A REVIEW OF THE SPECIES STATUS OF THE ANGAYUKAKSURAK CHARR (*SALVELINUS ANAKTUVUKENSIS*) OF NORTHERN ALASKA: PERSPECTIVES FROM MOLECULAR AND MORPHOLOGICAL DATA

By

Scott David Ayers

RECOMMENDED:

Dr. Nicola Hillgruber

Dr. Amy Larsen

Dr. Eric Taylor

Dr. Amanda Rosenberger, Advisory Committee Chair

Dr. Shannon Atkinson,
Interim Director, Fisheries Division

APPROVED:

Dr. Denis Wiesenburg
Dean, School of Fisheries and Ocean Sciences

Dr. Lawrence Duffy, Dean of the Graduate School

Feb 3, 2010
Date

A REVIEW OF THE SPECIES STATUS OF THE ANGAYUKAKSURAK CHARR (*SALVELINUS ANAKTUVUKENSIS*) OF NORTHERN ALASKA: PERSPECTIVES FROM MOLECULAR AND MORPHOLOGICAL DATA

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Scott David Ayers, B.S.

Fairbanks, Alaska

May 2010

Abstract

The Arctic, known for its dynamic past, is a significant place to examine drivers of and spatial variation in diversity of life history strategies in fishes. Diversity in heritable life history traits can lead to speciation, as may be the case for the putative Angayukaksurak charr (*Salvelinus anaktuvukensis*). The goal of this study was to determine the species status of this fish, the only described freshwater species endemic to Alaska. I examined and compared the morphology and genetics of Angayukaksurak charr and its most closely related species, the Dolly Varden (*Salvelinus malma*). Meristic characters divided the specimens into three forms by major river drainage. Morphological analysis divided the specimens into two forms along nominal species lines based on differences that could also be attributed to differences between life history forms. Sequences from a 550 bp section of mitochondrial d-loop failed to segregate the putative Angayukaksurak charr into a separate lineage, rather placing specimens into two previously resolved lineages of holarctic Arctic charr. In addition, analysis of microsatellite loci showed no clear differentiation between species. Based on these results, I concluded that the Angayukaksurak charr is not a separate species, but rather a resident life history form of the Dolly Varden.

Table of Contents

List of Figures

Page

List of Tables

Page

Dedication

To Gordon R. Haas for inspiration and starting me on the path. To Amanda E. Rosenberger for helping me pick up the pieces and find the path again.

Acknowledgments

This project would not have been possible without the tireless help, knowledge, and enthusiasm of many people. Many thanks to my committee members, Amanda Rosenberger, Nicola Hillgruber, Amy Larsen, and Eric B. Taylor (University of BC), for agreeing to serve on my committee and for providing insight and guidance at numerous points throughout this project. I would like to thank the community of Anaktuvuk Pass for allowing me to work in an amazing area of Alaska. Additional thanks go to F. DeCicco for field assistance and thoughtful discussions on the life of fishes, J.A. Lopez for assistance with specimens, methodology, and reviews, B. Sidlauskas for assistance with methodology, J. Burch for assistance with field collections, T. Rosenberger and T. Simmons for assistance with artwork, K. Hildebrandt for sharing expertise in many areas, J. Reist for thoughtful advice, P. Crane and M. Osborne for samples, and N.F. Hughes for sharing his time. The U.S. National Park Service Arctic Network and the School of Fisheries and Ocean Sciences at the University of Alaska Fairbanks (UAF) provided financial support for this project. The National Museum of Natural History provided access to specimens vital to this project. The UAF Life Science Informatics Portal was instrumental in computation of genetic analyses.

Lastly, many thanks go to family, friends, and especially Danielle, for support and encouragement throughout this project.

Chapter 1 Introduction

1.1 General Introduction and Study Approach

The opening chapter of this thesis is an introduction to the ideas and background material relevant to the larger project within, a review of the species status of the Angayukaksurak charr, *Salvelinus anaktuvukensis*. In this section I begin with the topics of evolution, endemism, and plasticity in species traits, as they pertain to my work and this region. The primary study area and study species are presented, as are the taxonomic issues associated with the genus *Salvelinus*. Species concepts in regards to this project, and taxonomy in general, are discussed. Finally, the goals and objectives of the study, as well as the direction of the subsequent chapters, are introduced.

1.1.1 Evolution and Endemism versus Phenotypic Plasticity

Species endemic to restricted areas, such as single drainages or regions, are most common in isolated situations, such as islands, where gene flow from new migrants is limited, or in stable areas with a long evolutionary history, such as the southeastern United States. With little to no gene flow from other populations, isolated populations have the opportunity to evolve via directional selective pressures. Alternatively, genetic drift in small, isolated populations can result in a shift in the location of populations on the adaptive landscape to new adaptive optima with a new set of traits (Wright, 1932), such that later introgression with the original species or population from which they are derived would be maladaptive. Under these circumstances, populations remain differentiated. As such, places that are considered hotspots of endemism are often sought

out for conservation or preservation to retain these unique, locally adapted species and distinct environments that represent opportunities to better understand evolutionary processes and factors that contribute to biodiversity (Waples, 1995; Reid, 1998; Myers et al., 2000).

Alaska has a dynamic geologic history. For example, much of the region was submerged below marine waters as recently as the Mississippian period (~360 mya); periods of massive mountain building took place during the Cretaceous period (~135 mya); and ice sheets and glaciers covered large regions of Alaska during the Pleistocene period (2 mya; Wahrhaftig, 1965). From a biogeographical perspective, the most notable event in recent geologic history was the formation of the Bering land bridge at the height of the last (Wisconsinan) Pleistocene glaciation, which linked the Asian and North American continents, creating refugia areas. The potential dispersal between and within the two continents facilitated by the land bridge, together with the dynamic history of the region, however, are unlikely to lead to high levels of endemism. Instead, this highly variable environment may favor plasticity in species traits (Wright, 1932; Via et al., 1995; Lema, 2008).

Alaska, although known for its pristine wilderness and great expanses, is not known as a hotspot for endemism in freshwater fishes. Rather, the region contains few native species relative to its vast size. Instead, biodiversity and adaptation to the diverse and fluctuating environment are displayed through variability and plasticity in life histories and ecology within fish species, notably within the family Salmonidae. As such, recognition of an endemic freshwater fish here would present a unique and surprising

find. This is not to say that there are no freshwater fish endemic to this region. The Alaska blackfish (*Dallia pectoralis*) is endemic to Beringia, with populations on both the North American and Eurasian continents. However, distribution of this species appears to be largely a result of dispersal following glacial retreat and not a case of a localized speciation event (Meldrim, 1968).

Whereas endemism is brought about by directional selection, isolation, and stability (Price, 2008), plasticity in trait space is adaptive under harsh selection pressures, combined with instability in environmental conditions (Via et al., 1995). Specifically, a mixture of environmental conditions and genomic composition can control plastic responses (Lema, 2008). A population of organisms must be developmentally plastic to have the ability to respond differentially to the influences of variable inputs from the environment on the genome. Alteration of environmental conditions can lead to expression of new traits in the population, and the resulting differences produce dissimilarities in reproductive success or survival within the population. This variability in reproductive success is particularly evident in situations of so-called hard selection, where intermediary phenotypes would be severely maladaptive, producing distinct phenotypic variants (Wright, 1932).

1.1.2 Plasticity in the Family Salmonidae

Plasticity in species traits is commonly found in members of the family Salmonidae in the form of multiple life history variants. For example, Dolly Varden charr (*Salvelinus malma*) populations display diadromous (overwinter and spawn in freshwater, feed during summers in marine waters), resident (life cycle in freshwater only), and

residual (one sex, typically male, in freshwater for entire life cycle, never attains large size) variants within single populations (DeCicco, 1985). Multiple life histories are also expressed in Pacific salmon, most notably within Chinook (*Oncorhynchus tshawytscha*) and sockeye salmon (*O. nerka*). Males display different reproductive strategies, namely a standard anadromous variety, a "jack" that returns a year or two before the other members of its cohort, and a "precocious parr" (similar to the residual male above) that matures without an oceanic migration (Quinn, 2005). Within the whitefishes (sub-family Coregoninae), populations of Inconnu (*Stenodus leucichthys*) from the Yukon River drainage display both anadromous and resident fish from at least one population in the Tanana River (Brown et al., 2007). Plastic expression of these multiple life history variants allows these groups the flexibility to respond to a variety of environmental conditions that they may encounter in this region, resulting in a form of "bet hedging," such that no one environmental catastrophe will destroy all variants, and the genetic structure of offspring will persist. This type of life history variability is thought to contribute to the long-term stability and sustainability of salmonid populations to environmental variability and large-scale perturbations (Hilborn et al., 2003).

Life history can be expressed as a heritable but plastic trait in response to environmental variation, or as a solution to an ecological problem (Stearns, 1976). Arctic charr (*Salvelinus alpinus*) have shown that life histories can be heritable—anadromous fish typically beget anadromous fish. They have also displayed plasticity in expression of those traits—resident fish can give rise to anadromous fish (Nordeng, 1983). Sympatric life history variants can remain genetically distinct in salmonid populations; genetic

differentiation was found in brook charr (*Salvelinus fontinalis*) between sympatric anadromous and resident forms (Boula et al., 2002). However, plasticity and gene flow between life histories also takes place: e.g., greater relatedness between morphotypes of Arctic charr within a lake than to corresponding morphotypes from other local lakes (Hindar et al., 1986) and interbreeding between freshwater resident and anadromous brown trout (*Salmo trutta*) in sympatry (Charles et al., 2006).

The Arctic is proving an interesting place to investigate evolutionary and ecological processes that lead to diversity in fish form and function. The continual shaping and reshaping of the terrestrial environment through time has led to isolation, extirpation, and repeated recolonizations of fish populations. These events have, in turn, led to diversification of life-history strategy and morphology within species in response to selective pressures. Thorough reviews of the geologic history have provided insights into how the current landscape was formed (e.g. Wahrhaftig, 1965; Miller et al., 2002); a similar review of the genetic landscape of fish populations in the north may help to provide insights into biogeography and the adaptive landscape.

1.2 Study Area

The primary study area that encompasses the described range of the Angayukaksurak charr lies within the central Brooks mountain range of northern Alaska. The Brooks Range is a continuation of the Rocky Mountain range of North America and the continental divide between the Pacific and the Arctic/Atlantic drainages. Its ontogeny dates to the Cretaceous period (~135 mya), and it was, as recently as the Pleistocene period (~2 mya), fully glaciated (Figures 1.1, 1.2; Wahrhaftig, 1965; Hamilton, 1986).

Underlying much of the range are large porous limestone deposits, vitally important to this region as reservoirs of groundwater for perennial springs (Yoshikawa et al., 2007). Through even the coldest months of the year, these springs produce flowing water, creating refugia for overwintering fishes and invertebrates. Specimens for this project, including whole fish, genetic fin clips, and photographs, were collected across northern Alaska. Angayukaksurak charr specimens were collected from drainages on either side of the village of Anaktuvuk Pass in the Anaktuvuk and John rivers, both within the primary study area. Comparative Dolly Varden specimens were collected from the Hula hula River in the Arctic National Wildlife Refuge of northeastern Alaska, the lower Anaktuvuk River (north of the primary study area), and from the Wulik, Avan, and Kugururok rivers of northwestern Alaska.

1.3 Study Species

The Angayukaksurak charr was formally described in 1973 by James Morrow, then Curator of Fishes at the University of Alaska Fairbanks Museum, and represents the only freshwater fish species endemic to Alaska. In comparison to related species in Alaska, this species is a dwarf form, with black background coloration, fiery red spots, and most closely related to the Dolly Varden charr (Morrow, 1973). The two species variants were described as distinct based on differences in color, several meristic characters, and timing of spawning (i.e., the Angayukaksurak charr purportedly spawn in spring, while the Dolly Varden spawn in autumn). This was, however, not the first

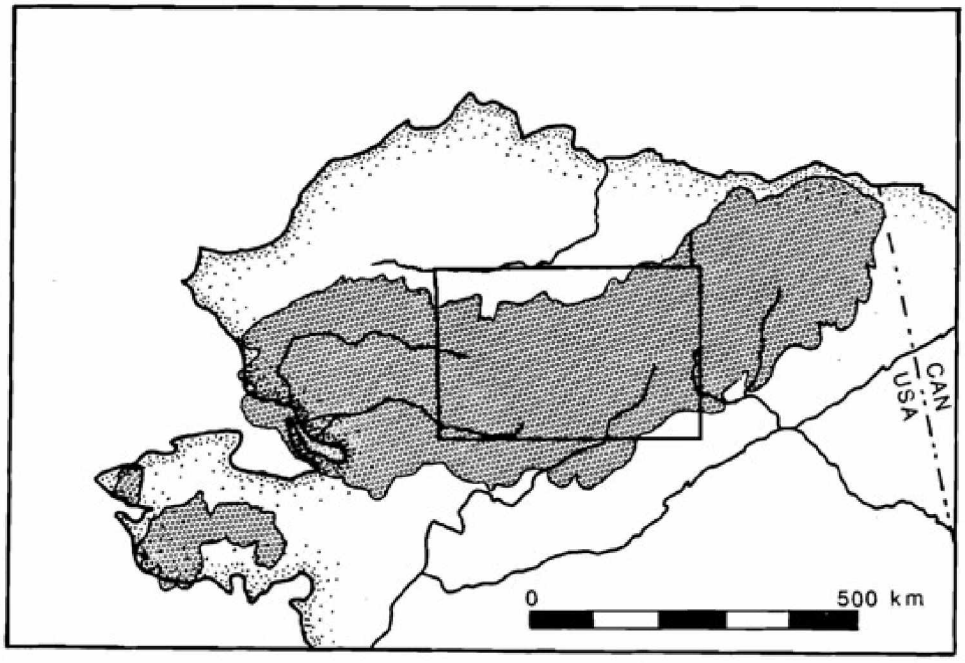

Figure 1.1 Extent of glaciation in northern Alaska during the last glacial maximum (shaded regions refer to areas of glacial influence; inset area shown in Figure 1.2) Large shaded area roughly coincides with Brooks Mountain Range. Used and edited with permission (Hamilton, 1986).

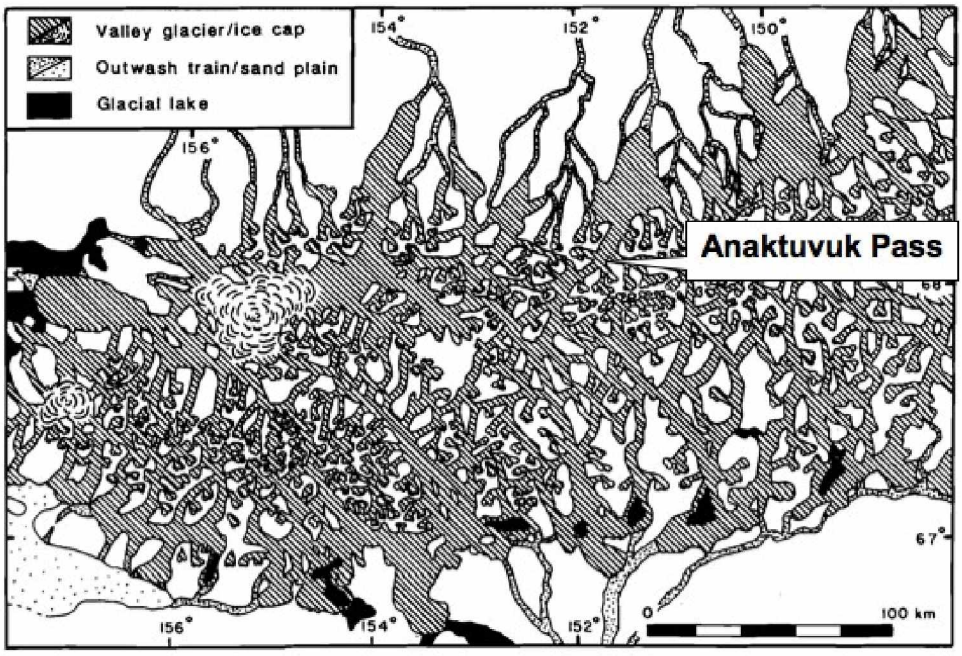

Figure 1.2 Extent of glaciation in the central Brooks Range during the last glacial maximum (area encompassed is outlined in Figure 1.1). Figure displays glaciaion within the drainages around Anaktuvuk Pass, in the primary study area. Used and edited with permission (Hamilton, 1986).

published account of this species. Angayukaksurak, or "old man fish," a name given to it by the indigenous people of the area, was noted in a publication on the taxonomy and zoogeography of fishes in the region (Walters, 1955). In this publication, the fish is described as stocky in appearance, similar to brook trout (*Salvelinus fontinalis*) of eastern North America (Walters, 1955). This species might have survived Pleistocene glaciations by using the perennial spring areas in this region of the central Brooks Range (Morrow, 1973); thus, the species might have been sufficiently isolated to become specifically adapted to this habitat and therefore unable to expand its range, given competition from the re-invading Dolly Varden (Morrow, 1973).

1.4 Taxonomic Issues

Species validity has been a contentious issue for species of *Salvelinus*, particularly within the '*alpinus-malma*' complex. In an attempt to clarify this issue, McPhail (1961) studied sympatric populations of *Salvelinus malma* (Dolly Varden) and *S. alpinus* (Arctic charr) in Karluk and Fraser lakes, Kodiak Island, Alaska, based on reports that two species of charr coexisted in one of the lakes (DeLacy and Morton, 1943). Discriminant analysis of meristic counts and measures suggested that hybridization rarely occurred, supporting the existence of two distinct species. Subsequently, however, the systematic relationship between *S. malma* and *S. alpinus* has been a topic of extensive debate (McPhail and Lindsey, 1970; Cavender, 1978; Armstrong and Morrow, 1980; Behnke, 1980; McCart, 1980; Cavender, 1984; Behnke, 1989; Cavender and Kimura, 1989; Reist, 1989; Grewe et al., 1990; Haas and McPhail, 1991; Crane et al., 1994; Phillips et al., 1994; Phillips et al., 1995; Everett et al., 1997; Phillips et al., 1999; Brunner et al., 2001;

Radchenko, 2002; Westrich et al., 2002; Crane et al., 2004; Oleinik et al., 2005; Taylor et al., 2008). Although both species exhibit a large degree of phenotypic plasticity, it is currently accepted that Arctic charr and Dolly Varden are two distinct species (Taylor et al., 2008).

In allopatry, Dolly Varden display unique and complex adaptive features that make them difficult to characterize. These include multiple life history forms (anadromy, residual male, and residency), variable breeding behaviors (summer and fall spawning runs and non-consecutive spawning), and migration patterns that range from resident populations to non-natal stream overwintering, to populations undertaking large scale (>1500km) migrations (DeCicco, 1985, 1989, 1992, 1997; Crane et al., 2005a, 2005b). In addition, occasional differences in meristics and morphology occur between sympatric life history forms, particularly within isolated populations. For instance, isolated populations from the North Slope of Alaska and the Northwest Territories, Canada, display significant variation in size, growth rate, fecundity, spawning timing, gill raker counts, coloration, parr marks (number and retention), pyloric caeca, and vertebrae compared to non-isolated populations within the same system (McCart and Craig, 1973; McCart and Bain, 1974). Differences in genetic markers have also been documented between presently isolated and anadromous populations within the same drainage (Leder, 2001), suggesting the presence of substantial selective forces capable of rapidly manipulating the genome of this species in the relatively short time period since glacial retreat. Because many of these same meristic characters that show so much variation within Dolly Varden were used to differentiate the Angayukaksurak charr from Dolly

Varden for its species description, it is reasonable to be skeptical of the validity of the full species designation for *S. anaktuvukensis*. A combined approach of meristic and morphometric characters with genetic markers would more accurately determine species designation.

