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Phylogeography and local adaptation in prickly sculpin, *Cottus asper*

by

Stefan Dennenmoser

A THESIS

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Abstract

This thesis examined historical and contemporary effects on genetic population structuring of prickly sculpin (*Cottus asper*) in British Columbia, Canada. A phylogeographic study was conducted, which disproved the traditional view that prickly sculpin survived the Last Glacial Maximum in only southern coastal and inland refugia, favoring allopatric divergence and giving rise to coastal and inland "prickling" phenotypes, which vary in the degree to which spine-like scales cover the body of the fish. Instead, analyses of mitochondrial DNA and microsatellite markers suggest parallel evolution of highly prickled inland populations, which presumably have evolved independently across three glacial lineages, originating from highly differentiated ancestral coastal populations. Postglacial colonization from coastal towards inland regions and associated founder effects resulted in decreased genetic diversities, which was also evident in a subsequent study on conservation genetics of a recently discovered peripheral population in the Peace River in Alberta. While no divergent "conservation designation unit" was detected in the Peace River, a for freshswater sculpins unusual high genetic connectivity over large geographic scales was found, which could reflect high dispersal capacities of an extended, planktonic larval stage that might have been at least partially retained from an amphidromous life cycle of the putative ancestral coastal populations. While such assumptions on life history characteristics of inland populations remain speculative, the divergence between coastal, amphidromous and inland, purely freshwater life cycles is young (< 14,000 years), and may be in an early, incomplete stage of a life history transition. To better understand life history transitions from amphidromous to freshwater life cycles in *C. asper*, a population genetics study was

conducted in the Lower Fraser River system, which identified sympatric life history ecotypes represented by amphidromous sculpins in the river main channel, and genetically differentiated freshwater sculpins in the river tributaries. These results indicate that that isolation-by-environment to discrete freshwater habitats could play a role for the early stage of amphidromy-freshwater transitions, which may be further favored by the presence of large tributary streams and lakes allowing for large effective population sizes.

Preface

The individual data chapters have been published or submitted for publication as follows:

Chapter 2:

Dennenmoser S, Nolte A, Vamosi SM, and Rogers SM (under review) Phylogeography of prickly sculpin (*Cottus asper*) in the Pacific Northwest reveals parallel phenotypic evolution across multiple glacial lineages. *Submitted to: Journal of Biogeography*

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Chapter 1: General Introduction

Within this thesis, I explore the role of demographic history and local adaptation in shaping population genetic structure of prickly sculpin (*Cottus asper* Richardson), a small and widely distributed freshwater fish of the Pacific Northwest. Following a general introduction, historical effects are addressed in a phylogeographic study, while subsequent chapters investigate the possible outcomes of range expansions and local adaptation on population genetic structure. A final conclusion chapter is provided to synthesize the main findings, and to outline possible future research avenues.

1.1 Phylogeography of the Pacific Northwest

1.1.1 Phylogeography

Phylogeography is an interdisciplinary research area concerned with explaining the biogeographic distribution of divergent genetic lineages within or between closely related species (Avise 1987, 2000). Understanding how phylogeographic patterns of neutral genetic variation reveal the signature of a species' demographic past remains the first step in efforts to elucidate how geological events, environmental influences, and geographical factors interact with aspects of a species ecology and natural history in shaping its evolution (Avise et al. 1987, Knowles 2009). Since Avise coined the term "phylogeography" by merging existing theory in systematics, biogeography and population genetics (Avise et al. 1987), it has rapidly evolved into a highly integrative research area, where recent advances combine genome scans and ecological niche models with newly developed techniques in statistical phylogeography (Hickerson et al. 2010), which increasingly integrate phenotypic data to uncover evolutionary consequences of

