap-159																																				1	**1**
Hap-160						1																															**1**
Hap-161																																				1	**1**
Hap-162																1																					**1**
Hap-163																						1															**1**
Hap-164																						1															**1**
Hap-165					1																																**1**
Hap-166						1																															**1**
Hap-167																																	1				**1**
Hap-168																										1											**1**
Hap-169																							1														**1**
Hap-170					1																																**1**
Hap-171																										1											**1**
Hap-172																						1															**1**
Hap-173																																	1				**1**
Hap-174		1																																			**1**
Hap-175					1																																**1**
Hap-176																									1												**1**
Hap-177																						1															**1**
Hap-178																						1															**1**
Hap-179																										1											**1**
Hap-180																								1													**1**
Hap-181																								1													**1**
Hap-182																								1													**1**
Hap-183																								1													**1**
Hap-184																								1													**1**
Hap-185																								1													**1**
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Hap-188																				1																	**1**
Hap-189																				1																	**1**
Hap-190																				1																	**1**
Hap-191																				1																	**1**
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Hap-193																				1																	**1**
Hap-194																				1																	**1**
Hap-195																				1																	**1**
Hap-196																				1																	**1**
Hap-197						1																															**1**
Hap-198																										1											**1**
Hap-199																																	1				**1**
Hap-200					1																																**1**
Hap-201																					1																**1**
Hap-202																								1													**1**
Hap-203									3																												**3**
Hap-204									1																												**1**
Hap-205																																				1	**1**
Hap-206															1																						**1**
Hap-207															1																						**1**
Hap-208															1																						**1**
Hap-209															1																						**1**
Hap-210																									1												**1**
Hap-211															1																						**1**
Hap-212												2																									**2**
Hap-213																	1																				**1**
Hap-214																	1																				**1**
Hap-215																		1																			**1**
Hap-216			1																																		**1**
Hap-217			1																																		**1**
Hap-218																																	1				**1**
Hap-219																		1																			**1**
Hap-220				1																																	**1**
Hap-221				1																																	**1**
Hap-222			1																																		**1**

Appendix 3.4 continued

	Estero Topocalma	Estero Nilahue	Estero Reloca	Rio Tolten	Rio Queule	Rio Lingue	Lago Calafquen	Lago Panguipulli	Lago Rinihue	Lago Neltume	Rio Valdivia	Rio Bueno	Rio Contaco	Lago Rupanco	Lago Llanquihue	Rio Paredes	Rio Maullin	Lago Huillinco	Lago Tarahuin	Lago Natri	Rio Blanco	Rio Negro	Rio Yelcho	Lago Yelcho	Rio Palena	Rio Cisnes	Lago Quillen	Lago Espejo	Lago Morenito	Lago Hess	Lago Martin	Lago Steffen	Rio Chico	Lago Argentino	Lago Roca	Beagle Channel	**Total**
Hap-223																			1																		**1**
Hap-224	1																																				**1**
Hap-225													1			1																					**2**
Hap-226																	1																				**1**
Hap-227																	4																				**4**
Hap-228																	1																				**1**
Hap-229																		1																			**1**
Hap-230																	1	1																			**2**
Hap-231				1																																	**1**
Hap-232				1																																	**1**
Hap-233																		1																			**1**
Hap-234																			1																		**1**
Hap-235																			1																		**1**
Hap-236																			1																		**1**
Hap-237																			1																		**1**
Hap-238																			1																		**1**
Hap-239																			1																		**1**
Hap-240																			1																		**1**
Hap-241																			1																		**1**
Hap-242																		1																			**1**
Hap-243																			1																		**1**
Hap-244																	1																				**1**
Hap-245											1																										**1**
Hap-246											1																										**1**
Hap-247											1																										**1**
Hap-248											1																										**1**
Hap-249											1																										**1**
Hap-250											1																										**1**
Hap-251						1																															**1**
Hap-252											1																										**1**
Hap-253											1																										**1**
Hap-254											1																										**1**
Hap-255												2																									**2**
Hap-256												1																									**1**
Hap-257												2																									**2**
Hap-258												1																									**1**
Hap-259														1																							**1**
Hap-260														1																							**1**
Hap-261														1																							**1**
Hap-262														1																							**1**
Hap-263														1																							**1**
Hap-264														1																							**1**
Hap-265														1																							**1**
Hap-266														1																							**1**
Hap-267												1																									**1**
Hap-268												1																									**1**
Hap-269																											1										**1**
Hap-270																											1										**1**
Hap-271																											1										**1**
Hap-272				3																																	**3**
Hap-273				1																																	**1**
Total	**1**	**4**	**3**	**8**	**10**	**10**	**10**	**5**	**10**	**10**	**9**	**10**	**4**	**8**	**10**	**5**	**10**	**7**	**10**	**10**	**9**	**10**	**7**	**9**	**10**	**10**	**10**	**9**	**2**	**9**	**9**	**13**	**9**	**10**	**9**	**10**	**299**

Appendix 4.1 Estimated population divergence time across the Patagonian *Central Drainage Boundary* (*CDB*) in millions of years before present (MY BP) for *Galaxias maculatus* and *Percichthys trucha* based on control region sequence variation. Population genetic divergence time in *G. maculatus* was estimated using the two most divergent haplotype groups [1 and 7c in Zemlak et al. (2010)] and a molecular substitution rate of 0.01876 changes/site/MY (Burridge et al. 2008). Population genetic divergence time in *P. trucha* contrasted all Chilean versus Argentinean samples available from Ruzzante et al. (2006) and employed a rate of molecular substitution of 0.05 changes/site/MY as recommended by Ruzzante et al. (2008).