1.5 Species Concepts

Research projects concerning species designations must also consider which species concept best applies to their particular circumstances. The Biological Species Concept (BSC) and the Phylogenetic Species Concept (PSC) are two of the primary concepts used. The most common interpretation of the BSC is that of Mayr (1942), which states that species are groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups. Taxonomists have relied on this concept as it provides an "acid test" for sexually reproducing species that persist in sympatry (Avise, 1994). However, the BSC has been problematic for asexual species or for examining speciation in allopatric sexual populations. The PSC has defined species as the smallest aggregation of (sexual) populations or (asexual) lineages diagnosable by a unique combination of character states (Wheeler and Platnick, 2000). This concept allows for the ability to distinguish species as monophyletic clades without the problems of division into populations or other assemblages of individuals, as those smaller assemblages no longer manifest constantly distributed characters with the ability to indicate their affinity to other such assemblages (Wheeler, 1999). The PSC best applies to the present study because I am using unique character states for differentiation purposes, and because the populations of nominal species I am examining do not exist in sympatry.

1.6 Objectives

The goal of this study was to re-examine the species designation of the Angayukaksurak charr using both genetic and traditional morphological and meristic tools. The objectives were to: 1) examine fish for differences in meristic (as in the original study) and morphometric characters, as well as genetic differentiation between populations (based on microsatellite nuclear DNA and mitochondrial DNA); and 2) compare specimens collected from locations in the original description with other populations of Dolly Varden from northern Alaska.

Chapter two of this document is formatted as a manuscript to be submitted for journal publication, and, as such, some of the introductory information presented in detail in this chapter will be summarized there. The appendices that follow chapter two contain data from this project and others, such as meristic counts and principal component scores, not otherwise reported in this document. Further detail of the data recovered, analyses performed, and conclusions garnered will also be presented in chapter two. A summary of study conclusions will be presented in chapter three, with further ideas regarding future research.

1.7 Literature Cited

Armstrong, R. H., and J. E. Morrow. 1980. The Dolly Varden charr, *Salvelinus malma*, p. 99-140. *In:* Charrs: Salmonid Fishes of the Genus *Salvelinus*. E. K. Balon (ed.). Dr. W. Junk Publishers, The Hague, the Netherlands.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Oxford University Press, New York.

Behnke, R. J. 1980. A review of the genus *Salvelinus*, p. 441-480. *In:* Charrs: Salmonid Fishes of the Genus *Salvelinus*. E. K. Balon (ed.). Dr. W. Junk Publishers, The Hague, the Netherlands.

—. 1989. Interpreting the phylogeny of *Salvelinus*. Physiological Ecology Japan. Special Volume 1:35-48.

Boula, D., V. Castric, L. Bernatchez, and C. Audet. 2002. Physiological, endrocrine, and genetic bases of anadromy in the brook charr, *Salvelinus fontinalis*, of the Laval River (Quebec, Canada). Environmental Biology of Fishes. 64:229-242.

Brown, R. J., N. Bickford, and K. Severin. 2007. Otolith trace element chemistry as an indicator of anadromy in Yukon River drainage coregonine fishes. Transactions of the American Fisheries Society. 136:678-690.

Brunner, P. C., M. R. Douglas, A. Osinov, C. C. Wilson, and L. Bernatchez. 2001. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus L.*) inferred from mitochondrial DNA sequences. Evolution. 55:573-586.

Cavender, T. M. 1978. Taxonomy and distribution of the bull trout *Salvelinus confluentus* (Suckley), from the American northwest. California Fish and Game. 64:139-174.

—. 1984. Cytotaxonomy of North American *Salvelinus*, p. 431-455. *In:* Biology of the Arctic Charr: Proceedings of the International Symposium on Arctic Charr. L. Johnson and B. Burns (eds.). The University of Manitoba Press, Winnipeg, Manitoba.

Cavender, T. M., and S. Kimura. 1989. Cytotaxonomy and interrelationships of Pacific basin *Salvelinus*. Physiological Ecology Japan. Special Volume 1:49-68.

Charles, K., J.-M. Roussel, J.-M. Lebel, J.-L. Bagliniere, D. Ombredane. 2006. Genetic differentiation between anadromous and freshwater resident brown trout

(*Salmo trutta* L.): insights obtained from stable isotope analysis. Ecology of Freshwater Fish. 15:255-263.

Crane, P. A., A. L. DeCicco, B. Spearman, and J. K. Wenburg. 2005a. Genetic diversity patterns of Dolly Varden in Norton and Kotzebue Sounds. U.S. Fish and Wildlife Service, Alaska Fisheries Technical Report, Vol. 80, Anchorage, Alaska.

Crane, P. A., C. J. Lewis, E. J. Kretschmer, S. J. Miller, W. J. Spearman, A. L. DeCicco, M. J. Lisac, and J. K. Wenburg. 2004. Characterization and inheritance of seven microsatellite loci from Dolly Varden, *Salvelinus malma*, and cross-species amplification in Arctic char, *S. alpinus*. Conservation Genetics. 5:737-741.

Crane, P. A., L. W. Seeb, and J. E. Seeb. 1994. Genetic relationships among *Salvelinus* species inferred from allozyme data. Canadian Journal of Fisheries and Aquatic Sciences. 51:182-197.

Crane, P. A., T. Viavant, and J. K. Wenburg. 2005b. Overwintering patterns of Dolly Varden *Salvelinus malma* in the Sagavanirktok River in the Alaska North Slope inferred using mixed-stock analysis. U.S. Fish and Wildlife Service, Alaska Fisheries Technical Report, Vol. 84, Anchorage, Alaska.

DeCicco, A. L. 1985. Inventory and cataloging of sport fish and sport fish waters of western Alaska with emphasis on Arctic char life history studies. Alaska department of Fish and Game, Federal Aid in Fish Restoration, Annual Performance Report 1984-1985. Project F-9-17, (G-I):41-134.

—. 1989. Movements of spawning adult Dolly Varden char (*Salvelinus malma*) in Chukchi Sea drainages of northwestern Alaska: evidence for summer and fall spawning populations. Physiological Ecology Japan. Special Volume 1:229-238.

—. 1992. Long-distance movements of anadromous Dolly Varden between Alaska and the U.S.S.R. Arctic. 45:120-123.

—. 1997. Movements of postsmolt anadromous Dolly Varden in northwest Alaska. American Fisheries Society Symposium. 19:175-183.

DeLacy, A. C., and W. M. Morton. 1943. Taxonomy and habits of the charrs *Salvelinus malma* and *Salvelinus alpinus* of the Karluk drainage system. Transactions of the American Fisheries Society. 72:79-91.

Everett, R. J., R. L. Wilmot, and C. C. Krueger. 1997. Population genetic structure of Dolly Varden from Beaufort Sea drainages in northern Alaska and Canada. American Fisheries Society Symposium. 19:240-249.

Grewe, P. M., N. Billington, and P. D. N. Hebert. 1990. Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. Canadian Journal of Fisheries and Aquatic Sciences. 47:984-991.

Haas, G. R., and J. D. McPhail. 1991. Systematics and distributions of Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America. Canadian Journal of Fisheries and Aquatic Sciences. 48:2191-2211.

Hamilton, T. D. 1986. Late Cenozoic glaciation of the central Brooks Range, p. 9–49. *In:* Glaciation in Alaska: the geologic record. T. D. Hamilton, K. M. Reed, and R. M. Thorson (eds.). Alaska Geological Society, Anchorage.

Hilborn, R., T. P. Quinn, D. E. Schindler, and D. E. Rogers. 2003. Biocomplexity and fisheries sustainability. Proceedings of the National Academy of Sciences. 100:6564-6568.

Hindar, K., N. Ryman, and G. Ståhl. 1986. Genetic differentiation among local populations and morphotypes of Arctic charr, *Salvelinus alpinus*. Biological Journal of the Linnean Society. 27:269-285.

Leder, E. H. 2001. Phylogenetic analysis of the Arctic char-Dolly Varden species complex. Unpubl. Ph.D. Diss, The University of Wisconsin-Milwaukee, Milwaukee, Wisconsin.

Lema, S. C. 2008. The phenotypic plasticity of Death Valley's pupfish: desert fish are revealing how the environment alters development to modify body shape and behavior. American Scientist. 96:28-33.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York, New York.

McCart, P. J. 1980. A review of the systematics and ecology of the Arctic char, *Salvelinus alpinus*, in the western Arctic. Canadian Technical Report of Fisheries and Aquatic Sciences. 935.

McCart, P. J., and H. Bain. 1974. An isolated population of Arctic char (*Salvelinus alpinus*) inhabiting a warm mineral spring above a waterfall at Cache Creek, Northwest Territories. Journal of the Fisheries Research Board of Canada. 31:1408-1414.

McCart, P. J., and P. C. Craig. 1973. Life history of two isolated populations of Arctic char (*Salvelinus alpinus*) in spring-fed tributaries of the Canning River, Alaska. Journal of the Fisheries Research Board of Canada. 30:1215-1220.

McPhail, J. D. 1961. A systematic study of the *Salvelinus alpinus* complex in North America. Journal of the Fisheries Research Board of Canada. 18:793-816.

McPhail, J. D., and C. C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Fisheries Research Board of Canada. 173.

Meldrim, J. W. 1968. The ecological zoogeography of the Olympic mudminow, *Novumbra hubbsi* (Schultz). Unpubl. Ph.D. Diss, The University of Washington, Seattle, Washington.

Miller, E. L., A. Grantz, and S. L. Klemperer. 2002. Tectonic evolution of the Bering shelf – Chukchi Sea – Arctic Margin and adjacent landmasses. Geological Society of America. Special Paper 360.

Morrow, J. E. 1973. A new species of *Salvelinus* from the Brooks Range, northern Alaska. Biological Papers of the University of Alaska. 13:1-8.

Myers, N., R. A. Mittermeier, G. A. B. DaFonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature. 403:853-858.

Nordeng, H. 1983. Solution to the "char problem" based on Arctic char (*Salvelinus alpinus*) in Norway. Canadian Journal of Fisheries and Aquatic Sciences. 40:1372-1387.

Oleinik, A. G., L. A. Skurikhina, V. A. Brykov, P. A. Crane, and J. K. Wenberg. 2005. Differentiation of Dolly Varden char *Salvelinus malma* from Asia and North America inferred from PCR-RFLP analysis of mitochondrial DNA. Russian Journal of Genetics. 41:501-508.

Phillips, R. B., L. I. Gudex, K. M. Westrich, and A. L. DeCicco. 1999. Combined phylogenetic analysis of ribosomal ITS1 sequence and new chromosome data supports three subgroups of Dolly Varden char (*Salvelinus malma*). Canadian Journal of Fisheries and Aquatic Sciences. 56:1504-1511.

Phillips, R. B., S. A. Manley, and T. J. Daniels. 1994. Systematics of the salmonid genus *Salvelinus* inferred from ribosomal DNA sequences. Canadian Journal of Fisheries and Aquatic Sciences. 51:198-204.

Phillips, R. B., S. L. Sajdak, and M. J. Domanico. 1995. Relationships among charrs based on DNA sequences. Nordic Journal of Freshwater Research. 71:378-391.

Price, T. 2008. Speciation in birds. Roberts and Company Publishers, Greenwood Village, Colorado.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle, Washington.

Radchenko, O. A. 2002. Genetic differentiation inferred from data on restriction analysis of mitochondrial DNA in northern and southern forms of the Dolly Varden char. Russian Journal of Genetics. 38:421-428.

Reid, W.V. 1998. Biodiversity hotspots. Trends in ecology and evolution. 13:275-280.

Reist, J. D. 1989. Genetic structuring of allopatric populations and sympatric life history types of charr, *Salvelinus alpinus/malma*, in the Western Arctic, Canada. Physiological Ecology Japan. Special Volume 1:405-420.

Stearns, S. C. 1976. Life-history tactics: a review of the ideas. The Quarterly Review of Biology. 51:3-47.

Taylor, E. B., E. Lowery, A. Lilliestrale, A. E. Elz, and T. P. Quinn. 2008. Genetic analysis of sympatric char populations in western Alaska: Arctic char (*Salvelinus alpinus*) and Dolly Varden (*Salvelinus malma*) are not two sides of the same coin. Journal of Evolutionary Biology. 21:1609-1625.

Via, S., R. Gomulkiewicz, G. DeJong, C. D. Schlicting, and P. H. Van Tienderen. 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends in Ecology and Evolution. 10:212-217.

Wahrhaftig, C. 1965. Physiograhic divisions of Alaska. U.S. Geological Survey, Washington, D.C.

Walters, V. 1955. Fishes of western Arctic America and eastern Arctic Siberia: taxonomy and zoogeography. Bulletin of the American Museum of Natural History. 106:255-368.

Waples, R. S. 1995. Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. American Fisheries Society Symposium. 17:8-27.

Westrich, K. M., N. R. Konkol, M. P. Matsuoka, and R. B. Phillips. 2002. Interspecific relationships among charrs based on phylogenetic analysis of nuclear growth hormone intron sequences. Environmental Biology of Fishes. 64:217-222.

Wheeler, Q. D. 1999. Why the phylogenetic species concept? – Elementary. Journal of Nematology. 31:134-141.

Wheeler, Q. D., and N. I. Platnick. 2000. The phylogenetic species concept (*sensu* Wheeler and Platnick), p. 55-69. *In:* Species concepts and phylogenetic theory: a debate. Q. D. Wheeler and R. Meier (eds.). Columbia University Press, New York.

Wright, S. 1932. The role of mutation, inbreeding, crossbreeding, and selection on evolution. Proceedings of the Sixth International Congress on Genetics 1: 356-366. Reprinted, p. 161-171. *In:* Sewall Wright: evolution, selected papers. W. B. Provine (ed.). University of Chicago Press, Chicago, Illinois.

Yoshikawa, K., L. D. Hinzman, and D. L. Kane. 2007. Spring and aufeis (icing) hydrology in the Brooks Range, Alaska. Journal of Geophysical Research. 112, G04S43:1-14.

Chapter 2 A Review of the Species Status of *Salvelinus anaktuvukensis* (Teleostei: Salmoniformes) of Northern Alaska[1]

2.1 Abstract

In a region known for its complex and dynamic past, the Arctic is a unique place to examine drivers of diversity in life history strategies, particularly in species that colonize new or disturbed habitats. Of particular interest is how diversity in life history can contribute to speciation or endemism, as may be the case for the putative Angayukaksurak charr (*Salvelinus anaktuvukensis*). The goal of this project is to determine the species status of *S. anaktuvukensis*; the only described freshwater fish endemic to Alaska. We examined the morphology (meristics and geometric morphometrics) and genetics (microsatellite and mitochondrial DNA) of Angayukaksurak charr and its closest relative, the Dolly Varden (*Salvelinus malma*) of northern Alaska. Meristic characters divided the specimens into three groups that corresponded to river drainage, primarily based on orbit width and anal fin ray count. Geometric morphometric analysis divided the specimens into two forms along nominal species lines based on body depth and minor shape differences that could, however, also

---

[1] Ayers, S.D., A.E. Rosenberger, and E.B. Taylor. 2009. A review of the species status of *Salvelinus anaktuvukensis* (Teleostei: Salmoniformes) of Northern Alaska. Prepared for submission to Copeia.

be attributed to differences between anadromous and resident life history forms. Sequences from a 550bp section of the mitochondrial d-loop failed to segregate the putative Angayukaksurak charr into a single separate lineage, rather placing specimens into two previously resolved lineages (Arctic and Bering). In addition, differentiation between species was not evident based on analysis of microsatellite loci. We conclude that the Angayukaksurak charr of northern Alaska is a resident life history form (and junior synonym) of the Dolly Varden.

2.2 Introduction

The harsh extremes that characterize the Arctic, including limitations in productivity, habitat diversity, and temperature extremes, have created a narrow niche of tolerance for fish species (Reynolds, 1997). Arctic freshwater systems, such as systems with large lakes, are typically low in diversity (Power, 1997). The dynamic history and severe conditions that characterize the freshwaters of high latitudes have prohibited the high species diversity and endemism that characterize more stable, productive hotspots for fish diversity around the globe (Weider and Hobæk, 2000). Biological diversity in Arctic ecosystems is instead more commonly represented through life history diversity, particularly among and within salmonid species.

Fishes of the family Salmonidae display a variety of life history forms; the most dominant are anadromy, amphidromy, and residency. Under the strategies of anadromy and amphidromy, individuals presumably maximize lifetime fitness by attaining a body size conducive to high fecundity and/or reproductive success at the expense of increased rates of mortality in the marine environment, while also using the less productive

freshwater environment for shelter of eggs and rearing juveniles (Gross, 1987). Variability in environmental conditions resulting in differential selective pressures and sources of mortality may lead to variability in life history strategy within as well as among species (Stearns, 1976). Examples include the presence of both "jack" and dominant alpha males in a spawning population or portions of diadromous populations remaining as freshwater residents for their entire lives (often as dwarf forms). As with jacks, residency can be confined to the male portion of the population (as residual males) or, alternatively, can constitute an independent population of both sexes. This within-species life history diversity is thought to contribute to the long-term resilience of populations and species to the harsh, dynamic conditions characteristic of the Arctic (Hilborn et al., 2003).

Differentiating between probable species or life history diversity in response to environmental variation in the Arctic can lead to taxonomic dilemmas. Taxonomic identification is imparted when identifying genetically distinct groups (Evolutionary Significant Units – ESU's) for conservation purposes (e.g., the U.S. Endangered Species Act; Waples, 1991), when investigating if spawning aggregations for different (but spatially close) drainages are distinct (e.g., Hendry et al., 2000), or if runs at different times of the season are distinct (i.e., summer and fall runs of chum salmon, *Oncorhynchus keta*, in the Yukon River of Alaska; Sato et al., 2004). The issue is particularly complex for the genus *Salvelinus*, a member of the salmonid family, one of the most successful groups of freshwater and diadromous Arctic fishes. More than 250 years after the original description of the Arctic charr by Linnaeus in 1758, species

relationships within this genus are not yet resolved satisfactorily, and questions of species status within the genus remain.

Species of *Salvelinus* in North America inhabit a range south from the southern extent of ice cover from the last glaciation to high into the Canadian high Arctic and include the most northerly freshwater species, *S. alpinus* (Johnson, 1980; Reist et al., 2006). Within *Salvelinus*, as within the rest of Salmonidae, variation in life history is great: e.g., three different forms of Arctic charr (*Salvelinus alpinus*), dwarf resident, non-dwarf (standard) sized resident, and anadromous, were identified in the Salangen River of Norway (Nordeng, 1983). After further observation, however, it was determined that dwarf forms occasionally grew to standard sized fish after a few years, while standard sized resident fish occasionally became anadromous. Progeny from each group was more likely to, but did not always follow the parental life history strategy, even though spawning sites appeared to be distinct among the different forms (Nordeng, 1983). It is theorized that this plasticity in life history of charrs is what has enabled them to be successful in harsh conditions and to quickly recolonize postglacial environments (Wilson et al., 1996; Wilson and Hebert, 1998; Milner et al., 2000; Haas and McPhail, 2001).