complex demographic histories (e.g., Dinca et al. 2011). Central themes of phylogeographic studies are the identification of glacial refugia, postglacial colonization routes and secondary contact zones among glacial lineages (e.g., Avise 2000, Hewitt 2000), all of which can have profound impacts on genetic variation, diversity and structuring across a species' distribution range (Shafer et al. 2010). While phylogeographic studies traditionally attempted to infer demographic histories directly from gene genealogies (usually derived from a single mitochondrial DNA marker, Avise 2000), modern phylogeography has switched to multi-locus, coalescent modeling techniques in order to account for stochasticity of genetic processes (i.e., random loss of gene lineages by genetic drift, Knowles 2009). Such advances have largely facilitated interpretations of phylogeographic patterns, which can sometimes be confounded by over-interpretation or confirmation bias in favor of existing hypotheses (Nickerson 1998, Knowles and Maddison 2002).

1.1.2 The Pacific Northwest

The Pacific Northwest, which encompasses the western coast of North America from southern Alaska to northern California, to the east of the Rocky Mountains of British Columbia, western Montana and central Idaho, has been dramatically affected by the Pleistocene glaciations (~2.5 Myr – 12,000 years BP; Shafer et al. 2010), which resulted in complex biogeographic histories influenced by multiple cycles of advancing and retreating ice sheets (e.g., Brunsfeld et al. 2001). Phylogeographic patterns in the Pacific Northwest were recently reviewed by Shafer et al. (2010) who underlined the importance of Pleistocene glaciations, during which many taxa survived in isolated, cryptic refugia, as well as in separate locations within major refugia. Other important

biogeographic factors include geomorphological barriers like rivers and glaciers, and more ancient geological events such as the orogeny of the Cascade-Sierra chain around 5-2 Myr ago (Brunsfeld et al. 2001). Hence, the biogeographic history of the Pacific Northwest presents a complex mixture of vicariance events (emergence of physical barriers) such as mountain ranges separating coastal and inland areas, as well as dispersal processes along or across mountain ranges, often following the retreat of ice-sheets during interglacial periods, and leading to secondary contact between divergent genetic lineages originating from different glacial refugia (Shafer et al. 2010).

Glacial-induced vicariance has been proposed for explaining a north-south divergence found in numerous plants and animals, resulting from historical separation in northern and southern glacial refugia (Soltis et al. 1997, Shafer et al. 2010). Additionally, disjunct east-west distributions of the mesic coniferous forests and associated organisms, such as the widely distributed lodgepole pine (*Pinus contorta*) have been explained by vicariance caused by the Cascade Ranges and the Rocky Mountains during periods of glaciation (Godbout et al. 2008). For example, vicariance through the formation of a Cascade Range rain shadow is thought to have separated western (coastal) from eastern (inland) distributions of the forest dwelling yellow-pine chipmunk (*Tamias amoenus*) (Demboski and Sullivan 2003). Vicariance was also inferred in the blue-grey taildropper (*Prophyason coeruleum*), with divergence estimates around 2.6 - 5.9 Myr predating the Pleistocene and falling within the time of the orogeny of the Cascade Ranges (Wilke and Duncan 2004). While a biogeographic split between coastal and inland regions has been long recognized and presumably includes hundreds of taxa (Findley 1955, Nielson et al. 2001), this pattern does not necessarily imply vicariance as the only mechanistic

explanation. For example, a comparative study conducted by Carstens et al. (2005) revealed deep divergence between coastal and inland populations in three amphibian lineages (*Ascaphus, Plethodon, Dicamptodon*) which is consistent with vicariance due to xerification of the Columbian Basin, while a similar pattern in species such as water vole (*Microtus richardsoni*), dusky willow (*Salix melanopsis*) or whitebark pine (*Pinus albicaulis*) could be better explained by recent, post-Pleistocene dispersal from inland towards coastal regions through periodically available dispersal corridors (Carstens et al. 2005). Similarly, Brunsfeld et al. (2001) summarized biogeographic patterns of twelve plant and animal species showing a disjunct distribution associated with the mesic forest, and found that both ancient vicariance (five species) and recent dispersal (seven species) could best explain the finding of disjunct distribution patterns. Hence, dispersal as an explanation for biogeographic pattern is common, and colonization pathways often appear to follow geographic features such as mountain ranges or river valleys (Shafer et al. 2010).