G. maculatus			***P. trucha***		
MEGA % sequence divergence	=	**0.07**	MEGA % sequence divergence	=	**0.123**
fragment size	=	**803** sites	fragment size	=	**364** sites
D, population divergence	=	0.07 x 803 sites	*D*, population divergence	=	0.123 x 364 sites
	=	**56.42** sites		=	**44.77** sites
μ, mutation rate	=	(0.01876 changes/site/MY) x (803 sites)	μ, mutation rate	=	(0.05 changes/site/MY) x (364 sites)
	=	**16.13** sites/MY		=	**18.2** sites/MY
t, time since divergence	=	$D / 2\,\mu$	*t*, time since divergence	=	$D / 2\,\mu$
	=	(56.42 sites) / (16.13 sites/MY) x (2)		=	(44.77 sites) / (18.2 sites/MY) x (2)
	=	**1.75** MY		=	**1.23** MY

Appendix 4.2. The number of alleles per locus sampled from each of the targeted collection locations. Each site is grouped according to drainage orientation, Atlantic or Pacific, and sub-categorized by basin classification based on headwater position: Argentinean, trans-Andean, Chilean. CR is the abbreviated form of control region mtDNA and ANL represents anonymous nuclear locus.

Ocean drainage	**Basin Classification**	**Collection location**	**Number of Individuals**	**No. Alleles**							
				CR*	**ANL8**	**ANL9**	**ANL18**	**ANL20**	**ANL21**	**ANL22**	**ANL23**
Atlantic	*Argentinean*	*Espejo*	2	2	2	4	4	4	4	4	4
		Fonck	2	2	4	4	4	4	4	4	4
		Moscos	2	2	2	4	4	4	2	4	4
		La Plata	2	2	4	4	4	4	4	4	4
		Viedma	1	-	2	2	2	2	2	2	2
Pacific	*Trans-Andean*	*Puelo*	2	2	4	4	4	4	4	4	4
		Rivadavia	2	2	4	4	4	4	4	4	4
		Pueyrredon	2	2	4	4	4	4	4	4	4
		Belgrano	2	2	4	4	4	4	4	4	2
		Yehuin	2	2	4	4	4	4	4	4	4
	Chilean	*Llanquihue*	2	2	4	2	4	4	4	4	4
		Cudil	2	2	4	4	4	4	4	4	2
		Risopatron	2	2	4	4	4	4	4	4	4
		Thompson	2	2	-	4	2	4	4	2	2
		General Carrera	2	2	4	4	4	4	4	4	4
			29	**28**	**50**	**56**	**56**	**58**	**56**	**56**	**52**

**Haplotypic data (mtDNA)*

Appendix 4.3 Locus-specific methodological details for isolating each genetic marker employed in the current study. Listed are the expected sizes of each product, the primer sequences and annealing temperatures for the mitochondrial control region (CR) and each anonymous nuclear locus (ANL).

Locus	**Expected size (*bp*)**	**Forward Primer (*5′ to 3′*)**	**Reverse Primer (*5′ to 3′*)**	**Annealing Temp. (*°C*)**
CR	822	GCT TTA GTT AAG CTA CG	AAC TTC CAT CCT CAA CTC CCA AAG	50
ANL8	749	GGG CCA TTC AAG CAG TTT TA	TGA TGG GTA AAA GCA AAG AGC	63
ANL9	929	AAA AAC AAC GAC ACC CGT TT	CGA AGT CCA CCA TTG GTA AAG	63
ANL18	498	GAA TCG TGA GCA ACA AAC CA	TGC TCC TTG GGA AAA CTT CA	63
ANL20	649	TTC ATG ATA CCA CCG GAA CC	CTG GCT GTC TTC TGT GTG GA	63
ANL21	603	GGC CTG GCT GAA ATG ATA AA	CTT CCA AAG TTA CTC CAC GAG A	63
ANL22	529	TGT TTG GCT TCT ATG CAG GA	TGC GAT CCA TCA TCA ACT TT	63
ANL23	617	GCG GTT AAT GTT TGG TCG AA	ACC AGT TAC CAG GCT GAA GG	63

Appendix 4.4 Locus-specific characteristics and summary statistics for the control region (CR) and each anonymous nuclear locus (ANL). Details include the total number of alleles, the actual total overlap of all contigs recovered from each locus, the average guanine and cytosine (G+C) content, haplotype diversity (h), nucleotide diversity (π), the average pairwise distance between alleles (k), and Tajima's D (Tajima 1989).

Locus	**No. Alleles**	**Overlap (bp)**	**G+C**	***h***	***π***	***k***	***D***
CR*	28	768	0.410	0.892 ± 0.043	0.02286 ± 0.00447	17.55291	0.59928 *(P>0.10)*
ANL8	50	739	0.424	0.647 ± 0.038	0.00287 ± 0.00025	2.11755	1.47871 *(P>0.10)*
ANL9	56	929	0.484	0.235 ± 0.075	0.00070 ± 0.00025	0.6474	-1.48894 *(P>0.10)*
ANL18	56	483	0.447	0.558 ± 0.070	0.00512 ± 0.00066	2.47208	0.71772 *(P>0.10)*
ANL20	58	629	0.370	0.498 ± 0.055	0.00344 ± 0.00042	2.16334	1.65418 *(P>0.10)*
ANL21	56	596	0.440	0.465 ± 0.055	0.00151 ± 0.00018	0.90130	0.76556 *(P>0.10)*
ANL22	56	476	0.371	0.610 ± 0.042	0.00325 ± 0.00325	1.54545	1.71576 *(P>0.10)*
ANL23	52	558	0.398	0.906 ± 0.033	0.00451 ± 0.00046	2.51885	-0.15124 *(P>0.10)*

**Haplotypic data (mtDNA)*