In contrast to life history diversity, members of *Salvelinus* have experienced a relatively small number of species radiations, with little overall change in morphology. Minor morphological differences have, however, allowed these fish to use different portions of the aquatic environment, allowing species within the genus to co-occur over large regions rather than exist as allopatric populations. Large groups of variable

populations within nominally single taxa are termed "species complexes" (i.e., Arctic charr complex), and only with exhaustive meristic studies and the advent of molecular techniques has it become possible to more closely examine these complexes. The Arctic charr complex has yielded two distinct species, Arctic charr and Dolly Varden (DeLacy and Morton, 1943; McPhail, 1961). Fish that were previously thought to be Dolly Varden due to their remarkable similarity in appearance have now been accurately described as bull charr (*S. confluentus*; Cavender, 1978; Haas and McPhail, 1991; Reist et al., 2002), which are actually more closely related to the white spotted charr (*S. leucomaenis*) of eastern Eurasia than to Dolly Varden (Phillips and Pleyte, 1991; Crane et al., 1994). A species controversy within the genus *Salvelinus* that remains unresolved is the species status of the Angayukaksurak charr, *S. anaktuvukensis*.

First observed by Walters (1955), the Angayukaksurak charr was formally described in 1973 by James Morrow (Morrow, 1973), then curator of fishes at the University of Alaska Museum. Angayukaksurak charr (also known as the old man charr) are dwarf-sized, resident fish that live within headwater tributaries of the central Brooks Mountain Range of northern Alaska. Bearing closest similarity to the Dolly Varden, Angayukaksurak charr were differentiated from populations of Dolly Varden and Arctic charr in Alaska, Canada, and Siberia using a discriminant analysis of meristic characters (Frohne, 1973). Both Morrow (1973) and Walters (1955) also note that these fish reportedly spawn in the spring, which would temporally segregate them from the fall spawning season of other charr in Alaska.

As a species, the Angayukaksurak charr would represent the only freshwater fish endemic to Alaska. Two of the possible scenarios that endemism could arise in Alaska are (1) the presence of an unknown stable environment that persisted over a long enough period for species differentiation, and/or (2) extraordinarily fast radiation within a genus. The stability required for the first scenario is unlikely and would require the presence of unknown refugia during past glacial events in Alaska, such as perennial springs. Alaska's past is dynamic, from its origins as a landmass—accrual of land from passing tectonic plates, (Gates and Gryc, 1963)—to the extent of glaciation during past ice ages (Hamilton, 1986; Hamilton et al., 1986; Lindsey and McPhail, 1986). In contrast, endemism characterizes regions of extended periods of stability (Price, 2008). Fast species radiation appears unlikely within *Salvelinus* based on historic taxonomic divisions in this genus. Species tend to differentiate according to habitat type (e.g., in Alaska, Dolly Varden specialize within stream environments, while Arctic charr specialize within lakes; Davis and Webb, 1993). Within species, life history forms differ according to access to migratory corridors and/or distance to the marine environment, particularly within Dolly Varden (e.g., dwarf, resident forms above barriers to migration, anadromous forms below; McCart and Craig, 1973; McCart and Bain, 1974; McCart, 1980; Morrow, 1980). Angayukaksurak charr, in contrast, are described as existing in only headwater lotic environments as a single life history form (dwarf resident; Morrow, 1973).

Accordingly, species designation for the Angayukaksurak charr has undergone scrutiny and is not universally accepted (McCart, 1980; Lindsey and McPhail, 1986;

Mecklenburg et al., 2002). While some authors have given it species designation (e.g., Page and Burr, 1991), others have categorized it with temporary, sub-specific status (Nelson et al., 2004). Although species designation has yet to be clarified, conservation status has been conferred. The Bureau of Land Management (BLM) considers it a species of concern (BLM Instruction Memorandum No. AK 2006-003). Also, the American Fisheries Society's endangered species committee lists it as vulnerable, defined as in imminent danger of becoming threatened throughout all or a significant portion of its range (Jelks et al., 2008). The lack of agreement on species designation, and therefore conservation status, for this nominal species has led reviewers (e.g., Nelson et al., 2004) to defer a decision until further research has been conducted that takes advantage of the advent of modern genetic tools to resolve species designation for the Angayukaksurak charr.

Research towards the determination of phylogenetic structure within *Salvelinus* (and fishes in general) has undergone recent, significant changes. Whereas initial relationships were inferred from meristic characters, morphological measurements, or ecology (e.g., McPhail, 1961; Cavender, 1978; McCart, 1980; Nordeng, 1983; Haas and McPhail, 1991), recent investigations have used different types of genetic data. Coding mitochondrial DNA (mtDNA) appears particularly useful for determining larger scale (global) relations and non-coding microsatellite loci useful for smaller scale (within and among population) variation. Using a combined approach by examining both meristic/morphological and genetic characteristics in the same study may be particularly

valuable to attain a more comprehensive evaluation of change within or between groups (e.g., Brunner et al., 1998; Reist et al., 1997; Taylor et al., 2008).

Previous mtDNA research has shown that circumpolar Arctic charr populations fit into five discreet lineages, two of which are in Alaska (the Arctic and Bering lineages; Brunner et al., 2001). The Arctic lineage ranges north of the Brooks Range in Alaska and encompasses all of Arctic Canada; the Bering lineage ranges south and west of the Brooks Range and includes all fish in the region considered to be *S. alpinus, S. malma,* or *S. a. taranetzi*. Microsatellite loci from nuclear DNA have been used within Alaska to examine and differentiate sympatric populations of Arctic charr and Dolly Varden (Taylor et al., 2008) and to characterize overwintering patterns and stock identification of North Slope Dolly Varden populations (Crane et al., 2005).

To address the taxonomic designation and species status uncertainties of the Angayukaksurak charr, this study will use mitochondrial and nuclear (microsatellite) DNA, meristic counts, and morphometric measures to resolve species status and determine *S. anaktuvukensis* placement within *Salvelinus*. For the purposes of this inquiry, the phylogenetic species concept (PSC) as defined by Wheeler and Platnick (2000) will be used to ultimately determine species status. Under the PSC, species are defined as the smallest aggregation of (sexual) populations or (asexual) lineages diagnosable by a unique combination of character states. This definition is particularly useful in taxonomic situations such as this, where the species of interest is not sympatric with its closest relative. The information garnered from this study will give insight into the evolutionary forces and processes at work in the central Brooks Range and contribute

to the determination of the conservation status of the Angayukaksurak charr. Our objectives are to:

1. Determine the morphological and/or meristic characteristics of the Angayukaksurak charr and compare with those of the Dolly Varden.
2. Determine the placement of the Angayukaksurak charr within the genus *Salvelinus*, specifically within the Arctic charr lineages determined by Brunner et al. (2001).
3. Use microsatellite (nuclear) DNA to determine if populations of Angayukaksurak charr differ genetically from Dolly Varden.
4. Determine congruence between the characters investigated (meristic counts, morphometric measures, mtDNA, and microsatellite DNA).

2.3 Materials and Methods

2.3.1 Study Area

The focal study area for this project is the central Brooks Range of northern Alaska. The Brooks Range is a continuation of the Rocky Mountain Range of North America and is situated in an east-west direction, proceeding from the western edge of the Mackenzie River basin to the Bering Sea. It also divides the Pacific Ocean-Bering Sea drainages and the Arctic Ocean-Beaufort Sea drainages. This section of the mountain range dates to the Cretaceous period (Wahrhaftig, 1965) and is underlain by large sections of porous sedimentary limestone rock containing numerous groundwater springs (Yoshikawa et al., 2007).

The primary study systems are on the north and south sides of the village of Anaktuvuk Pass (68°8′58.31″N, 151°43′33.51″W), situated at the continental divide. The

Anaktuvuk River drains north from the divide into the Colville River, which drains into the Beaufort Sea (Arctic Ocean). The John River flows south from the divide into the Koyukuk River, feeding into the lower Yukon River, which empties into the Bering Sea. Specimen collections in the original description occurred in both drainages (Morrow, 1973). The collection sites for this project were within the headwater tributaries of these two river systems, low- and medium-gradient systems prone to flash-flooding in summer months, with predominantly gravel substrate.

This region has been instable on the geologic time-scale, with at least four major glacial episodes in the past 3 million years. In the central section of the Brooks Range (including the study systems), glacial lobes extended beyond the mountains to the plains in both north- and south-sloping valleys, with the last glacial advance occurring between 11.5 and 13 thousand years ago (Hamilton, 1986). This glacial activity would likely have extirpated aquatic organisms within these valleys or forced relocation to potential refugia; retreats and advances would have led to repeated recolonizations of this area through time.

A large refuge existed during the height of glacial advancement that shaped the distributions and diversity of freshwater fishes in this region (Lindsay and McPhail, 1986). The Bering refugia (Beringia), which ranged eastward from northwestern Canada to the western Kamchatka peninsula of Russia, encompassed a large ice-free region that acted as a source for re-populating glacially influenced systems on both sides of the Bering Sea during warming periods (Lindsey and McPhail, 1986). The existence of this refuge through multiple glacial episodes allowed exchange of freshwater fish within the

region as ocean levels dropped, exposing the Bering land bridge, while exchange between Beringia and other continental refugia in North America or Eurasia were limited or completely cut off. A Faunal Resemblance Index (FRI) of 85% (23 of 30 freshwater fish species in common) within Beringia, which drops sharply as comparisons are made with drainages outside of Beringia, supports this theory (Lindsay and McPhail, 1986). Thus, current distribution patterns or levels of relatedness between populations should strongly reflect past glacial activities and drainage systems.

2.3.2 Charr Collections

Samples from the primary study area were collected from four locations in the Anaktuvuk River drainage (three upper river and one lower river) and from two locations in the upper John River drainage. Comparative samples were obtained from the Avan, Kugururok, and Wulik rivers, all of which drain into the Chukchi Sea of northwestern Alaska, as well as from photographic images of charr collected from the Hulahula River in northeastern Alaska (Table 2.1; Figure 2.1). Fish collection methods included baited minnow traps, dip nets, angling, gill nets, and electrofishing. All fish collected for morphological analyses were fixed in a 10% buffered formalin solution immediately after capture, returned to the lab, and subsequently stored in a 70% ethanol solution.

Table 2.1 Location of specimen collection and number of samples used for different analyses (meristic counts / geometric morphometrics / microsatellite DNA / mitochondrial DNA).

Site[1]	River	Drainage	Latitude	Longitude	Nominal Taxon	Sample Size
ADT	Anaktuvuk	Beaufort Sea	68.13563	-151.52900	*S. anaktuvukensis*	41 / 41 / 30 / 2
ADTA	Anaktuvuk	Beaufort Sea	68.87550	-151.14769	*S. malma*	-- / -- / 32 / 2
AVAN	Avan	Chukchi Sea	68.27500	-161.80667	*S. malma*	-- / -- / 22 / 2
AUT	Anaktuvuk	Beaufort Sea	68.13225	-151.51882	*S. anaktuvukensis*	42 / 42 / 32 / 2
EK	John	Bering Sea	68.01700	-152.33900	*S. anaktuvukensis*	11 / 11 / 11 / 2
GLC	Anaktuvuk	Beaufort Sea	68.10193	-151.05465	*S. anaktuvukensis*	18 / 18 / 18 / 2
GTC	John	Bering Sea	68.07830	-151.70987	*S. anaktuvukensis*	-- / -- / -- / 1
KUG	Kugururok	Chukchi Sea	68.52833	-160.49500	*S. malma*	-- / -- / 18 / 2
WUL	Wulik	Chukchi Sea	67.75600	-164.44200	*S. malma*	14 / 6 / 23 / 2
HULA	Hulahula	Beaufort Sea	69.47858	-144.38815	*S. malma*	-- / 9 / -- / --
Holo-type	John	Bering Sea	68.01700	-152.33900	*S. anaktuvukensis*	1 / 1 / -- / --
Para-type	John	Bering Sea	68.01700	-152.33900	*S. anaktuvukensis*	21 / -- / -- / --
					Total	148 / 128 / 186 / 17

[1]Collection sites located in northern Alaska. Populations ADT, AUT, GLC, and ADTA, are all from the Anaktuvuk River drainage; populations AVAN and KUG are from the Noatak River drainage; populations EK and GTC are from the Koyukuk River drainage; and population WUL is from the Wulik River drainage. Specimens all collected between 2006 and 2008, except for ADTA (2002), holotype (1968), and paratypes (1968).

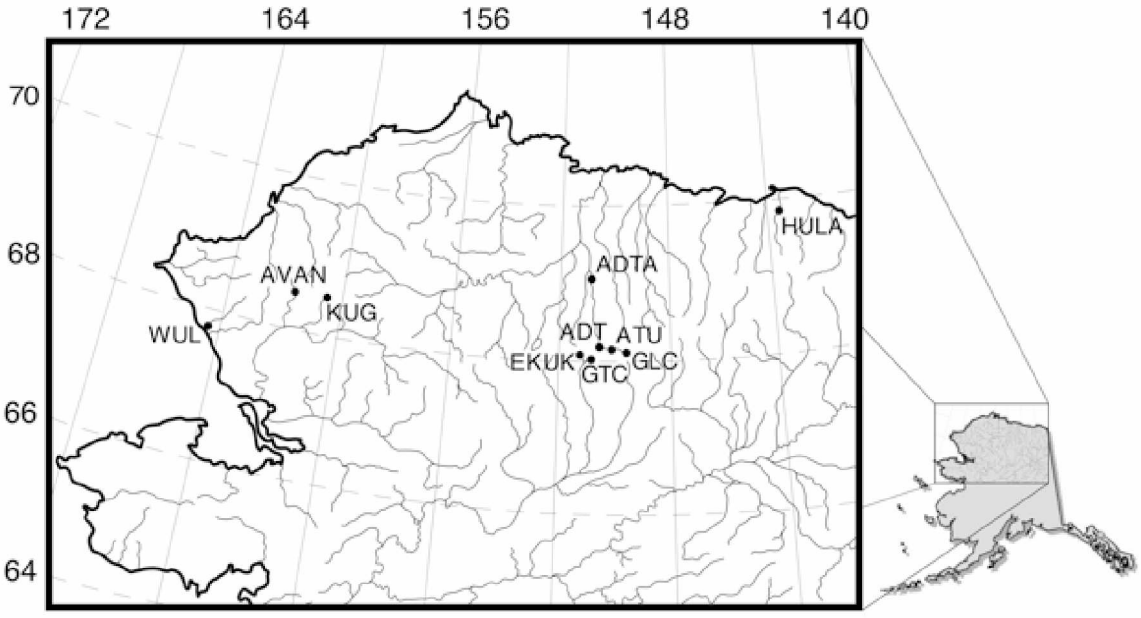

Figure 2.1 Sites of charr specimen collection in northern Alaska (●; Table 2.1). Sites ADTA, ADT, ATU, and GLC are all located in the Anaktuvuk River drainage. Sites EKUK and GTC are from the John River drainage. Sites AVAN and KUG are from the Noatak River drainage. Site HULA is from the Hulahula River. Site WUL is from the Wulik River.

Specimen collection within the primary study area occurred almost entirely during winter months when capture success rate was much higher. This success is attributed to a major constriction in available river habitat due to ice formation and winter stream-drying events throughout much of the study area (Craig, 1989). Remaining fish concentrate in deep pools or groundwater upwelling (spring) areas (Reynolds, 1997). Specimen collection at this time of year, while habitat is limited, increases the chance of collecting fish from distant locations. This, however, was considered to be a negligible factor as the type specimens for *S. anaktuvukensis* were also collected during midwinter, and the summer range for these fish remains unknown.

2.3.3 Meristic Counts and Morphometric Measures

Relevant meristic counts and morphological measures for species of *Salvelinus* were compiled from a review of similar studies (Morrow, 1973; Haas and McPhail, 1991, Reist et al., 1995; 1997; Scanlon, 2000). Meristics and morphology were the primary means of constructing phylogenies prior to the advent of genetic technology (Patterson et al., 1993) and the methods used for the original description of the Angayukaksurak charr (Morrow, 1973). Linear measurements are straight-line distances. Counts and measures were taken from the left hand side of fish after fixation in formalin and preservation in ethanol (Hubbs and Lagler, 1964; Morrow, 1973; Scanlon, 2000; Table 2.2). Body measurements were made with Fowler dial calipers accurate to 0.02 mm. A binocular dissecting microscope was used when necessary for measurements or counts.

Table 2.2 Literature sources and abbreviations for all meristic counts and measures used in this project (excluding geometric morphometric measures).

Abbreviation	Measure	Literature Source
STDL	Standard length	Hubbs and Lagler (1964)
DOFR	Dorsal fin rays	Hubbs and Lagler (1964)
ANFR	Anal fin rays	Hubbs and Lagler (1964)
PCFR	Pectoral fin rays	Hubbs and Lagler (1964)
PVFR	Pelvic fin rays	Hubbs and Lagler (1964)
BRAN	Branchiostegal rays	Strauss and Bond (1990)
LATL	Lateral line scale count	Hubbs and Lagler (1964)
PEDW	Peduncle width	Hubbs and Lagler (1964)
SNTL	Snout (pre-orbital) length	Scanlon (2000)
PRDL	Predorsal length	Hubbs and Lagler (1964)
DOAD	Dorsal to Adipose length	Hubbs and Lagler (1964)
PEFL	Pectoral fin length	Reist et al. (1995)
ORBW	Orbit (inter-orbital) width	Hubbs and Lagler (1964)
POBL	Post orbital length	Morrow (1973)
HEAW	Head width	Hubbs and Lagler (1964)
MAXL	Maxillary (upper jaw) length	Reist et al. (1995)
UGRC	Upper gill raker count	Hubbs and Lagler (1964)
LGRC	Lower gill raker count	Hubbs and Lagler (1964)
GLRC	Total gill raker count	Hubbs and Lagler (1964)
PYCC	Pyloric caeca count	Strauss and Bond (1990)

To ensure consistency, *S. anaktuvukensis* data from this study were compared to the original study (Morrow, 1973). Meristic counts from 21 specimens listed in Morrow's publication (holotype plus 20 paratypes) were added to the data collected as a separate group. A holotype is a single physical example of an organism, known to have been collected when the species was formally described, while paratypes are members of the type series that are collected at the same time in the same location as the holotype. Additionally, for corroboration, the holotype of *S. anaktuvukensis* (NMNH catalog #210965) was examined. Meristic and morphometric data collected from the holotype specimen were compared to counts in the original description to validate measurement accuracy and to check for potential biases.

Morphological shape was also used to examine differences between nominal species. This method has been used with success to differentiate cryptic species in prior studies (Strauss and Fuiman, 1985; Sidlauskas et al., 2007). Morphological measurements were obtained using the geometric morphometric (GM) method, which allows users to place landmark points that are discrete anatomical loci that can be located and recognized as the same on all specimens, on images of each specimen. GM changes the points on a three-dimensional specimen into two-dimensional coordinates, which can then be used to identify differences in shape. Variation due to differences in position, scale, and orientation are removed from each set of landmark coordinates using a General Procrustes Analysis (GPA), allowing all specimens to be compared simultaneously (Zelditch et al., 2004). Sixteen landmark locations were chosen to best display shifts in body form between groups (Figure 2.2).