During the last glacial maximum (LGM), around 21,000 – 17,000 years ago, large parts of Canada and northern United States were covered under the Laurentide and Cordilleran ice sheets (Denton and Hughes 1981). The weight of the Cordilleran ice sheets caused up to hundreds of meters of crustal depression along coastal areas in southwestern British Columbia (James et al. 2005), causing relative sea level to be higher than present, despite global sea level being around 120 m lower (Hutchinson et al. 2004). Major deglaciation and a subsequent fall in sea level occurred between 15,000 and 8000 years ago (Dyke and Prest 1987, Hutchinson et al. 2004), facilitating postglacial dispersal and adaptive radiation of aquatic organisms into newly formed lakes and water drainages.

Periods of de-glaciations were often accompanied by complex geomorphologic changes of river drainages, which temporarily opened and closed connections between river basins, enabling species to disperse to neighboring drainages. For example, with the retreat of the Cordilleran Ice Sheet, the Columbia River became a dispersal corridor into British Columbia for freshwater fish that survived glaciation in the southern parts of the Columbia Basin (McPhail 2007). Temporary connections created through glacial lakes between the Fraser and the Columbia system allowed a number of fish species to colonize the Fraser River from the Columbia Basin, eventually dispersing further north through ephemeral connections to northern drainages such as the Peace River (McPhail 2007). The high variability of distribution ranges that can be found in freshwater fishes, such as sculpins of the genus *Cottus,* indicates species-specific dispersal abilities as well as differences in the number and locations of glacial refugia. For instance, the widely distributed slimy sculpin (*C. cognatus*) has presumably survived glaciation in (at least) three different refugia, while most other sculpin species are assumed to have originated from only one glacial refugium (i.e., either the Pacific refugium, or the Great Plains refugium, McPhail 2007). On the other hand, fish species that have survived in additional, northern refugia (e.g., the Beringia refugium) exhibit genetic signatures of glacial-induced divergence. For example, Witt et al. (2011) reported a range disjunction for pygmy whitefish (*Prosopium coulterii*) that results from isolation in at least two glacial refugia located in the northern Beringia and the southern Pacific refugium. Postglacial dispersal through ephemeral connections between different river systems (e.g., proglacial lakes connecting Fraser River with Peace River) is thought to explain present-day distribution patterns of divergent glacial lineages (Witt et al. 2011). While

glacial-induced vicariance and survival in separated, glacial refugia has contributed to the genetic divergence of many freshwater fishes in the Pacific Northwest, postglacial dispersal appears to be equally or more important in determining present-day biogeographic patterns and genetic differentiation among glacial lineages, particularly of species that survived glaciations in just one refugium.

1.1.3 Evolutionary Implications

The increasing knowledge of the postglacial history of numerous taxa in the Pacific Northwest creates unique opportunities to investigate evolutionary consequences of allopatric separation in glacial refugia, rapid range expansions into recently deglaciated areas, and mixing of genetic lineages in zones of secondary contact (e.g., Shafer et al. 2010). Such investigations contribute to a better understanding of the relative importance of historical and ecological effects on intraspecific geographic variation (Shikano et al. 2010), and therefore are necessary to make inferences about adaptive divergence of local populations (Knowles 2009). For example, both *in situ* ecological divergence and secondary contact of differentiated glacial lineages have contributed to the sympatric occurrence of "limnetic dwarf" and "benthic normal" whitefish ecotypes across a number of lakes in eastern Canada (e.g., Lu and Bernatchez 2001), which differ in the degree of genomic divergence depending on a complex interplay of natural selection, strength of ecological niche divergence and demographic characteristics of each lake (Gagnaire et al. 2013). Both mixing of glacial lineages in secondary contact zones, and sympatric ecological divergence fall within a "speciation-with-gene-flow" framework (e.g., Smadja

and Butlin 2011), which cannot be complete without knowledge of historical effects on genetic differentiation.