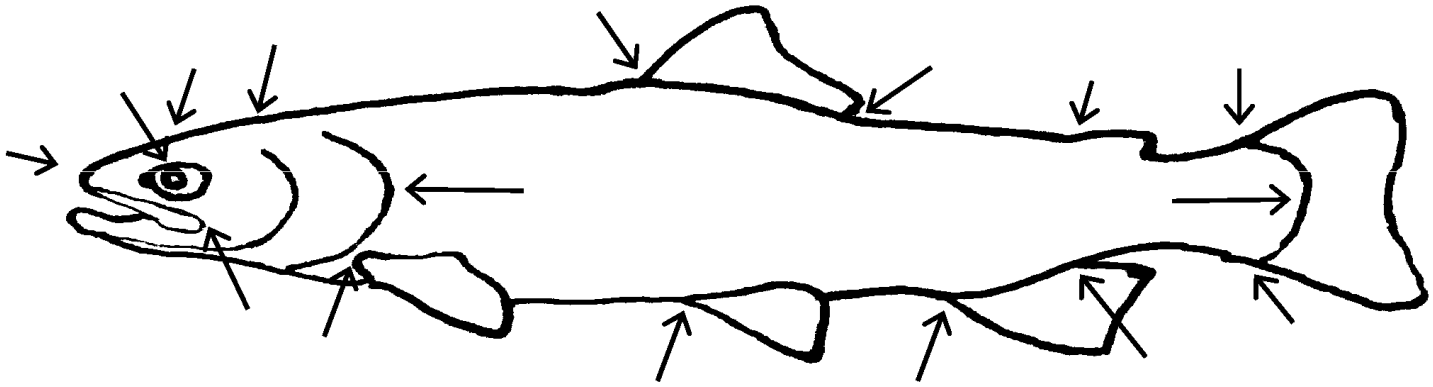

Figure 2.2 Locations of 16 landmark points on a representative charr specimen for geometric morphometric analysis of body shape to differentiate morphology among groups (e.g., life stages or species).

Digital images of specimens were captured using a Samsung GX10 DSLR camera mounted on a stand with multiple light sources. Fish were pinned to a foam board to ensure that all landmark points were clearly visible. Images were digitized and formatted using Rohlf's TpsUtil Program Version 1.38. Landmark points and a scale were added to each specimen image using the TpsDig2 Program Version 2.10 (both TPS programs available at the website of FJ Rohlf at SUNY Stony Brook, NY http://life.bio.sunysb.edu/morph/). MorphoJ software (Klingenberg, 2008) was used to scale landmark configurations to unit centroid size, align configurations to their consensus with a GPA, calculate partial warps of aligned specimens, and run a Principal Component Analysis (PCA) of the partial warps to display variations in shape. Classifier variables of nominal species and drainage were added to all specimens for interpretation of PCA figures.

2.3.4 Analysis of Meristic and Morphological Data

Principal component analysis was used to determine which, if any, variables discriminate between putative species groups based on meristic counts or morphometric measures using PRIMER (v.6; Clarke and Gorley, 2006). Groups were pre-assigned by study site, watershed, and region. Data were checked for normality, homogeneity of variances and covariances, and for correlations between means and variances. In an attempt to remove the confounding factor of size from morphometric measures, all measures were analyzed as proportions of standard length (SL) following Haas and McPhail (1991). Pretreatments of standardization by variable and transformation by square root were performed on the data prior to analysis.

Computation of a PCA of the covariance matrix of partial warps generated for the GM data was performed with MorphoJ (Klingenberg, 2008). PCA of the partial warps displays any groupings based on shape changes between populations and indicates those changes that account for the majority of the difference.

2.3.5 Mitochondrial and Microsatellite DNA Analysis

To understand the placement of the Angayukaksurak charr among members of *Salvelinus* and its relationship with Dolly Varden in close geographic range, two types of genetic loci (mitochondrial and microsatellite DNA) were examined (Brunner et al., 1998). The first objective was to sequence a portion of the control region of the mtDNA to place this nominal species within the holarctic Arctic charr lineages of Brunner et al. (2001). MtDNA is a useful marker for examining phylogenetic relationships among closely related species due to lack of recombination and rate of nucleotide substitution (in higher vertebrates) 5 to 10 times greater than within the nuclear genome (Billington, 2003). These characteristics allow genealogies to be tracked through time, with completely linked homologous markers and with rates of differentiation (nucleotide substitution) that display enough change to clarify relatively recent phylogenetic relationships.

Secondly, nuclear microsatellite DNA genotyping was used to clarify the associations between regional populations. These markers are usually highly polymorphic, even in small populations, as a result of high mutation rates, which make them advantageous for population differentiation (Allendorf and Luikart, 2007). Analysis

of microsatellite loci allows for identification of possible reproductive isolation between groups and can provide a test of species status.

Tissue samples for genetic analysis were obtained primarily from pectoral fin clips from the right-hand side of the specimens and, in the case of very small fish, whole organisms. Sample tissues were stored in a 95% ethanol solution until DNA extraction was performed using Qiaquick spin columns (QIAGEN, Inc., Valencia, California). The DNA was then stored at -20°C until analysis.

All sequencing of DNA materials follows the methods of Taylor et al. (2008). An approximately 1-kilobase pair fragment of the control region was amplified in an initial polymerase chain reaction (PCR) using the primers HN20 and Tpro2 (Brunner et al., 2001). PCR conditions included 0.1mM of each dNTP, 2mM $MgCl_2$, 0.2 μM of each primer, 1 unit of Invitrogen (Carlsbad, California) *Taq* polymerase, and 1X of the associated buffer (final concentrations in 50 μl final volumes). All PCR amplifications were run under the following conditions: 1 X (95°C for 3 min, 50°C for 1 min, 72°C for 1 min); 4 X (95°C for 1 min, 50°C for 1 min, 72°C for 1 min); 30 X (92°C for 30 s, 50°C for 30 s, 72°C for 30 s); and 1 X 72°C for 10 min. The fragment was purified using Qiagen columns, ethanol precipitated, air-dried, and cycle sequenced using the TPro-2 primer on an ABI automated sequencer using BigDye Terminator methods. The mtDNA sequences examined consist of a 550 base pair portion of the mtDNA control region. Two specimens were randomly selected from each collection location for sequencing, except for the Giant Creek site, where only one specimen was collected.

Individuals were scored for 9 microsatellite loci in this study, isolated from Dolly Varden (Smm22; Crane et al., 2004), bull trout (Sco106, 204, 206, 215, 216, 218, and 220; DeHaan and Ardren, 2005; S. Young, Wash. Dept. Fish and Wildlife, Olympia, WA, unpublished data), and brook charr (Sfo18, Angers et al., 1995). Amplification of loci from the samples was performed using fluorescent tagging and assaying on a Beckman-Coulter CEQ 8000 automated genotyper (Beckman Coulter, Inc., Fullerton, CA, USA). Microsatellite analyses were performed on all individuals from whom tissue samples were collected.

2.3.6 Genetic Data Analysis

Mitochondrial DNA sequences were examined from a range of divergences of previously resolved major lineages from holarctic *Salvelinus*, as well as several sequences from other taxa within the genus. Sequence data from specimens collected for this study were examined in comparison to the 41 haplotypes resolved by Brunner et al. (2001) and data from Redenbach and Taylor (2003) and Elz (2003). Homologous sequences were used from brook trout, white-spotted charr, and lake trout (*S. namaycush*), for phylogenetic analyses. The program BIOEDIT (Version 7.0.9.0; Hall, 1999; available at http://www.mbio.ncsu.edu/BioEdit/bioedit.html) was used to align the control region sequences of mtDNA. The programs MODELTEST (Version 3.7; Posada and Crandall, 1998) and MRMODELTEST (version 2.3; Nylander, 2004) were used to select nucleotide substitution models most appropriate for the data and analysis type. The Akaike Information Criterion (AIC) method of model selection was used to choose a model of sequence evolution because it appears better suited for evolutionary models

than the hierarchical likelihood ratio test due to greater comparison and selection power (Posada and Buckley, 2004). The model chosen by the AIC method was the General Time Reversible with among site rate heterogeneity and invariant sites (GTR+G+I) model. Measures of sequence divergence were calculated using this model, and these estimates were clustered with Parsimony using PAUP* 4.0 (Macintosh beta version 10; Swofford, 2002) under 1000 bootstrap replicates.

A Bayesian estimation of tree topology was obtained using the Markov Chain Monte Carlo (MCMC) implementation in MRBAYES (version 3.1.2; Ronquist and Huelsenbeck, 2003). Two replicates were run for 10,000,000 generations based on the (HKY+G+I) model selected by MRMODELTEST (version 2.3; Nylander, 2004), using random starting trees, with trees saved every 2000 generations. Sample plots of the posterior probability distribution suggested that the MCMC reached stationarity after 450 generations; therefore, the first 450 generations were discarded as burn-in. Tree diagrams from the bootstrap and MCMC analyses were produced using DENDROSCOPE (version 2.2.1, Huson et al., 2007) and then examined to determine whether samples collected for this study fit into the previously constructed lineage framework of Brunner et al. (2001) or constitute their own lineage. Lastly, divergence distances of mtDNA sequences (excluding outgroups) were examined using MESQUITE (Version 2.6; Maddison and Maddison, 2009) based on taxon pairs from the character matrix using uncorrected distance.

Raw microsatellite data were processed with the program MICRO-CHECKER (version 2.2.3; Van Oosterhout et al., 2004), to check for null alleles and scoring errors

due to stuttering and large allele drop-out. GENEPOP (version 4.0; Rousset, 2008) was used to examine Hardy-Weinberg equilibrium (random mating and null alleles), linkage disequilibrium (loci linkage), population differentiation (using an *exact G- test* to examine distribution of alleles), and allele and genotypic frequencies per locus and sample for microsatellite data. The program GENETIX (version 4.05; Belkhir et al., 2004) was used for calculation of *F*-statistics to examine levels of heterozygosity for population pairs, as well as to analyze allele frequency data using a Factorial Correspondence Analysis (FCA) to illustrate variation among populations by finding associations between allele frequencies at different loci.

2.4 Results

2.4.1 Meristic and Morphologic Variation

Principal component analysis of size-adjusted meristic characters produced five primary components that described approximately 87% of the total variation in the data set (Table 2.3), based primarily on the character of orbit width in the first axis and snout length, pyloric caeca counts, and gill raker counts from the upper gill arch in the second axis. Individual principal component scores for the two primary axes, when plotted, displayed groupings based on the classifier variable nominal species (Figure 2.3a). Groupings were also apparent, however, when the data were examined by the variable drainage basin (Figure 2.3b).

Table 2.3 Principal components analysis of meristic characteristics of 148 specimens of *Salvelinus malma* and putative *S. anaktuvukensis* from Bering, Beaufort, and Chukchi Sea drainages in northern Alaska. Percentage variability explained by each principal component axis (PC1-PC5) is presented individually and cumulatively. Loadings are presented for each meristic character measured.

Variable	PC1	PC2	PC3	PC4	PC5
Dorsal fin rays	-0.18	0.303	0.446	0.194	0.148
Anal fin rays	-0.226	0.348	0.478	0.2	0.227
Pectoral fin rays	-0.04	-0.004	0.102	-0.041	-0.074
Pelvic fin rays	-0.081	0.108	0.111	0.01	-0.01
Branchiostegal rays	0.087	-0.097	-0.029	-0.145	-0.093
Lateral line pores	-0.09	0.053	0.035	0	0.029
Snout length/SL	-0.054	-0.478	0.295	0.575	-0.034
Pre-dorsal length/SL	0.085	-0.079	-0.053	0.136	0.031
Orbit width/SL	0.882	0.21	0.105	0.285	-0.057
Post orbital length/SL	0.136	-0.042	-0.029	0.259	0.1
Head width/SL	-0.164	-0.184	-0.499	0.475	0.431
Upper gill Rakers	-0.02	-0.419	0.268	0.01	-0.361
Lower gill Rakers	-0.058	-0.301	0.169	-0.02	-0.311
Pyloric caeca	0.234	-0.427	0.31	-0.42	0.695
Variation per axis (%)	50.4	11.6	9.7	8.4	7.1
Cumulative variation (%)	50.4	62.0	71.7	80.0	87.1

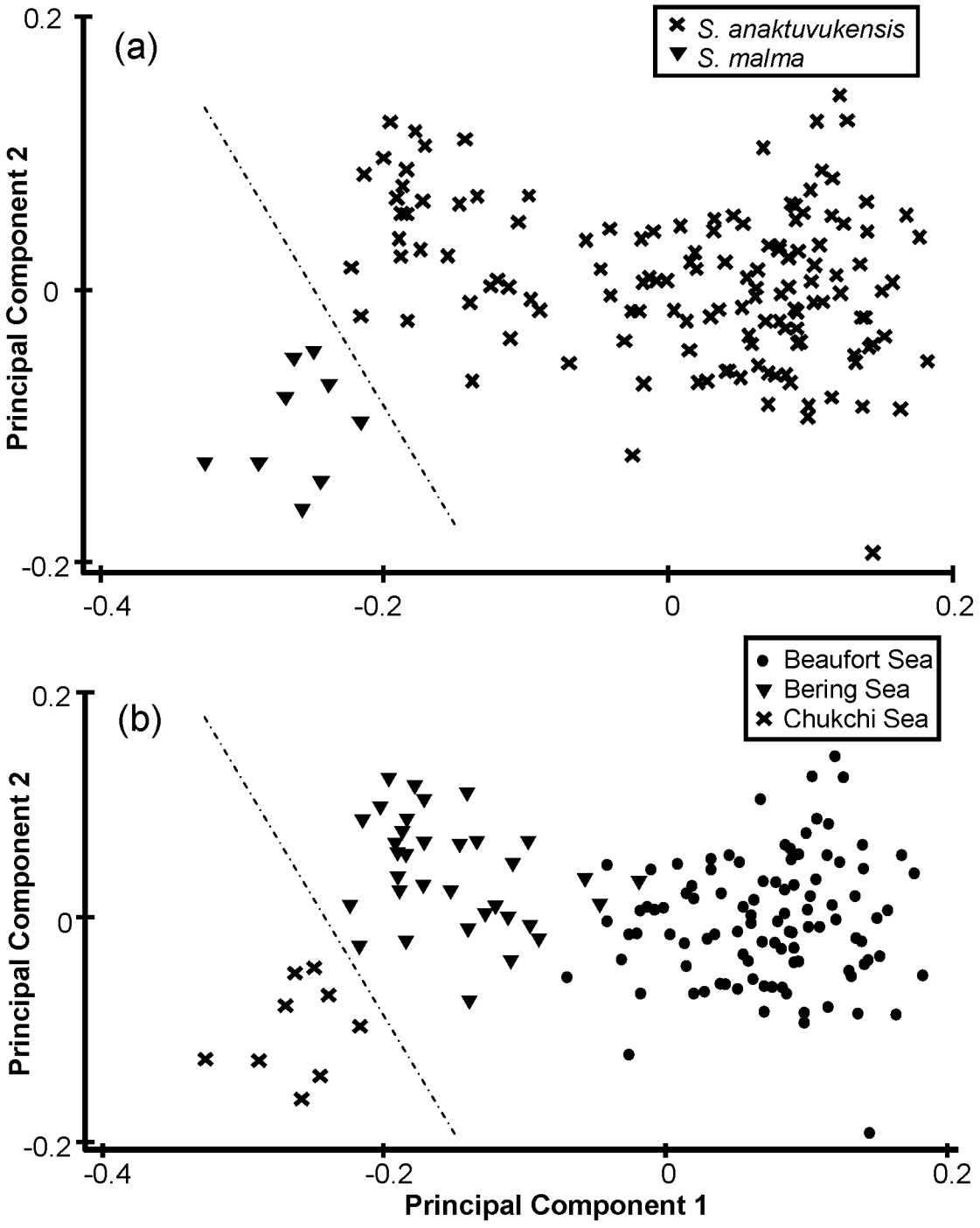

Figure 2.3 Graphical representation of principal component analysis of size adjusted meristic measures. Principal scores are plotted by (a) nominal species (*Salvelinus malma* and *S. anaktuvukensis*) and (b) major drainage basin of northern Alaska. Hatched lines indicate separation between groups.

Putative species grouping were also apparent along PCA axes based on geometric morphometric data. PCA performed on the covariance matrix of partial warps generated five axes that accounted for approximately 81% of the total variation within these groups (Table 2.4). Separation among groups was primarily along the PC1 axis (Figures 2.4a, 2.5), based upon changes in head shape (points 1 & 16), location of anterior insertion of the pelvic fin (point 13), and dorsal insertion of the caudal fin (point 8). These differences in shape are observed between these groups after initial removal of differences in position, scale, and orientation prior to analysis. Groupings were also examined by drainage (Figure 2.4b). No clear differentiation was evident; however, this may be confounded by a small sample size (Table 2.1) when samples are split in this manner.

Table 2.4 Principal components analysis of geometric morphometric measurements of fish landmarks (x1 – y16; Figure 2.3) from 128 specimens of *Salvelinus malma* and putative *S. anaktuvukensis* from Bering, Beaufort, and Chukchi Sea drainages in northern Alaska. Percentage variability explained by each principal component axis (PC1-PC5) is presented both individually and cumulatively. Loadings are presented for each morphometric landmark.