Phylogeographic studies can be invaluable for uncovering instances of parallel evolution following the repeated colonization of similar habitats by independently evolving glacial lineages, which is generally assumed to indicate adaptive divergence resulting from natural selection (e.g., Simpson 1953, Schluter 2000, Elmer and Meyer 2011). While parallel phenotypes could also emerge either by chance, genetic drift, evolutionary constraints, indirect selection on a linked adaptive trait, or through "exaptation", referring to a fitness advantage despite an evolutionary origin unrelated to the environment where it currently occurs, natural selection remains the strongest explanation for the finding of consistent and repeated pattern of phenotype-environment correlations (Schluter et al. 2004, Losos 2011). Intriguingly, the genetic basis of parallel phenotypes may vary among populations, ranging from identical or different mutations in the same gene to mutations in different genes (Elmer and Meyer 2011). This suggests an important role of genomic architecture underlying the genetic basis of adaptive change, such as pleiotropic constraints, linkage relationships, epistatic effects, variable mutation rates and the amount of available standing genetic variation (e.g., Conte et al. 2012). For example, historical differentiation in standing genetic variation may lead to different genetic pathways causing parallel evolution across a species distribution range (Prunier et al. 2012, Kokita et al. 2013). It is therefore increasingly important to consider the historical context of parallel evolution and its underlying genetic basis, in order to better understand targets and mechanisms of natural selection. Such investigations will also help to clarify the relative contribution of historical standing genetic variation, new

mutations and adaptive introgression to the presence of adaptive genetic variants, which can affect the speed of adaptive responses to environmental change (Barrett and Schluter 2008, Hedrick 2013). Accordingly, the availability of standing genetic variation allows faster fixation of alleles compared to low-frequency new mutations, which may enhance the probability of finding parallel phenotypes in recently deglaciated, postglacial environments such as those found in the Pacific Northwest (e.g., Colosimo et al. 2004, Barrett and Schluter 2008).

Standing genetic variation of high adaptive potential could also facilitate rapid adaptation during range expansions following climatic shifts, such as the colonization of recently de-glaciated areas after the last glacial maximum (Barrett and Schluter 2008). Population genetic consequences of range expansions can be complex and are often affected by strong genetic drift due to repeated founder effects at the expansion front, which is usually expected to cause increased genetic divergence and decreased genetic diversity, and sometimes may lead to the fixation of alleles over large geographic areas, which has been coined "allele surfing" (e.g., Excoffier and Ray 2008, Excoffier et al. 2009, Nullmeier and Hallatschek 2013). While a decrease of genetic diversity at neutral genetic markers has often been associated with reduced adaptive genetic variability for selection to act on (Reed and Frankham 2003), neutral genetic diversity does not necessarily predict adaptive potential (Hendry and Taylor 2004, Kawecki 2008), and recent research has shown that strong selection acting during range expansions can promote local adaptation despite a loss of genetic diversity due to genetic drift (White et al. 2013). Despite such recent advances, it often remains difficult to disentangle selective mechanisms from genetic drift and historical effects on the degree of adaptive divergence

and potential reproductive isolation between populations (e.g., Hofer et al. 2009), underlining the importance of considering historical demographic factors for the interpretation of patterns of adaptive divergence.

1.2 Divergence with gene flow during the early stages of speciation

In the light of complex historical processes that may affect genetic divergence and potentially accumulate reproductive isolation in numerous ways, the question "Which processes favor the emergence of new species?" becomes a fundamental challenge to evolutionary biology and our understanding of contemporary biodiversity (Coyne and Orr 2004). Therefore, a key towards the development of advanced speciation scenarios is the study of ecological and genetic mechanisms during the early stages of speciation, before reproductive isolation is complete and the build-up of additional genetic divergence confounds the processes initiating speciation (e.g., Via 2009, Langerhans and Riesch 2013). Past work has largely focused on allopatric speciation and the build-up of Dobzhansky-Muller genetic incompatibilities (i.e., negative epistasis between alleles at different loci; Dobzhansky 1936, Muller 1940) as a major factors promoting reproductive isolation (e.g., Mayr 1947, Coyne and Orr 2004), which likely has promoted diversification of postglacial fishes during Pleistocene glacial cycles (April et al. 2012). However, speciation mechanisms in fishes are diverse (reviewed in Bernardi 2013), and more recent research has demonstrated that sympatric speciation may be common, without a preceding period of geographic separation of populations (e.g., Smadja and Butlin 2011). Thus, current research places much emphasis on ecological causes of divergent selection ("ecological speciation", e.g., Schluter 2001, Rundle and Nosil 2005,