Variable	PC1	PC2	PC3	PC4	PC5
x1	0.357	0.168	0.155	-0.115	-0.173
y1	-0.024	0.298	0.169	0.090	0.288
x2	0.134	0.073	0.085	-0.008	0.087
y2	-0.158	0.126	-0.002	0.092	0.028
x3	0.119	0.117	0.126	0.022	0.149
y3	-0.218	0.087	0.074	0.065	0.229
x4	-0.154	0.093	0.092	0.353	-0.630
y4	-0.167	-0.061	0.088	-0.084	-0.101
x5	-0.143	-0.074	-0.074	-0.274	0.010
y5	0.080	-0.440	0.357	-0.264	-0.018
x6	-0.274	-0.144	0.040	-0.173	-0.241
y6	0.125	-0.376	0.340	0.022	0.021
x7	0.159	-0.058	0.204	0.191	0.123
y7	-0.051	-0.049	0.208	0.121	-0.071
x8	0.299	0.002	-0.080	0.076	0.085
y8	-0.200	0.136	0.027	-0.097	-0.048
x9	0.117	0.065	-0.159	0.094	-0.066
y9	-0.172	0.295	-0.071	-0.291	0.006
x10	0.183	0.170	-0.152	-0.170	0.084
y10	0.015	0.242	-0.067	-0.039	0.031
x11	-0.107	0.072	0.078	-0.120	-0.095
y11	0.049	0.058	0.026	0.185	0.027
x12	-0.048	0.118	0.136	0.036	-0.068
y12	0.184	-0.065	-0.140	0.347	0.128
x13	-0.292	-0.180	-0.083	0.356	0.047
y13	0.208	-0.300	-0.380	0.180	-0.048
x14	-0.113	-0.176	-0.304	-0.065	0.195
y14	0.132	-0.039	-0.230	-0.153	-0.180
x15	0.112	-0.066	0.114	-0.225	0.186
y15	0.077	0.188	-0.060	0.065	-0.048
x16	-0.349	-0.180	-0.177	0.024	0.308
y16	0.121	-0.101	-0.337	-0.237	-0.243
% Variation	37.0	26.1	10.2	4.8	3.6
% Cumulative Variation	37.0	63.2	73.3	78.1	81.7

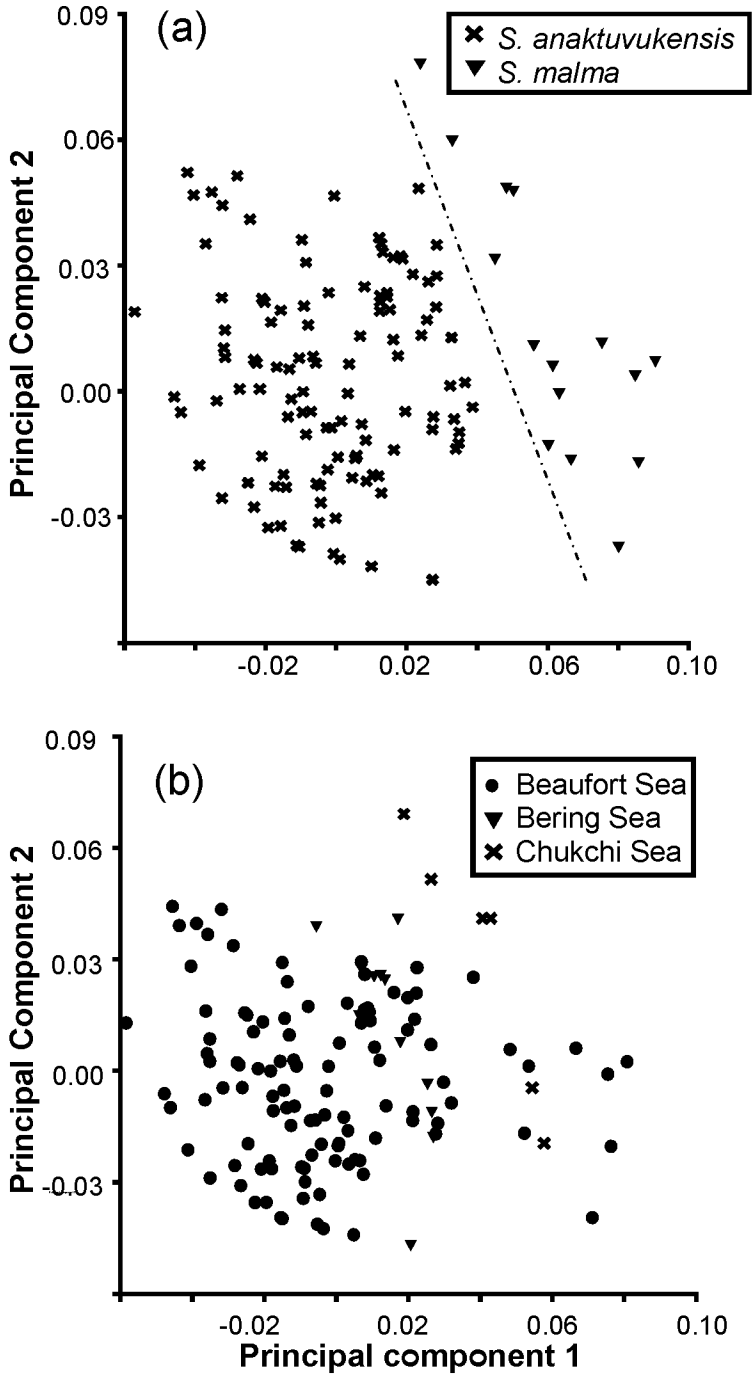

Figure 2.4 Principal component analysis results of geometric morphology based on 16 landmark points taken from digital images of *Salvelinus* specimens of northern Alaska. Each specimen is represented by one point on the graphs. Principal scores are plotted by (a) nominal species (*Salvelinus malma* and *S. anaktuvukensis*) and by (b) major drainage basin of northern Alaska.

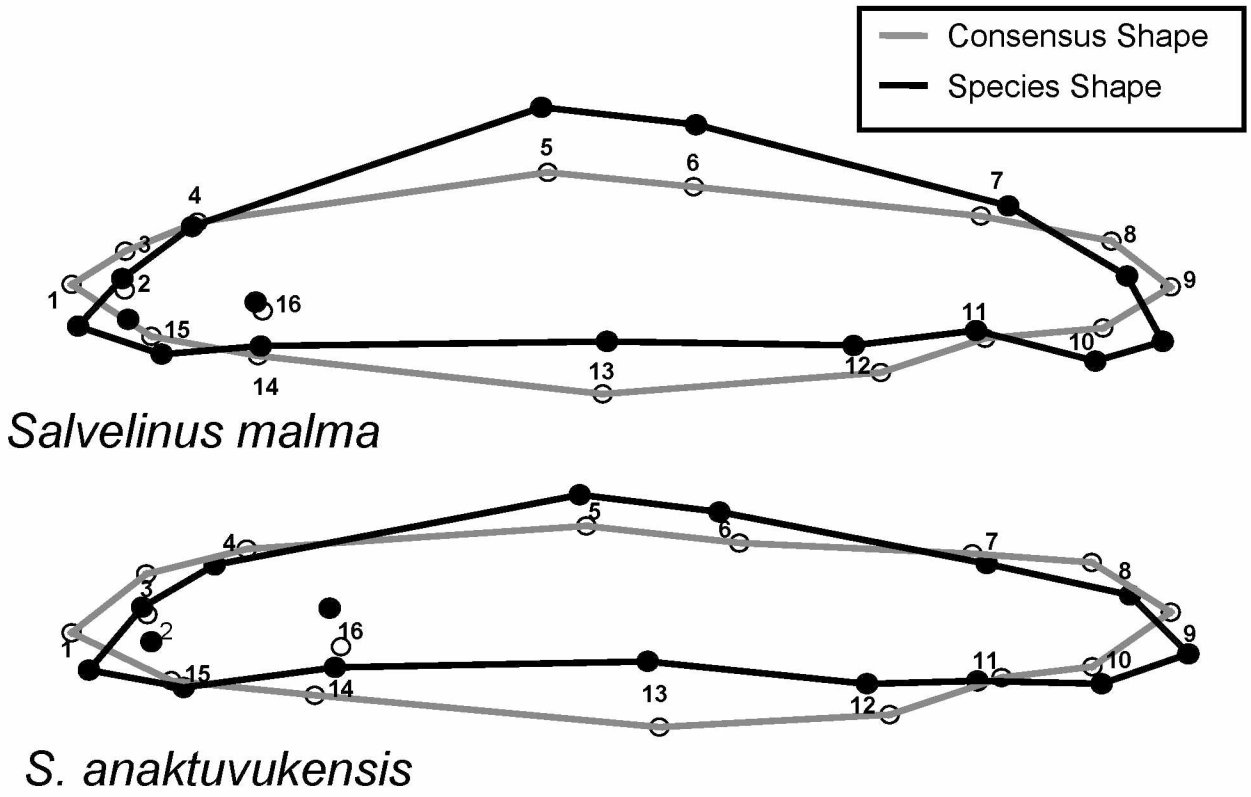

Figure 2.5 Consensus of shape changes (grey, based on the first 16 landmark points) between the nominal species *Salvelinus malma* and *S. anaktuvukensis* of northern Alaska. Displayed in black is the first principal component shape change, when *S. malma* (above) and *S. anaktuvukensis* (below) are considered separately.

2.4.2 MtDNA Phylogeography

Addition of mtDNA sequences from this project with sequences used in prior assessments of mtDNA phylogeny of the genus *Salvelinus* (Brunner et al., 2001; Taylor et al., 2008) indicate that there is no single distinct lineage for the Angayukaksurak charr (Figure 2.6). Rather, sequences from Angayukaksurak charr fall into the Bering and Arctic sister lineages of Arctic charr and Dolly Varden as resolved by Brunner et al. (2001) and Taylor et al. (2008). Although strong support is found for the out-groups (*Salvelinus namaycush*, *S. leucomaenis*, *S. fontinalis*) and other lineages (Acadian, Arctic, Atlantic, Siberian), there appears to be too little informative variation within this section of the mtDNA sequence to clearly differentiate the Bering lineage, which contained most of the Angayukaksurak charr sequences, from other lineages. Two Angayukaksurak charr sequences (haplotypes 2 and 4), however, did cluster with a distinctive group of "Arctic" lineage charr (Figure 2.6). Bootstrap and posterior probabilities (Figure 2.6) do support several of the individual sequences together as smaller groups within these lineages.

2.4.3 Population Differentiation (Microsatellite DNA)

Genetic differentiation at the population level was examined using microsatellite loci. Total number of alleles resolved ranged from 1 (Sfo18) to 25 (Sco204). Initial examination of population differentiation was based on 8 populations (or sampling locations) using 8 loci; however, one population (EK, John River, Bering Sea Drainage) was dropped from this analysis due to small sample size (n=11). Several violations of

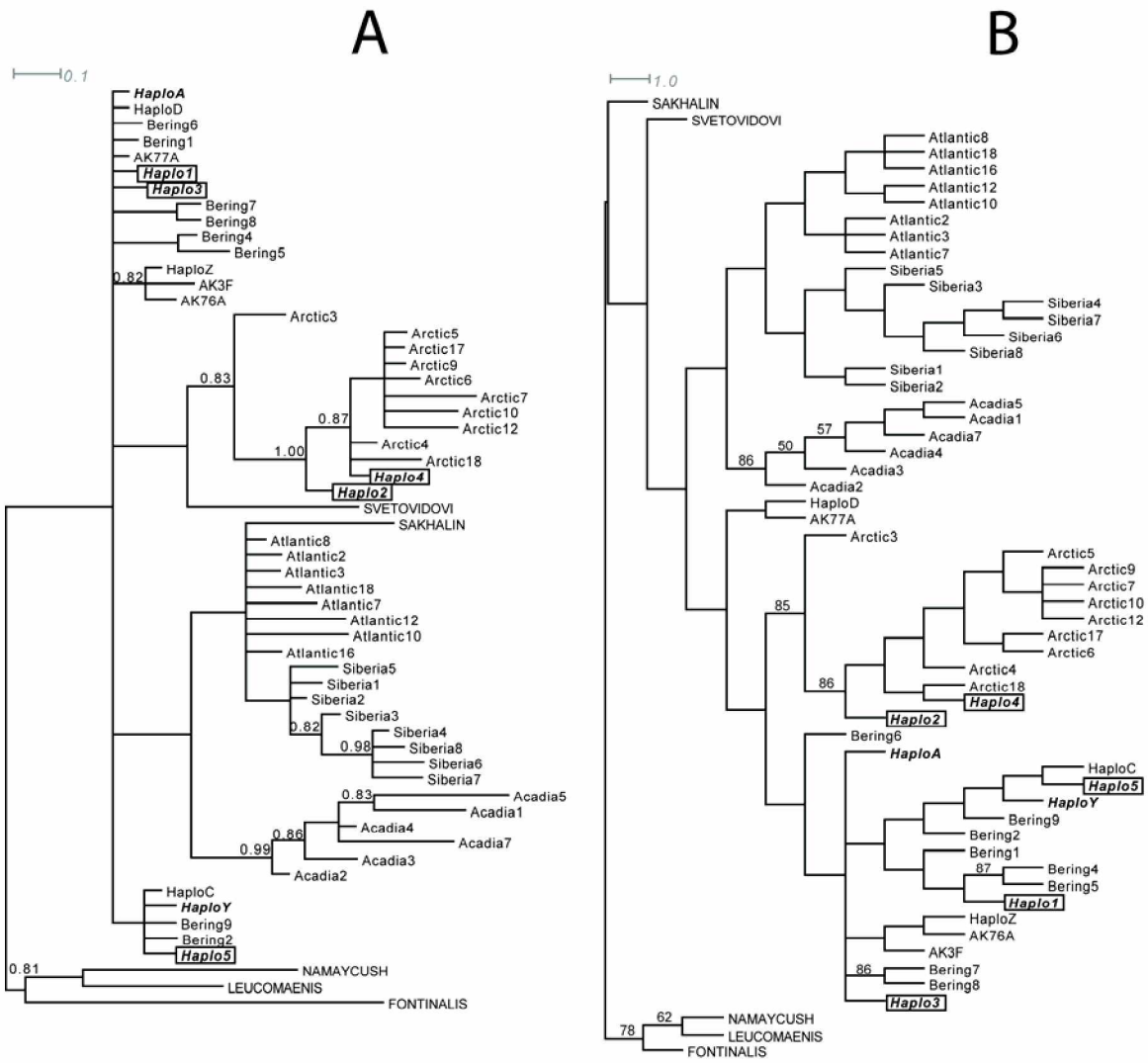

Figure 2.6 Mitochondrial DNA phylogenetic trees summarizing relationships of holarctic *Salvelinus alpinus* haplotypes and outgroups combined with *S. anaktuvukensis* and *S. malma* samples collected for this project derived from 550 bp section of the control region. Trees constructed using (a) Bayesian Marcov chain Monte Carlo approach, and (b) Parsimony clustering of pairwise distances among haplotypes. Posterior probabilities (>80) are noted above branch points (a), parsimony bootstrap scores for 1000 replications (>60%) indicated above branch points (b). Haplotypes found in samples collected for this project are italicized and bold, new haplotypes (not in previous papers) are also inside boxes. Identity and locality of each haplotype as well as references are given in Appendix 2B.

Hardy-Weinberg Equilibrium (HWE) were noted after sequential Bonferroni testing (Rice, 1989), particularly within the locus Sco109 (Table 2.5). This was due to heterozygote deficiencies and necessitated removal of this locus from further analysis (see Appendix 2.A for further details). Following these modifications, analysis of microsatellite loci showed no evidence of linkage disequilibrium, implying independence of loci across all populations. Variability displayed between groups (species) was similar to variability displayed between populations within groups. Between group variation (F_{CT}), after accounting for variation among populations and within groups, was small (3%) but statistically significant according to Analysis of Molecular Variance (AMOVA) results (Table 2.6). Variability among populations within groups (F_{ST}) showed that populations closest geographically are also the least genetically different (Table 2.7). Also, as expected, the largest amount of allelic variability was from within populations (<92%). These results imply that differentiation between populations is as great as between putative species. This is also displayed by the FCA (Figure 2.7), which shows that different populations of nominal species do not spatially segregate from each other, nor do they show distinct segregation between species.

Table 2.5 Sequential Bonferroni adjusted Hardy Weinberg Equilibrium (HWE) *p*-values for each population of the nominal species *Salvelinus malma* and *S. anaktuvukensis*, at each locus (*p*-value / sequential Bonferroni α/k value). ** denotes population out of HWE.

Popn[1]	Sfo18	Smm22	Sco106	Sco109	Sco218	Sco202	Sco204	Sco220
ADT	-	0.459 / 0.017	0.177 / 0.010	0.00 / 0.007**	0.405 / 0.017	1.000 / 0.050	0.322 / 0.013	0.053 / 0.008
AUT	-	0.140 / 0.008	0.381 / 0.010	0.026 / 0.007	0.746 / 0.017	0.425 / 0.013	0.980 / 0.050	0.850 / 0.025
GLC	-	0.273 / 0.010	0.502 / 0.017	0.017 / 0.007	0.799 / 0.025	1.000 / 0.050	0.412 / 0.013	0.025 / 0.008
KUG	-	0.319 / 0.010	0.022 / 0.008	0.499 / 0.017	0.467 / 0.013	1.000 / 0.050	0.601 / 0.025	0.009 / 0.007
EK	-	0.107 / 0.010	0.512 / 0.017	0.404 / 0.013	-	1.000 / 0.025	0.102 / 0.008	1.000 / 0.050
WUL	-	0.088 / 0.013	0.014 / 0.008	0.005 / 0.007**	0.451 / 0.017	0.527 / 0.050	0.506 / 0.025	0.029 / 0.010
AVAN	-	0.008 / 0.008	0.00 / 0.007**	0.838 / 0.025	0.176 / 0.010	1.000 / 0.050	0.355 / 0.017	0.206 / 0.013
ADTA	-	0.888 / 0.050	0.00 / 0.071**	0.00 / 0.008**	0.024 / 0.010	0.558 / 0.025	0.091 / 0.013	0.334 / 0.017

[1]Collection sites located in northern Alaska. Populations ADT, AUT, GLC, and ADTA, are all from the Anaktuvuk River drainage; populations AVAN and KUG are from the Noatak River drainage; population EK is from the Koyukuk River drainage; and population WUL is from the Wulik River drainage.

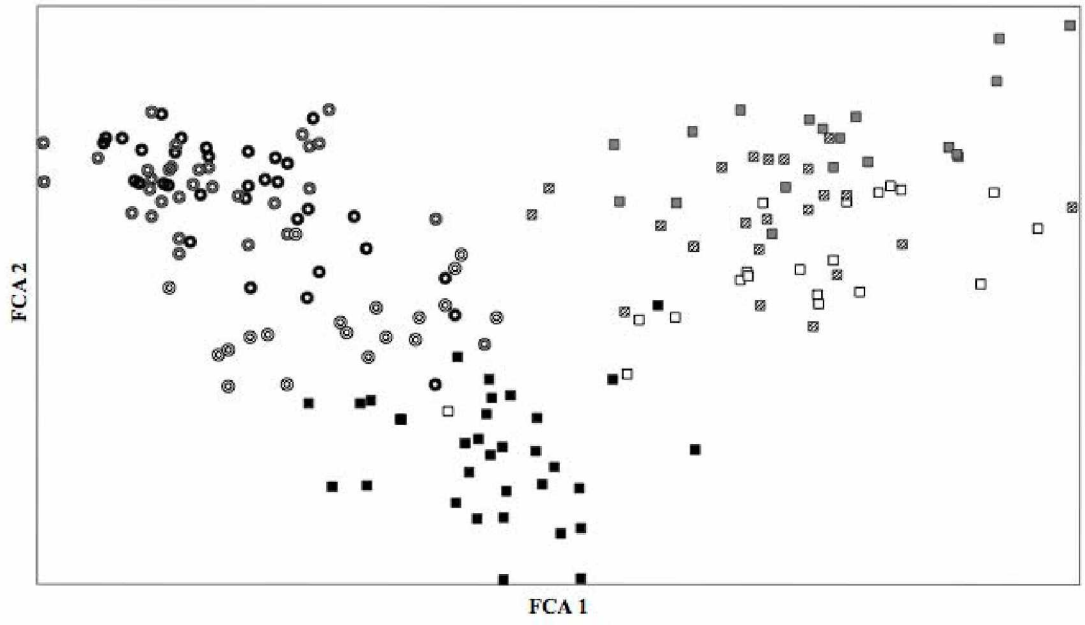

Figure 2.7 Display of factorial correspondence analysis performed on microsatellite locus data. Circles represent individuals of *Salvelinus anaktuvukensis* and squares represent individuals of *S. malma*. Populations are denoted by unique colors associated with symbols (squares [4 populations] or circles [3 populations]).

Table 2.6 Results of AMOVA test on microsatellite markers from populations of the nominal species *Salvelinus malma* and *S. anaktuvukensis* in northern Alaska. Analysis based on 2 groups, 7 populations, and 7 microsatellite loci.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Components	Percentage of Variation
Among groups	1	8.997	0.04891 Va	2.25
Among populations within groups	5	22.836	0.10721 Vb	4.93
Within populations	164	331.267	2.01992 Vc	92.83
Total	170	363.099	2.17604	
Fixation indices	FSC: 0.0504	FST: 0.07174	FCT: 0.02248	
Significance tests	1023 Permutations			
Vc and FST	P(rand. value	< obs. value)	=	0.00000
	P(rand. value	= obs. value)	=	0.00000
			P-value	0.00000+-0.00000
Vb and FSC	P(rand. value	< obs. value)	=	0.00000
	P(rand. value	= obs. value)	=	0.00000
			P-value	0.00000+-0.00000
Va and FCT	P(rand. value	< obs. value)	=	0.00000
	P(rand. value	= obs. value)	=	0.03030
			P-value	0.03030=-0.00487

Table 2.7 F_{ST} scores for population pairs based on seven microsatellite DNA loci (Sfo18, Smm22, Sco106, Sco202, Sco204, Sco218, and Sco220) on top right; pairwise *P*-values for the F_{ST} scores (chance of Type I error) on bottom left. Angayukaksurak charr populations are bold and underlined.

Popn[1]	**ADT**	**AUT**	**GLC**	KUG	WR	AR	ADTA
ADT	------	0.022	0.058	0.047	0.043	0.060	0.068
AUT	0.000	------	0.052	0.044	0.040	0.057	0.062
GLC	0.000	0.016	------	0.049	0.051	0.062	0.053
KUG	0.000	0.000	0.020	------	0.011	0.016	0.055
WR	0.000	0.000	0.000	0.000	------	0.013	0.030
AR	0.000	0.000	0.000	0.000	0.000	------	0.044
ADTA	0.000	0.000	0.000	0.000	0.000	0.000	------

[1]Collection sites are located in northern Alaska. Populations ADT, AUT, GLC, and ADTA, are all from the Anaktuvuk River drainage; populations AVAN and KUG are from the Noatak River drainage; population EK is from the Koyukuk River drainage; and population WUL is from the Wulik River drainage.

2.5 Discussion

Previous research concerning the Angayukaksurak charr has resulted in a lack of consensus regarding its taxonomic status (Morrow, 1973; Behnke, 1980; McCart, 1980; Page and Burr, 1991; Mecklenburg et al., 2002). Until now, it has been unclear whether the Angayukaksurak charr is a valid species or a form of Dolly Varden. Previous conclusions on the status of the Angayukaksurak charr were based entirely on studies of meristic characters (Morrow, 1973; McCart, 1980) and have been considered insufficient to determine its status (Nelson et al., 2004). Our comprehensive analysis of meristic, morphological, and genetic characteristics of the species largely indicates that species status is unwarranted for the Angayukaksurak charr; instead *S. anaktuvukensis* represents a life history variant of a species well known for plasticity in form and life history, the Dolly Varden.

2.5.1 Evidence of Phenotypic Differentiation

The meristic characters cited by Morrow (1973), in his opinion, substantiate differentiation of the Angayukaksurak charr as an independent taxa of species rank. This conclusion resulted mainly from establishing a significant difference from other forms of *S. malma* and *S. alpinus* based on dorsal ray and anal fin ray counts (McCart, 1980). These characters, however, are less distinct within this population than from within other populations of *S. malma* located above barriers to migration in this same region (McCart, 1980; Appendix 2.C). Thus, the argument has been made that as phenotypic expression within *S. malma* is plastic, it should not be used to designate species status. To this point,

molecular markers were used in this study in an attempt to clarify relations in this phenotypically variable group.

PCA analyses of meristic characters from *S. malma* and *S. anaktuvukensis* separate these two groups in multivariate space based primarily on orbit width. Growth rate, however, increases differentially in terms of morphology over ontogeny (Nicieza, 1995). Given the differences in the size range of specimens of the two groups examined (standard length: *S. anaktuvukensis* 62-195 mm; *S. malma* 363-603 mm), this grouping may represent ontogenetic shifts in proportion, even after length adjusting all measurements. Interestingly, although the groups segregate when examined by species designation, they also segregate (more distinctly) when examined by drainage basin (Figure 2.3a, b). Although the headwaters of the drainages for *S. anaktuvukensis* are less than 1 km from each other, they drain into entirely different basins over a broad region (Anaktuvuk River into the Beaufort Sea, and John River into the Bering Sea). This spatial segregation may allow for enough minor differentiation to occur in morphology such that these populations would group by drainage. For a genus that displays high variability in phenotypic expression, this differentiation is not surprising.

Morphology also showed differentiation between *S. anaktuvukensis* and *S. malma* based on body shape. PCA results displayed two groups (Figure 2.4). Differences in overall body shape, however, are not unexpected between resident and anadromous fish (Nicieza, 1995). Anadromous (*S. malma*) fish images were captured directly upon return to freshwater (after summer feeding in the marine environment) in an attempt to decrease the amount of secondary sexual characteristics present; however, any development of

these characters, together with the overall size differences between the two groups, could drive the observed differences in body shape.

Ultimately, although evidence of grouping from both the meristic counts and the morphometric measures exists, it does not support differentiating *S. malma* and *S. anaktuvukensis* into two separate species. This conclusion is strengthened by the assertion that other isolated populations of *S. malma* in the region display even larger differences in meristic counts than those used to differentiate *S. anaktuvukensis* from *S. malma* and *S. alpinus* in the species description (McCart, 1980). We concluded that minor differences between these groups are most likely the result of different life history patterns and specimen lengths and do not warrant segregating them as separate species.

2.5.2 Phylogenetic Structure Based on Mitochondrial DNA

Phylogeny trees produced from the 53 unique sequences of holarctic *Salvelinus* (Figure 2.6) from a 550 bp fragment of the control region of mtDNA show no structure based on specimens of *S. anaktuvukensis*. Rather, *S. anaktuvukensis* samples fit within two other lineages, the Bering lineage that covers much of Alaska and the Chukotka Peninsula of Russia south of the Bering Strait, and the Arctic lineage that covers most of Arctic Canada, Arctic Alaska, and the Chukotka Peninsula of Russia north of the Bering Strait. The fit of mtDNA sequences into these lineages appears to split in Alaska at the continental divide, with specimens on the north side of the divide part of the Arctic lineage and those from the south side part of the Bering lineage. Angayukaksurak charr specimens not only fit into these two lineages (Arctic and Bering), but also do not show a

unique lineage of their own and consequently refute the notion that they are a separate species based on this marker.

Further examination of this section of mitochondrial DNA based on pairwise sequence divergence distances of the *S. alpinus*/*S. malma* group (excluding outgroups) shows two minor peaks of divergence (Figure 2.8a). Examined again as within-lineage divergence (each lineage assessed separately), the first peak of very low divergence is predominately from the Bering lineage (Figure 2.8b), giving it the lowest power to detect differentiation. Although some *S. anaktuvukensis* specimens were placed within the Arctic lineage, most were located within the Bering lineage, which appears to be the least divergent from the common ancestor and may not show enough structure to segregate them into their own group. This result suggests that the use of more variable genetic markers, such as microsatellite DNA, is necessary to detect differentiation between *S. malma* and *S. anaktuvukensis*.

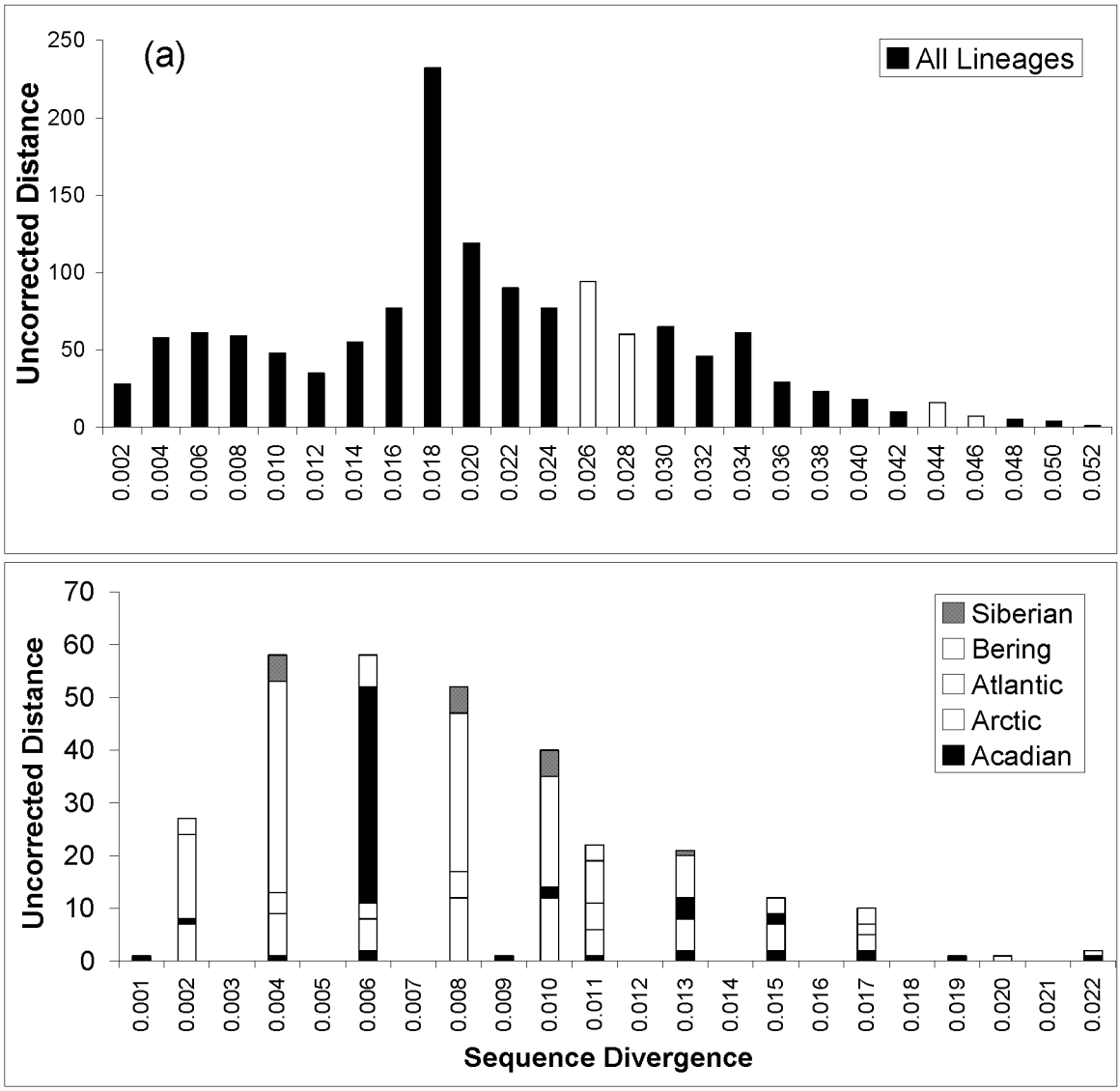

Figure 2.8 Pairwise comparisons of mitochondrial DNA sequence divergence among populations of holarctic *Salvelinus* (*S. alpinus* and *S. malma*, including *S. anaktuvukensis*) based on 53 unique haplotypes analyzed for this study. (a) Pairwise comparisons based upon haplotype sequences for all lineages (excluding outgroups); (b) Pairwise comparisons among lineages only.

2.5.3 Population Differentiation in Riverine Charrs of Northern Alaska

Microsatellite (nuclear) DNA did not differentiate between *S. malma* and *S. anaktuvukensis*. Fixation index values (F_{ST}) displayed heterozygosity levels expected from populations that were randomly mating. As would be expected in a single mixing group, populations closer geographically (by drainage terminus) were more closely related (Table 2.7), suggesting an isolation by distance structure (Wright, 1943) for Dolly Varden in northern Alaska. Examination of allele frequencies over all loci and populations failed to resolve any novel alleles that would segregate these two groups (e.g., Sfo18 has been proven in other studies to clearly differentiate between *S. malma* and *S. alpinus* populations; Taylor et al., 2008). This lack of novel alleles, combined with no clear change in allele frequency or number, suggest a lack of genetic differentiation between the two types of charr. Testing for variation between *S. malma* and *S. anaktuvukensis* (F_{CT}), after accounting for variation among populations within groups, showed a small (3%), but significant, differentiation, indicating some distinction (Table 2.6). This value is, however, likely an indication of the fact that the groups are from spatially (geographic) different regions and watersheds, and the *S. anaktuvukensis* specimens are likely resident (non-anadromous) fish. More population differentiation occurred among life history groups than between species; variation between groups, in fact, was far less than variation observed within groups. In summary, we conclude that these two putative species do not clearly segregate from each other based on microsatellite DNA markers.

No unique markers or traits were able to differentiate these two putative species as more than life history variants of the same species. Although some differentiation was observed within the meristic counts and the morphometric measures, it was not at levels greater than would be expected in a species that shows plasticity in phenotype and life history strategy. Mitochondrial DNA show that *S. anaktuvukensis* fit within the lineages of the *S. alpinus*/*S. malma* groups; however, they do not segregate by themselves, and microsatellite DNA suggests that our *S. anaktuvukensis* and *S. malma* specimens were all a part of a larger mixing population. Thus, while the different methods used vary in the manner in which they group specimens, they all lead us to the same conclusion, namely, that the Angayukaksurak charr is not a distinct species.

2.5.4 General Conclusions

Alaska has not proven to be an area of localized endemism; rather, it is inhabited by widespread species characterized by tolerance and a diversity of life history strategies. Even species with restricted ranges in Alaska, such as the Alaska blackfish *Dallia pectoralis*, are still widely dispersed, with native distributions in both Alaska and the far east of Russian Siberia (Gudkov, 1998; Mecklenburg et al., 2002). Diversity in Alaska's freshwater fish fauna is displayed in terms of plasticity, which contributes to the resilience and genetic diversity of populations, rather than large numbers of species. For example, an event that extirpates a cohort of young-of-year salmon from a single stream, an entire river, or series of drainages is overcome by adults from other cohort years retuning to spawn at differential rates (i.e., after 1-4 years), ensuring persistence of populations (Hilborn et al., 2003). In similar fashion, species that have anadromous and

resident populations in the same river system are buffered to some extent from events that may extirpate one of these runs, as the other can re-populate it in time. As environments in Alaska and the entire far north are dynamic over time, it is more advantageous for species to retain this plasticity rather than to adapt to tightly constrained niches.

Although the Angayukaksurak charr of the Anaktuvuk region of northern Alaska does not represent a species according to our results, or a reproductively isolated evolutionarily significant unit, it still represents an important source of biological diversity in Alaska. In essence, the life history variability within Dolly Varden and other salmonids of the Arctic is an important component of the species' evolutionary legacy, allowing them to persist in the variable and harsh environments of the far north. Even though species designation is not warranted, this component of life history diversity is worthy of conservation attention, given that the reservoir of genetic diversity that it represents is necessary for the species to respond to environmental variability, particularly in light of the heightened impact of global climate change on Arctic ecosystems (Reist et al., 2006; Wrona et al., 2006).

2.6 Acknowledgments

The authors would like to thank the following individuals for their invaluable assistance on this project: G. Haas for helping to initiate this project and advice; F. DeCicco for advice and field assistance; B. Sidlauskas for methodological assistance; J. Reist and N. Hughes for thoughtful advice; J.A. Lopez for assistance with lab work, methodology, and reviews; and committee members N. Hillgruber and A. Larsen for advice, assistance, and manuscript revisions.

Specimens were collected under permits from the Alaska Department of Fish and Game (SF2006-143, SF2007-047, and SF2008-015), under IACUC #06-14 from the University of Alaska Fairbanks (UAF), and with permissions from the U.S. National Park Service compliance team, the City of Anaktuvuk Pass, and the Arctic Slope Regional Corporation. Additionally, the National Museum of Natural History kindly lent the holotype specimen of *S. anaktuvukensis* for examination. Thanks to UAF Life Science Informatics Portal for computational assistance. Funding for this project came from the U.S. National Park Service Arctic Network Inventory and Monitoring Program (North and West Alaska CESU agreement H9910030024, task J9910324501) and the University of Alaska Fairbanks School of Fisheries and Ocean Sciences.

2.7 Literature Cited

Allendorf, F. W., and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Oxford.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology. 47:177-185.

Behnke, R. J. 1980. A review of the genus *Salvelinus*, p. 441-480. *In:* Charrs: Salmonid Fishes of the Genus *Salvelinus*. E. K. Balon (ed.). Dr. W. Junk Publishers, The Hague, the Netherlands.

Belkhir, K., P. Borsa, N. Chikhi, N. Raufaste, and F. Bonhomme. 2004. GENETIX 4.03, Logiciel Sous Windows TM Pour la Ge'ne'tique des Populations in Laboratoire Ge'nome, Populations, Interactions, CNRS UMR 5000. Universite' de Montpellier II, Montpellier, France.

Billington, N. 2003. Mitochondrial DNA, p. 50-100. *In*: Population Genetics: Principles and Applications for Fisheries Scientists. E. M. Hallerman (ed.). American Fisheries Society, Bethesda, MD.

Brunner, P. C., M. R. Douglas, and L. Bernatchez. 1998. Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr *Salvelinus alpinus* (Teleostei: Salmonidae) from central Alpine lakes. Molecular Ecology. 7:209-223.

Brunner, P. C., M. R. Douglas, A. Osinov, C. C. Wilson, and L. Bernatchez. 2001. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus L.*) inferred from mitochondrial DNA sequences. Evolution. 55:573-586.

Cavender, T. M. 1978. Taxonomy and distribution of the bull trout *Salvelinus confluentus* (Suckley), from the American northwest. California Fish and Game. 64:139-174.

Clarke, K. R., and R. N. Gorley. 2006. Primer v6: User Manual/Tutorial. PRIMER-E, Plymouth, UK.

Craig, P. C. 1989. An introduction to anadromous fishes in the Alaskan Arctic, p. 27-54. *In*: Research advances on anadromous fish in Arctic Alaska and Canada. D. W. Norton (ed.). Biological Papers of the University of Alaska, Vol. 24, Institute of Arctic Biology, Fairbanks, Alaska.

Crane, P. A., C. J. Lewis, E. J. Kretschmer, S. J. Miller, W. J. Spearman, A. L. DeCicco, M. J. Lisac, and J. K. Wenburg. 2004. Characterization and inheritance of seven microsatellite loci from Dolly Varden, *Salvelinus malma*, and cross-species amplification in Arctic char, *S. alpinus*. Conservation Genetics. 5:737-741.

Crane, P. A., L. W. Seeb, and J. E. Seeb. 1994. Genetic relationships among *Salvelinus* species inferred from allozyme data. Canadian Journal of Fisheries and Aquatic Sciences. 51:182-197.

Crane, P. A., T. Viavant, and J. K. Wenburg. 2005. Overwintering patterns of Dolly Varden *Salvelinus malma* in the Sagavanirktok River in the Alaska North Slope inferred using mixed-stock analysis. U.S. Fish and Wildlife Service, Alaska Fisheries Technical Report, Vol. 84, Anchorage, Alaska.

Davis, G. J., and J. F. Webb. 1993. A review of research on lake resident Arctic char, *Salvelinus alpinus*: 1980-1992. Bureau of Land Management, Kobuk District, Fairbanks, Alaska.

DeHaan, P. W., and W. R. Ardren. 2005. Characterization of 20 highly variable tetranucleotide microsatellite loci for bull trout (*Salvelinus confluentus*) and cross-amplification in other *Salvelinus* species. Molecular Ecology Notes. 5:582-585.

DeLacy, A. C., and W. M. Morton. 1943. Taxonomy and habits of the charrs *Salvelinus malma* and *Salvelinus alpinus* of the Karluk drainage system. Transactions of the American Fisheries Society. 72:79-91.

Elz, A. E. 2003. A hierarchical analysis of historical processes and phylogeographic patterns in *Salvelinus* (Pices: Salmonidae). Unpubl. M.S. thesis, University of British Columbia Department of Zoology, Vancouver, British Columbia.

Frohne, I. V. 1973. Statistical analyses of discrete morphology in northern populations of the fish genus *Salvelinus*. Biological Papers of the University of Alaska. 13:10-20.

Gates, G. O., and G. Gryc. 1963. Structure and tectonic history of Alaska, p. 264-277. *In:* Backbone of the Americas. O. E. Childs and B. W. Beebe (eds.). American Association of Petroleum Geologists, Tulsa, Oklahoma.