Langerhans and Riesch 2013), but lacks a coherent framework to explain how reproductive isolation is initiated and maintained in the presence of gene flow (e.g., Rogers et al. 2013). Gene flow, which can be defined as the movement of genes among population (Slatkin 1985), has traditionally been thought to impede local adaptation, unless strong selection pressures exist to overcome its homogenizing effects (e.g., Kawecki and Ebert 2004, Thibert-Plante and Hendry 2010, Yeaman and Whitlock 2011). Accordingly, a strong signal of habitat-dependent ecological divergence in neutrally evolving genetic markers is only expected when divergent selection is strong and more likely when migration rates are intermediate (Thibert-Plante and Hendry 2010). Recent "speciation with gene flow" models suggest that genomic divergence is restricted to some few genomic regions during the early stage of divergence, while allowing a free exchange of alleles from other genomic regions (e.g., Smadja and Butlin 2011, Nosil and Feder 2012). Subsequently, genetic hitchhiking around genomic regions under selection can increase differentiation and create so-called "genomic islands of speciation" (Turner et al. 2005), which eventually may lead to widespread genomic divergence and reproductive isolation among locally adapted populations (Nosil and Feder 2012, 2013). Genetic hitchhiking is assumed to proceed through local reductions in gene flow for regions physically linked to genomic regions under divergent selection ("divergence hitchhiking", DH), or due to genome wide reductions in gene flow because of selection on multiple unlinked loci ("genome hitchhiking", GH), the latter of which has been suggested being a major contributor to reproductive isolation (Flaxman et al. 2013). While DH and GH are not mutually exclusive, they differ in their predictions: DH should result in isolated clusters of tightly linked loci producing "islands of divergence",

whereas GH should act on a large number of selected loci and therefore elevate divergence across the whole genome, resulting in a more differentiated baseline differentiation and the occurrence of "continents of divergence" (e.g., Flaxman et al. 2013). Given that evidence for either scenario may also depend on the number of genetic markers along the genome, the increasing feasibility of using very high marker densities will likely facilitate the detection of GH, which might correct previous assumptions of DH based on low numbers of genetic markers in some cases. Moreover, commonly used genome scan approaches may bias against the detection of GH because weak selection can easily be overlooked (e.g., Feder et al. 2012), and clearly more empirical studies are needed to evaluate patterns of genomic divergence with gene flow.

Patterns of habitat-dependent divergence between populations or ecotypes, which are independent of isolation-by-distance (IBD, Wright 1943), have been termed "isolation-by-adaptation", "isolation-by-ecology" or "isolation-by-environment" (Nosil et al. 2005, 2008, Shafer and Wolf 2013), and may often reveal valuable natural settings to explore genome wide divergence with gene flow during the early stages of speciation. Isolation-by-adaptation usually refers to a correlation between adaptive phenotypic divergence and neutral genetic differentiation (Nosil et al. 2008), whereas isolation-by-environment focuses on environmental rather than phenotypic differences (Wang and Summers 2010), and both terms have been summarized as isolation-by-ecology (Shafer and Wolf 2013). Possible mechanisms leading to local adaptation and isolation-by-ecology include selection against immigrants or hybrid offspring, assortative mating, differences in reproductive timing, or divergent habitat preferences (e.g., Edelaar et al. 2008, Räsänen and Hendry 2008, Weissing et al. 2011). Studies aiming to demonstrate isolation-by-

ecology need to account for confounding effects of isolation-by-distance, the commonly observed positive relationship between neutral genetic differentiation and geographic distance (Meirmans 2012, Shafer and Wolf 2013). The detection of ecologically mediated genetic divergence among replicated population-pairs may often provide a first step towards investigations of mechanisms underlying incipient ecological speciation (e.g., Shafer and Wolf 2013), which has the potential to contribute to a more coherent framework of reproductive isolation.