Gross, M. R. 1987. Evolution of diadromy in fishes. American Fisheries Society Symposium. 1:14-25.

Gudkov, P. K. 1998. Bering Sea *Dallia pectoralis* in the Chukchi Peninsula. Journal of Ichthyology. 38:199-203.

Haas, G. R., and J. D. McPhail. 1991. Systematics and distributions of Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America. Canadian Journal of Fisheries and Aquatic Sciences. 48:2191-2211.

—. 2001. The post-Wisconsinan glacial biogeography of bull trout (*Salvelinus confluentus*): a multivariate morphometric approach for conservation biology and management. Canadian Journal of Fisheries and Aquatic Sciences. 56:2189-2203.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/97/NT. Nucleic Acids Symposium Series. 41:95-98.

Hamilton, T. D. 1986. Late Cenozoic glaciation of the central Brooks Range, p. 9–49. *In:* Glaciation in Alaska: The Geologic Record. T. D. Hamilton, K. M. Reed, and R. M. Thorson (eds.). Alaska Geological Society, Anchorage.

Hamilton, T. D., K. M. Reed, and R. M. Thorson. 1986. Glaciation in Alaska: introduction and overview, p. 1-8. *In:* Glaciation in Alaska: The Geologic Record. T. D. Hamilton, K. M. Reed, and R. M. Thorson (eds.). Alaska Geologic Society, Anchorage, Alaska.

Hendry, A. P., J. K. Wenburg, P. Bentzen, E. C. Volk, and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science. 290:516-518.

Hilborn, R., T. P. Quinn, D. E. Schindler, and D. E. Rogers. 2003. Biocomplexity and fisheries sustainability. Proceedings of the National Academy of Sciences. 100:6564-6568.

Hubbs, C. L., and K. F. Lagler. 1964. Fishes of the Great Lakes region. University of Michigan Press, Ann Arbor, Michigan.

Huson, D. H., D. C. Richter, C. Rausch, T. Dezulian, M. Franz, and R. Rupp. 2007. Dendroscope - An interactive viewer for large phylogenetic trees. BMC Bioinformatics. 8:460.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries. 33:372-407.

Johnson, L. 1980. The arctic charr, *Salvelinus alpinus*, p. 15-98. *In:* Charrs: Salmonid Fishes of the Genus *Salvelinus*. E. K. Balon (ed.). Dr. W. Junk, The Hague, the Netherlands.

Klingenberg, C. P. 2008. MorphoJ. Faculty of Life Sciences, University of Manchester, UK. http/flywings.org.uk/MorphoJ_page.htm.

Lindsey, C. C., and J. D. McPhail. 1986. Zoogeography of fishes of the Yukon and Mackenzie basins, p. 639-674. *In:* The Zoogeography of North American Freshwater Fishes. C. H. Hocutt and E. O. Wiley (eds.). John Wiley and Sons, New York, New York.

Maddison, W. P., and D. R. Maddison. 2009. Mesquite: a modular system for evolutionary analysis, v2.6. http://mesquiteproject.org.

McCart, P. J. 1980. A review of the systematics and ecology of the Arctic char, *Salvelinus alpinus*, in the western Arctic. Canadian Technical Report of Fisheries and Aquatic Sciences. 935.

McCart, P. J., and H. Bain. 1974. An isolated population of Arctic char (*Salvelinus alpinus*) inhabiting a warm mineral spring above a waterfall at Cache Creek, Northwest Territories. Journal of the Fisheries Research Board of Canada. 31:1408-1414.

McCart, P. J., and P. C. Craig. 1973. Life history of two isolated populations of Arctic char (*Salvelinus alpinus*) in spring-fed tributaries of the Canning River, Alaska. Journal of the Fisheries Research Board of Canada. 30:1215-1220.

McPhail, J. D. 1961. A systematic study of the *Salvelinus alpinus* complex in North America. Journal of the Fisheries Research Board of Canada. 18:793-816.

Mecklenburg, C. W., T. A. Mecklenburg, and L. K. Thorsteinson. 2002. Fishes of Alaska. American Fisheries Society, Bethesda, Maryland.

Milner, A. M., E. E. Knudson, and K. Magnasson. 2000. Colonization and development of stream communities across a 200-year gradient in Glacier Bay National Park, Alaska, U.S.A. Canadian Journal of Fisheries and Aquatic Sciences. 57:2319-2335.

Morrow, J. E. 1973. A new species of *Salvelinus* from the Brooks Range, northern Alaska. Biological Papers of the University of Alaska. 13:1-8.

—. 1980. Analysis of the Dolly Varden charr, *Salvelinus malma*, of northwestern North America and northeastern Siberia, p. 323–338. *In:* Charrs: Salmonid Fishes of the Genus *Salvelinus*. E. K. Balon (ed.). Dr. W. Junk, The Hague, the Netherlands.

Nelson, J. S., E. J. Crossman, H. Espinosa-Perez, L. T. Findley, C. R. Gilbert, R. N. Lea, and J. D. Williams. 2004. Common and scientific names of fishes from the United States, Canada, and Mexico. American Fisheries Society, Bethesda, Maryland.

Nicieza, A. G. 1995. Morphological variation between geographically disjunct populations of Atlantic salmon: the effects of ontogeny and habitat shift. Functional Ecology. 9:448-456.

Nordeng, H. 1983. Solution to the "char problem" based on Arctic char (*Salvelinus alpinus*) in Norway. Canadian Journal of Fisheries and Aquatic Sciences. 40:1372-1387.

Nylander, J. A. A. 2004. MrModeltest v2. Evolutionary Biology Centre, Uppsala University, Program distributed by the author.

Page, L. M., and B. M. Burr. 1991. Freshwater fishes: North America north of Mexico. The Easton Press, Norwalk, Connecticut.

Patterson, C., D. W. Williams, and C. J. Humphries. 1993. Congruence between molecular and morphological phylogenies. Annual Review of Ecological Systematics. 24:153-188.

Phillips, R. B., and K. A. Pleyte. 1991. Nuclear DNA and salmonid phylogenetics. Journal of Fish Biology. 39:259-275.

Posada, D., and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over Likelihood Ratio Tests. Systematic Biology. 53:793-808.

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

Power, G. 1997. A review of fish ecology in Arctic North America, p. 13-39. *In:* Fish ecology in Arctic North America. J. Reynolds (ed.). American Fisheries Society Symposium 19, Bethesda, Maryland.

Price, T. 2008. Speciation in birds. Roberts and Company Publishers, Greenwood Village, Colorado.

Redenbach, Z., and E. B. Taylor. 2003. Evidence for bimodal hybrid zones between two species of char (Pisces: *Salvelinus*) in northwestern North America. Journal of Evolutionary Biology. 16:1135-1148.

Reist, J. D., E. Gyselman, J. A. Babaluk, J. D. Johnson, and R. Wissink. 1995. Evidence for two morphotypes of Arctic char (*Salvelinus alpinus*(L.)) from Lake Hazen, Ellesmere Island, Northwest Territories, Canada. Nordic Journal of Freshwater Research. 71:396-410.

Reist, J. D., J. D. Johnson, and T. J. Carmichael. 1997. Variation and specific identity of char from northwestern Arctic Canada and Alaska. American Fisheries Society Symposium. 19:250-261.

Reist, J. D., G. Low, J. D. Johnson, and D. McDowell. 2002. Range extension of bull trout, *Salvelinus confluentus*, to the central Northwest Territories, with notes on identification and distribution of Dolly Varden, *Salvelinus malma*, in the western Canadian Arctic. Arctic. 55:70-76.

Reist, J. D., F. J. Wrona, T. D. Prowse, M. Power, J. B. Dempson, R. J. Beamish, J. R. King, T. J. Carmichael, and C. D. Sawatzky. 2006. General effects of climate change on Arctic fishes and fish populations. Ambio. 35:370-380.

Reynolds, J. B. 1997. Ecology of overwintering fishes in Alaskan freshwaters, p. 281-302. *In:* Freshwaters of Alaska: Ecological Syntheses. A. M. Milner and M. W. Oswood (eds.). Springer Publishers, New York, New York.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution. 43:223-225.

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

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources. 8:103-106.

Sato, S., H. Kojima, J. Ando, H. Ando, R. L. Wilmot, L. W. Seeb, V. Efremov, L. LeClair, W. Buchholz, D. H. Jin, S. Urawa, M. Kaeriyama, A. Urano, and S. Abe. 2004. Genetic population structure of chum salmon in the Pacific Rim inferred from mitochondrial DNA sequence variation. Environmental Biology of Fishes. 69:37-50.

Scanlon, B. P. 2000. The ecology of the Arctic char and the Dolly Varden in the Becharof Lake drainage, Alaska. Unpubl. M.S. thesis, University of Alaska Fairbanks School of Fisheries and Ocean Sciences, Fairbanks, Alaska.

Sidlauskas, B. L., J. C. Gravello, and J. Jellen. 2007. A new *Schizodon* (Characiformes: Anostomidae) from the Rio Orinoco system, with a redescription of *S. isognathus* from the Rio Paraguay system. Copeia. 2007:711-725.

Stearns, S. C. 1976. Life-history tactics: a review of the ideas. The Quarterly Review of Biology. 51:3-47.

Strauss, R. E., and C. E. Bond. 1990. Taxonomic methods: morphology, p. 109-140. *In:* Methods for fish biology. C. B. Schreck and P. B. Moyle (eds.). American Fisheries Society, Bethesda, Maryland.

Strauss, R. E., and L. A. Fuiman. 1985. Quantitative comparisons of body form and allometry in larval and adult Pacific sculpins (Teleostei: Cottidae). Canadian Journal of Zoology. 63:1582-1589.

Swofford, D. L. 2002. PAUP* Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.

Taylor, E. B., E. Lowery, A. Lilliestrale, A. E. Elz, and T. P. Quinn. 2008. Genetic analysis of sympatric char populations in western Alaska: Arctic char (*Salvelinus alpinus*) and Dolly Varden (*Salvelinus malma*) are not two sides of the same coin. Journal of Evolutionary Biology. 21:1609-1625.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes. 4:535-538.

Wahrhaftig, C. 1965. Physiographic divisions of Alaska. U.S. Geological Survey, Washington, D.C.

Walters, V. 1955. Fishes of western Arctic America and eastern Arctic Siberia: taxonomy and zoogeography. Bulletin of the American Museum of Natural History. 106:255-368.

Waples, R. S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. Marine Fisheries Review. 53:11-22.

Weider, L. J., and A. Hobæk. 2000. Phylogeography and arctic biodiversity: a review. Annales Zoologici Fennici. 37:217-231.

Wheeler, Q. D., and N. I. Platnick. 2000. The phylogenetic species concept (*sensu* Wheeler and Platnick), p. 55-69. *In:* Species concepts and phylogenetic theory: a debate. Q. D. Wheeler and R. Meier (eds.). Columbia University Press, New York.

Wilson, C. C., and P. D. N. Hebert. 1998. Phylogeography and postglacial dispersal of lake trout (*Salvelinus namaycush*) in North America. Canadian Journal of Fisheries and Aquatic Sciences. 55:1010-1024.

Wilson, C. C., P. D. N. Hebert, J. D. Reist, and J. B. Dempson. 1996. Phylogeography and postglacial dispersal of Arctic charr *Salvelinus alpinus* in North America. Molecular Ecology. 5:187-197.

Wright, S. 1943. Isolation by distance. Genetics. 28:114-138.

Wrona, F. J., T. D. Prowse. J. D. Reist, J. E. Hobbie, L. M. J. Levesque, and W. F. Vincent. 2006. Climate change effects on aquatic biota, ecosystem structure and function. Ambio 35:359-369.

Yoshikawa, K., L. D. Hinzman, and D. L. Kane. 2007. Spring and aufeis (icing) hydrology in the Brooks Range, Alaska. Journal of Geophysical Research. 112, G04S43:1-14.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric Morphometrics for Biologists: A Primer. Elsevier Academic Press, San Diego.

2.8 Appendices

2.8.1 Appendix 2.A Population abbreviations (as in Figure 2.8), nominal taxon, locality of collection, haplotype, and genebank accession numbers (if available) for mitochondrial control regions of *Salvelinus* specimens used in phylogenetic analysis. Haplotypes with Genebank accession numbers are from Brunner et al. (2001). Sequences from the current project are in bold, and new haplotypes are listed as Haplo1-5. All other haplotypes are from Redenbach and Taylor (2002), Elz (2003), and Taylor et al. (2008). Haplotypes denoted with (**) have had name changes in this project to signify that they are repeated sequences already listed in the table.

Population	Nominal Taxon	Locality	Haplotype	Genebank
Fontinalis	*S. fontinalis*			AF297987
Leucomaenis	*S. leucomaenis*			AF297988
Namaycush	*S. namaycush*	Nation River, upper Peace River, BC, Canada	Sn	
Acadia1	*S. alpinus oquassa*	Lac Sans Baie, Quebec, Canada	ACD1	AF298045
Acadia3	*S. alpinus oquassa*	Lac Chaudiere, Quebec, Canada	ACD3	AF298047
Acadia4	*S. alpinus oquassa*	Lac Rond, Quebec, Canada	ACD4	AF298048
Acadia5	*S. alpinus oquassa*	Walton Lake, New Brunswick, Canada	ACD5	AF298049
Acadia7	*S. alpinus oquassa*	Wassataquoik Lake, Maine	ACD7	AF298051
Atlantic2	*S. alpinus salvelinus*	Brienz Lake, Switzerland	ATL2	AF297992
Atlantic3	*S. alpinus salvelinus*	Lac Du Bourget, France	ATL3	AF297993
Atlantic7	*S. alpinus alpinus*	Femund Lake, Norway	ATL7	AF297997
Altantic8	*S. alpinus alpinus*	Gronningen Lake, Norway	ATL8	AF297998
Atlantic10	*S. alpinus alpinus*	Lake near Isortoq Fjord, Greenland	ATL10	AF298000
Atlantic12	*S. alpinus alpinus*	Luomusjarvi Lake, Finland	ATL12	AF298002

Appendix 2.A continued

Atlantic16	*S. alpinus erythrinus*	Gander Lake, Newfoundland, Canada	ATL16	AF298006
Atlantic18	*S. alpinus erythrinus*	Kugluktokoluk Brook, Labrador, Canada	ATL18	AF298008
Arctic3	*S. alpinus taranetzi*	Seutakan River, Cukotka Peninsula, Russia	ARC3	AF298029
Arctic4	*S. alpinus erythrinus*	Swan Lake, King William Island, Canada	ARC4	AF298030
Arctic5	*S. alpinus erythrinus*	Horizon Lake, Alaska, USA	ARC5	AF298031
Arctic6	*S. alpinus erythrinus*	Hall Lake, Melville Peninsula, eastern Arctic Canada	ARC6	AF298032
Arctic7	*S. alpinus erythrinus*	Nauyuk Lake, Mackenzie District, eastern Arctic Canada	ARC7	AF298033
Arctic9	*S. alpinus erythrinus*	Avamuktulik, Melville Peninsula, eastern Arctic Canada	ARC9	AF298035
Arctic10	*S. alpinus erythrinus*	Charr Lake, Cornwallis Island, central Arctic Canada	ARC10	AF298036
Arctic12	*S. alpinus erythrinus*	Resolute Lake, Cornwallis Island, central Arctic Canada	ARC11	AF298038
Arctic17	*S. malma*	Galbraith Lake, Alaska	ARC17	AF298043
Arctic18	*S. alpinus erythrinus*	P&N Lakes, western Arctic Canada	ARC18	AF298044
Bering1	*S. malma malma*	Kamchatka River, Kamchatka Peninsula, Russia	BER1	AF298018
Bering2	*S. malma malma*	Kamchatka River, Kamchatka Peninsula, Russia	BER2	AF298019
Bering4	*S. malma malma*	Kuma River, Paramushir Isle, eastern Siberia, Russia	BER4	AF298021
Bering5	*S. malma malma*	Kakhmauri River, Paramushir Isle, eastern Siberia, Russia	BER5	AF298022
Bering6	*S. malma malma*	Clear Hatchery, Alasks, USA	BER6	AF298023
Bering7	*S. malma lordi*	Prince William Sound, Alaska, USA	BER7	AF298024
Bering8	*S. malma lordi*	Prince William Sound, Alaska, USA	BER8	AF298025
Bering9	*S. malma*	Auke Creek, Alaska, USA	BER9	AF298026
HaploC	*S. malma malma*	Ayton Creek, Skeena River, BC, Canada	HaploC	
HaploD	*S. malma malma*	Pedro Ponds, Illiamna Lake, Alaska, USA	HaploD	
HaploB	*S. malma malma*	Togiak River, Bristol Bay, Alaska, USA	HaploA**	

Appendix 2.A continued

HaploA	*S. malma malma*	Aero Creek, Queen Charolette Island, BC, Canada	HaploA	
HaploY	*S. malma malma*	Paratuhka River, Kamchatka Peninsula, Russia	HaploY	
HaploZ	*S. malma malma*	Achen Lake, Chukotka, Russia	HaploZ	
AK77A	*S. malma malma*	Pedro Ponds, Illiamna Lake, Alaska, USA	AK77A	
AK76A	*S. malma malma*	Pedro Ponds, Illiamna Lake, Alaska, USA	AK76A	
AK3F	*S. malma malma*	Whitefish Creek, Lake Aleknagik, Alaska, USA	AK3F	
AK2B	*S. malma malma*	Hansen Creek, Lake Aleknagik, Alaska, USA	HaploA**	
Sakhalin	*S. malma krascheninnikovi*	Kuril Islands, Russia	Smk	
Siberia1	*S. alpinus alpinus*	Luolimo Lake, Finland	SIB1	AF298009
Siberia2	*S. alpinus alpinus*	Luolimo Lake, Finland	SIB2	AF298010
Siberia3	*S. alpinus alpinus*	Spitsbergen, Norway	SIB3	AF298011
Siberia4	*S. alpinus erythrinus*	Goltsovoe Lake, Baikal Region, Russia	SIB4	AF298012
Siberia5	*S. alpinus erythrinus*	Arilakh Lake, Taimyr Peninsula, central Siberia, Russia	SIB5	AF298013
Siberia6	*S. alpinus erythrinus*	Leprindo Lake, Baikal Region, Russia	SIB6	AF298014
Siberia7	*S. alpinus erythrinus*	Lama Lake, Taimyr Peninsula, Russia	SIB7	AF298015
Siberia8	*S. alpinus erythrinus*	Frolikha Lake, Baikal Region, central Siberia, Russia	SIB8	AF298016
Svetovidovi	*Salvethymus svetovidovi*	Elgygytgy Lake, Cukotka Peninsula, Russia	SVET	AF297990
ADT3	***S. anaktuvukensis***	**Anaktuvuk River, Alaska, USA**	**Haplo2**	
ADT4	***S. anaktuvukensis***	**Anaktuvuk River, Alaska, USA**	**Haplo2**	
ADTA1	***S. malma***	**Anaktuvuk River, Alaska, USA**	**HaploA**	
ADTA2	***S. malma***	**Anaktuvuk River, Alaska, USA**	**HaploA**	
AVAN1	***S. malma***	**Avan River, Noatak Drainage, Alaska, USA**	**HaploA**	
AVAN2	***S. malma***	**Avan River, Noatak Drainage, Alaska USA**	**Haplo5**	

Appendix 2.A continued

EK1	***S. anaktuvukensis***	**Ekokpuk Creek, John River, Alaska, USA**	**Haplo1**
EK2	***S. anaktuvukensis***	**Ekokpuk Creek, John River, Alaska, USA**	**Haplo1**
GLC1	***S. anaktuvukensis***	**Graylime Creek, Anaktuvuk River, Alaska, USA**	**Haplo4**
GLC2	***S. anaktuvukensis***	**Graylime Creek, Anaktuvuk River, Alaska, USA**	**HaploA**
GTC1	***S. anaktuvukensis***	**Giant Creek, John River, Alaska, USA**	**Haplo1**
KUG1	***S. malma***	**Kugururok River, Noatak Drainage, Alaska, USA**	**HaploY**
KUG2	***S. malma***	**Kugururok River, Noatak Drainage, Alaska, USA**	**HaploA**
UAT1	***S. anaktuvukensis***	**Anaktuvuk River, Alaska, USA**	**HaploA**
UAT2	***S. anaktuvukensis***	**Anaktuvuk River, Alaska, USA**	**Haplo3**
WUL1	***S. malma***	**Wulik River, Alaska, USA**	**HaploA**
WUL2	***S. malma***	**Wulik River, Alaska, USA**	**Haplo1**

2.8.2 Appendix 2.B Microsatellite DNA Variation in samples of *S. malm*a and the nominal species *S. anaktuvukensis* from populations[1] of northern Alaska. Shown are sample sizes (N), number of alleles (Na), Allelic richness (Ar), observed heterozygosity (Ho), and expected heterozygosity (He). Significant heterozygote deficiencies are underlined. Note that due to small sample size (N=11), specimens from the EK population have been removed.