1.3 Molecular markers used in this study

The choice of genetic markers in evolutionary biology studies is changing rapidly with the increasing availability of genomic data for non-model organisms, the often-successful cross-species amplification of genetic markers among related taxa, and the ever decreasing costs for developing new markers with the help of genome scans (e.g., Stapley et al. 2010, Glenn 2011). Accordingly, the method of choice depends largely on the current state of available genomic resources and costs for developing new markers, which means that well established approaches can get outdated over the course of a five-year PhD period. In this work, I used a combination of mitochondrial DNA sequences and microsatellite markers for investigating phylogeographic patterns, and microsatellite markers for population genetic analyses. While following a "traditional" choice of markers for such studies, this was justified by the availability of suitable markers through the extensive genetic work conducted by Dr. Arne Nolte's research group on European sculpin, *Cottus gobio*, allowing me to take advantage of existing microsatellites that could be successfully applied to my study organism, *C. asper*. In contrast, the time

consuming development of variable single-copy introns did not result in sufficiently informative genetic markers. At the end of my PhD, I started to join the new wave of genome scan enthusiasm surrounding "next-generation-sequencing" (NGS, e.g. Tautz et al. 2010), with the aim of characterizing single nucleotide polymorphisms (SNPs) located in teleost candidate genes underlying salinity tolerance and osmoregulation. In this section, genetic markers are explained, and benefits and limitations of genetic markers used are briefly discussed.

Since the early beginnings of phylogeography, the genetic marker of choice has been sequence fragments of the mitochondrial DNA (mtDNA; Avise et al. 1983, Avise et al. 1987, Beheregaray 2008), which still owns much of its popularity to the advantages of a purely maternally inherited marker. Because mtDNA is haploid and only maternally inherited, it is assumed to have a fourfold reduced effective population size (*Ne*) compared to the nuclear genome (Moritz et al. 1987, but see also Ballard and Whitlock 2004). Its strengths are lack of recombination, putative neutrality, high mutation rates, smaller effective population size and shorter expected time to reciprocal monophyly between geographic regions (Brito and Edwards 2009, Hickerson et al. 2010). Therefore, mtDNA was quickly embraced as a molecular tool for investigations of population structure and demographic history (Lee et al. 1995, Beheregaray 2008). However, mtDNA soon became criticized for several reasons, including being a single-locus marker not representative of the whole genome, non-neutrality, occurrence of recombination in some species, incorporation of mtDNA into nuclear genomes, and co-adaptation between mitochondrial and nuclear genomes (Ballard and Whitlock 2004, Dowling et al. 2008). Non-neutrality of mtDNA can lead to erroneous conclusions if no additional, nuclear

markers are used, because apparent phylogeographic breaks could arise if selection favours different mtDNA haplotypes in different environments (Irwin 2012), while on the other hand selective sweeps may cause the absense of mtDNA structuring over large geographic areas and thereby mask phylogeographic structuring present in the nuclear DNA (e.g., Rato et al. 2011). Following the recognition that discordance between mitochondrial and nuclear DNA based genealogies is common, it is now widely accepted that the use of mitochondrial markers should be complemented with multiple markers from the nuclear genome, which allows one to uncover otherwise undetectable patterns such as mitochondrial introgression, sex-biased dispersal or incomplete lineage sorting of the nuclear DNA (e.g., Brito and Edwards 2008, Godinho et al. 2008, Rato et al. 2011). Nevertheless, mtDNA still represents an important marker for phylogeographic studies (Zink and Barrowclough 2008), and will often be the first step in detecting historical associations among populations.