	ADT	AUT	GLC	KUG	EK	WUL	AVAN	ADTA	Mean
Sfo18									
N	30	32	18	18	11	19	22	32	
Na	1	1	1	1	1	1	1	1	1
Ar	1	1	1	1	-	1	1	1	1
Ho	0	0	0	0	-	0	0	0	0
He	0	0	0	0	-	0	0	0	0
Smm22									
N	30	32	18	18	11	19	22	32	
Na	11	15	9	12	5	15	19	14	13.57
Ar	9.30	10.18	8.39	11.04	-	13.11	15.22	10.25	11.07
Ho	0.900	0.844	0.591	0.833	-	0.944	0.773	0.844	0.818
He	0.892	0.888	0.708	0.925	-	0.982	0.951	0.811	0.880
Sco106									
N	30	32	18	18	11	19	22	32	
Na	15	14	13	16	3	17	19	12	15.14
Ar	10.89	10.69	11.44	14.48	-	14.34	15.34	8.90	12.29
Ho	0.700	0.844	0.667	0.722	-	0.632	0.682	0.438	0.669
He	0.791	0.878	0.744	0.832	-	0.834	0.816	0.693	0.798
Sco109									
N	30	32	18	18	11	19	22	32	
Na	15	19	13	14	6	14	18	15	15.43
Ar	12.92	12.94	12.12	14.00	-	13.03	15.65	12.67	13.33
Ho	0.700	0.875	0.778	0.667	-	0.632	0.773	0.500	0.703
He	0.896	0.935	0.882	0.757	-	0.769	0.779	0.725	0.821
Sco202									
N	30	32	18	18	11	19	22	32	
Na	2	2	3	2	-	2	2	2	2.14
Ar	4.60	2.65	4.60	8.12	-	6.22	5.19	6.75	5.45
Ho	0.467	0.531	0.333	0.389	-	0.474	0.091	0.375	0.380
He	0.499	0.448	0.351	0.322	-	0.371	0.089	0.310	0.341
Sco204									
N	30	32	18	18	11	19	22	32	
Na	19	15	12	20	7	25	5	15	15.86
Ar	15.19	11.76	12.49	16.62	-	16.18	15.23	10.77	14.03
Ho	0.900	0.906	0.667	0.889	-	0.895	0.955	0.625	0.834
He	0.880	0.877	0.781	1.027	-	0.920	0.946	0.735	0.881

[1]Collection sites located in northern Alaska. Populations ADT, AUT, GLC, and ADTA, are all from the Anaktuvuk River drainage; populations AVAN and KUG are from the Noatak River drainage; population EK is from the John River drainage; and population WUL is from the Wulik River drainage.

Appendix 2.B Continued

Sco218									
N	30	32	18	18	11	19	22	32	
Na	17	16	13	19	2	21	17	18	17.29
Ar	15.04	11.83	13.40	17.22	-	19.71	13.01	14.96	15.02
Ho	0.967	0.938	0.778	0.889	-	0.789	0.727	0.719	0.829
He	0.944	0.890	0.822	0.897	-	0.839	0.792	0.809	0.856
Sco220									
N	30	32	18	18	11	19	22	32	
Na	22	22	18	16	5	27	22	23	21.43
Ar	14.69	15.00	15.60	19.05	-	23.68	19.33	17.01	17.76
Ho	0.800	0.938	0.722	0.833	-	0.842	0.864	0.969	0.853
He	0.936	0.907	0.864	0.938	-	0.931	0.869	0.951	0.914

[1]Collection sites located in northern Alaska. Populations ADT, AUT, GLC, and ADTA, are all from the Anaktuvuk River drainage; populations AVAN and KUG are from the Noatak River drainage; population EK is from the John River drainage; and population WUL is from the Wulik River drainage.

2.8.3 Appendix 2.C Meristic counts of *Salvelinus* from northern Alaska and the Yukon Territory. Counts from the original description of the Angayukaksurak charr (Morrow, 1973) are listed on top (*S. anaktuvukensis*) of the table. All other counts are from isolated and non-isolated riverine charr (*S. malma*) populations along the North Slope of the Brooks Range in Alaska and the Yukon Territory (McCart, 1980).

	Gillraker Upper			Gillraker Lower			Pyloric Caeca		
	N	Mean	Range	N	Mean	Range	N	Mean	Range
S. anaktuvukensis		9.0	(7-11)		11.5	(9-13)		28.0	(24-32)
	21	9.1	(7-11)	21	12.0		20	27.0	(24-31)
ISOLATED									
Upper Babbage	159	8.4	(6-12)	159	11.7	(9-14)	149	28.5	(20-39)
Lower Babbage	24	8.5	(7-10)	24	12.1	(10-14)	24	28.4	(23-36)
Upper Cache Creek	25	9.7	(8-14)	25	11.8	(9-14)	25	26.0	(21-32)
CT-41 Spring	18	8.3	(7-9)	18	12.1	(11-13)	59	26.0	(16-35)
Shublik Spring	45	9.6	(8-11)	45	13.2	(12-15)	58	29.9	(23-39)
Sadlerochit Spring	48	7.5	(6-9)	48	11.3	(9-12)	48	25.8	(19-32)
	319	8.5	(6-14)	319	11.9	(9-15)	363	27.9	(16-39)
	Mean range (7.5-9.7)			Mean range (11.3-13.2)			Mean range (25.9-29.9)		
ANADROMOUS									
Hulahula River	29	9.1	(6-11)	29	12.4	(10-14)	29	30.8	(22-32)
Kavik River	43	9.9	(8-13)	43	12.4	(9-14)	43	29.2	(22-38)
Kongakut River	26	9.1	(8-12)	26	12.0	(10-13)	26	29.5	(20-38)
Echooka River	66	9.3	(7-11)	66	12.6	(11-15)	63	32.3	(25-42)
Shaviovik River	5	9.0	(8-11)	5	12.5	(12-13)	4	27.0	(20-30)
Canning River	96	9.2	(7-11)	96	12.6	(10-18)	97	31.4	(18-45)
Canning Spring-1	5	8.6	(8-9)	5	12.6	(12-13)	5	33.2	(29-39)
Firth River	68	9.8	(7-13)	69	12.3	(10-15)	73	30.0	(23-38)
Fish Creek	95	8.7	(7-11)	95	11.7	(9-14)	95	27.4	(19-40)
Fish Hole Creek	125	9.1	(7-12)	125	12.5	(9-14)	125	28.0	(21-39)
Joe Creek	24	9.4	(8-10)	24	12.4	(9-13)	24	29.1	(23-42)
Lower Cache Creek	35	8.9	(7-11)	35	12.3	(9-14)	34	28.3	(22-35)
	617	14.3	(6-13)	618	12.3	(9-18)	618	29.6	(18-45)
	Mean range (8.6-9.9)			Mean range (11.7-12.6)			Mean range (27.0-33.0)		

Appendix 2.C Continued

	Pectoral Rays			Anal Rays			Dorsal Rays		
	N	Mean	Range	N	Mean	Range	N	Mean	Range
S. anaktuvukensis		14.0	(13-15)		14.0	(13-15)		15.0	(14-17)
	21	14.3	(14-15)	21	13.4	(12-15)	21	15.2	(14-16)
ISOLATED									
Upper Babbage	57	13.9	(13-15)	58	12.7	(12-15)	58	14.6	(14-16)
Lower Babbage	22	14.0	(13-15)	22	12.9	(12-15)			
Upper Cache Creek	25	13.6	(13-15)	25	12.9	(12-14)	25	14.5	(14-16)
CT-41 Spring	18	13.9	(13-15)	18	13.0	(12-14)	18	14.4	(13-16)
Shublik Spring	16	14.4	(13-16)	11	12.7	(12-13)	9	14.6	(14-15)
Sadlerochit Spring	20	13.6	(13-15)	22	12.4	(12-13)	22	14.2	(13-15)
	158	13.9	(13-16)	156	12.8	(12-15)	132	14.6	(13-16)
	Mean range (13.6-14.4)			Mean range (12.4-13.0)			Mean range (14.2-14.6)		
ANADROMOUS									
Hulahula River	9	14.8	(13-16)						
Kavik River	20	14.5	(14-15)	20	12.8	(12-14)	20	14.5	(13-16)
Kongakut River									
Echooka River	20	14.6	(13-16)	41	13.6	(11-16)	21	14.4	(13-16)
Shaviovik River									
Canning River	40	14.2	(13-16)	35	13.1	(12-14)	35	14.5	(13-16)
Canning Spring-1	17	14.2	(13-15)	5	13.0	(12-14)	5	14.6	(14-15)
Firth River	20	14.3	(13-15)						
Fish Creek	29	14.1	(13-15)	30	13.3	(12-15)	25	14.7	(13-16)
Fish Hole Creek									
Joe Creek	10	14.5	(14-16)	10	12.9	(12-14)	10	14.1	(13-15)
Lower Cache Creek	14	14.2	(13-15)						
	179	14.3	(13-16)	141	13.2	(11-16)	116	14.5	(13-16)
	Mean range (14.1-14.8)			Mean range (12.8-13.6)			Mean range (14.1-14.7)		

Appendix 2.C Continued

	Vertebrae			Pelvic Rays			Lateral Line Pores		
	N	Mean	Range	N	Mean	Range	N	Mean	Range
S. anaktuvukensis		67.0	(65-70)		10.0	(9-10)		135.0	(127-152)
				21	9.9	(9-10)	21	135.8	(130-140)
ISOLATED									
Upper Babbage	58	67.4	(65-70)	57	9.1	(8-10)	58	129.6	(121-146)
Lower Babbage	22	67.0	(65-69)	22	9.2	(9-10)	22	129.7	(120-138)
Upper Cache Creek	24	64.7	(58-67)	25	9.0	(8-9)	25	131.8	(122-138)
CT-41 Spring	18	67.8	(66-70)	18	9.1	(9-10)	18	133.3	(122-141)
Shublik Spring	32	64.7	(63-66)	31	9.0	(9-10)	16	127.6	(120-135)
Sadlerochit Spring	20	66.9	(65-70)	40	8.9	(8-9)	22	126.2	(113-137)
	174	66.5	(58-70)	193	9.1	(8-10)	161	129.7	(112-146)
	Mean range (64.7-67.8)			Mean range (8.9-9.2)			Mean range (125.2-133.3)		
ANADROMOUS									
Hulahula River							9	135.7	(126-140)
Kavik River	10	68.3	(57-70)	10	9.1	(9-10)	20	139.2	(135-142)
Kongakut River									
Echooka River	20	67.7	(60-70)	30	9.1	(9-10)	20	139.2	(134-145)
Shaviovik River									
Canning River	40	67.8	(65-70)	40	9.1	(9-10)	40	133.2	(125-141)
Canning Spring-1	5	68.4	(67-59)	5	9.0	(9-9)	17	134.6	(120-146)
Firth River							20	139.2	(134-136)
Fish Creek	30	68.9	(67-71)	25	9.0	(8-10)	35	133.7	(122-144)
Fish Hole Creek	60	68.2	(66-70)				30	134.9	(122-143)
Joe Creek	9	68.4	(67-70)	10	8.9	(8-9)	10	138.3	(135-144)
Lower Cache Creek	27	67.1	(66-69)	28	9.0	(9-9)	14	136.4	(132-141)
	201	68.0	(60-70)	148	9.0	(8-10)	215	135.9	(122-146)
	Mean range (67.1-68.9)			Mean range (8.9-9.0)			Mean range (133.2-139.2)		

Chapter 3 Conclusions

Determination of why one species will express a diversity of life history forms while another will split into multiple species would help us better understand the evolutionary and selective processes at work in natural systems. These core issues of taxonomy and phylogeny are the basis of this project, in which I investigate a purported endemic species of charr of the genus *Salvelinus* in northern Alaska. The overall objective of the study was to determine whether the Angayukaksurak charr (*Salvelinus anaktuvukensis*) deserved species status or if it was a variant of the Dolly Varden (*S. malma*), following the criteria for a species under the phylogenetic species concept (sensu Wheeler and Platnick 2000). To make this determination, specimens of both nominal species from northern Alaska were examined for differences in meristic, morphological, and genetic characters. Meristic characters used in the original species description of the Angayukaksurak charr, in addition to other characters used in studies of this genus, were examined for differentiation. Digital images of both nominal species were used to examine differences in body shape using morphometrics. The relationship of the Angayukaksurak charr to other species and lineages of *Salvelinus*, based on the mitochondrial DNA framework of Brunner et al. (2001), was determined. Lastly, microsatellite loci were used to examine the relatedness of populations and to determine if any available novel alleles existed that might differentiate the Angayukaksurak charr from Dolly Varden.

The most important conclusions from this study are the following:

1. Differences in meristic characters were evident between samples nominally labeled as *S. anaktuvukensis* and *S. malma* in the study area. These differences, however, correspond equally with major drainage as with nominal species. Greater levels of diversity (range) of meristic counts within *S. malma* in the region have been reported than those used to originally define the Angayukaksurak charr (McCart, 1980), demonstrating the great range of phenotypic plasticity that this genus is able to display.
2. Nuclear (microsatellite) DNA analyses showed that samples of *S. anaktuvukensis* and *S. malma* collected from northern Alaska were part of a larger, mixed population, therefore related and better described as alternate life history forms, not separate species. This life history diversity, also displayed in phenotypic diversity, has potentially allowed for continued persistence of this species in this dynamic and harsh environment.
3. Complex relationships are evident in the phylogeny of the *S. alpinus*/*S. malma* group, when inferred from mtDNA sequences. Prior research showed that this marker divides the group into distinct lineages throughout the Arctic and brought to light potential issues regarding the species status of *S. malma* (Brunner et al., 2001). Interestingly, specimens originally considered *S. anaktuvukensis* fit into two of these lineages: the Arctic group of northern Alaska and the Canadian Arctic Archipelago (considered to be *S. alpinus*, based on this marker) and the Bering group of central and southern Alaska and western Siberia (considered to be *S. malma*). *S. anaktuvukensis* specimens split into these lineages in the middle

of their described range along the continental divide in the Brooks Mountain Range of northern Alaska. While this evidence disputes the idea that *S. anaktuvukensis* is a separate species, it highlights an interesting display of biogeographic divide and/or historic introgression of *S. alpinus* with *S. malma* in Arctic Alaska.

4. As small assemblages of resident fish (in comparison to the central anadromous population component of this larger regional group), these marginal populations of resident fish are vital to the genetic and phenotypic variability of the central populations, and are therefore of conservation significance. These marginal populations are subjected to extreme environmental conditions that could promote the evolution of novel alleles, which, when reincorporated back into the central populations, may increase the genetic variability of the entire group (Scudder, 1989).

This project, though resolving a taxonomic enigma for the genus *Salvelinus*, also illuminates multiple outstanding issues for this genus and future research directions. Of particular interest is the relationship between mitochondrial and microsatellite DNA results in defining *Salvelinus* species. Evidence presented in this thesis identifies the continental divide along the Brooks Range as the geographical divide between the Arctic and Bering lineages based on mitochondrial DNA. By this marker, most fish considered as *S. malma* fall into the Bering lineage, and most fish in the Arctic lineage are considered to be *S. alpinus*. Microsatellite loci, on the other hand, characterize a portion

of the Arctic lineage fish—those along the North Slope of Alaska and Canada to the Mackenzie River—as Dolly Varden, and not Arctic charr. This relationship, however, is based on a relatively small number of samples a from few locations in the central region of the Brooks Range. I believe it would be worth expanding the range and number of samples on both sides of the Brooks Range to determine if this relationship holds. It would also be instructive to examine the relatedness of the numerous isolated populations of Dolly Varden charr described from the literature on the North Slope of Alaska to the anadromous populations in those same river systems using microsatellite analyses. These studies could be used to examine the theory that external "marginal" populations act as sources of new genetic material for 'central' populations (Scudder, 1989). Finally, a review of museum specimens, to increase the number and size range of Dolly Varden specimens, would enhance the current study given the size differences between the specimens examined for meristic and morphometric characters.

Ultimately, given the effects of climate change apparent in the Arctic (Wrona et al., 2006), research to understand these systems and their inhabitants, as they currently exist, is of the highest conservation importance. Conservation of the diversity of the charrs of the genus *Salvelinus* will be impossible if we do not fully understand the processes that contribute to the development of evolutionary and ecological diversity within and among this unique Arctic group (Dunham et al., 2008).

Literature Cited

Brunner, P. C., M. R. Douglas, A. Osinov, C. C. Wilson, and L. Bernatchez. 2001. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus L.*) inferred from mitochondrial DNA sequences. Evolution. 55:573-586.

Dunham, J., C. Baxter, K. Fausch, W. Fredenberg, S. Kitano, I. Koisumi, K. Morita, T. Nakamura, B. Rieman, K. Savvaitova, J. Stanford, E. Taylor, and S. Yamamoto. 2008. Evolution, ecology, and conservation of Dolly Varden, white-spotted char, and bull trout. Fisheries. 33:537-50.

McCart, P. J. 1980. A review of the systematics and ecology of the Arctic char, *Salvelinus alpinus*, in the western Arctic. Canadian Technical Report of Fisheries and Aquatic Sciences. 935.

Scudder, G. G. E. 1989. The adaptive significance of marginal populations: a general perspective, p. 180-185. *In:* Proceedings of the National Workshop on Effects of Habitat Alteration on Salmonid Stocks. Vol. 105. C. D. Levings, L .B. Holtby, and M. A. Henderson (eds.). Canadian special publication of Fisheries and Aquatic Sciences.

Wheeler, Q. D., and N. I. Platnick. 2000. The phylogenetic species concept (*sensu* Wheeler and Platnick), p. 55-69. *In:* Species concepts and phylogenetic theory: a debate. Q. D. Wheeler and R. Meier (eds.). Columbia University Press, New York.

Wrona, F. J., T. D. Prowse. J. D. Reist, J. E. Hobbie, L. M. J. Levesque, and W. F. Vincent. 2006. Climate change effects on aquatic biota, ecosystem structure and function. Ambio. 35:359-369.