While the need for nuclear markers in phylogeographic studies has repeatedly been emphasized (e.g., Brito and Edwards 2008), it often remains challenging to find suitable markers that are variable and informative enough, in part because of their low mutation rates. Single copy introns – non-coding sections within a single-copy gene – have been a popular marker of choice (Brito and Edwards 2008), but can be difficult to develop and are often not variable enough. Intron markers are usually developed by designing primers on adjacent exon regions (coding region of a gene) to amplify across the non-coding intron region, which results in so-called exon-primed intron-crossing (EPIC) markers that can often be used for related taxa for which no genomic information is available (e.g., Li et al. 2010). However, my own endeavors into EPIC marker development resulted in only

a few single-copy introns that showed negligible variation over large geographic scales, and finally were deemed not informative enough to be included in the phylogeographic study (see also Chapter II).

Due to the need for a higher number of sufficiently variable nuclear loci, amplified fragment length polymorphisms (AFLPs) soon became popular genetic markers in phylogeography and population genetics (Bensch and Akkesson 2005, Meudt and Clarke 2007). AFLPs are differences in restriction fragment lengths, and are scored as presence-absence polymorphisms (e.g., Blears et al. 1998). The main strength of AFLPs is the ability to rapidly generate large numbers (hundreds to thousands) of polymorphic molecular markers randomly scattered throughout the genome, without the need for prior genetic information, which made it the method of choice for many "non-model" organisms (e.g., Meudt and Clarke 2007). Through analyzing thousands of markers across the genome, the confounding effects of selection, incomplete lineage sorting and recombination are assumed to be balanced (Albertson et al. 1999). While AFLPs are relatively easy to develop and highly efficient in detecting shallow population structures, their main weaknesses are that homozygotes and heterozygotes cannot be distinguished, the occurrence of fragment size homoplasy (co-migration of non-homologous AFLP fragments), and ascertainment bias in the choice of polymorphic markers (Fink et al. 2010). Consequently, the popularity of AFLP markers has been steadily decreasing in favor of co-dominant markers that allow distinguishing homo- from heterozygotes, such as single nucleotide polymorphisms (SNPs) or microsatellites.

In this thesis, I largely worked with microsatellites – short tandem repeats of one to six (usually two to four) nucleotide sequences (Tautz and Renz 1984), which have proven

particularly useful for population genetic analyses because of their high mutation rates, high variability and apparent selective neutrality (Selkoe and Toonen 2006). The repeat strings of microsatellite loci are commonly in the size range of five to 40 repeats, which can change rapidly due to slippage effects during DNA replication, resulting in the loss or additions of repeat units at often high mutation rates between 10^{-2} and 10^{-6} mutations per locus per generation (Schlötterer 2000, Selkoe and Toonen 2006). The resulting high polymorphism in fragment size provides a rich allelic diversity well suited for population genetic studies. Microsatellite markers have also been frequently applied in phylogeographic studies, often in conjunction with mitochondrial markers (Beheregaray 2008). The main advantage is that many (tens to hundreds) unlinked and highly variable loci can be analyzed. An advantage over dominant markers is the ability to distinguish homozygotes from heterozygotes, allowing for additional analyses such as testing for Hardy-Weinberg equilibrium. However, working with microsatellites can be challenging due to extremely high and variable mutation rates, complex mutation processes, size homoplasy and genotyping artefacts such as null alleles (failed to amplify in a PCR) and large allele dropouts (i.e., the longer allele in a heterozygote can amplify poorly and be missed) (Selkoe and Toonen 2006, Brito and Edwards 2009, Guichoux et al. 2011). Besides the notion that microsatellites are not always selectively neutral (e.g., Li et al. 2002), a common criticism is that alleles with the same repeat number occurring in separate geographic areas may be convergent rather than homologous due to their high mutation rates, casting doubt on the ability of microsatellites to produce a valid phylogenetic analysis and suggesting a poor performance in phylogeographic studies (e.g., Zink 2010). However, despite high mutation rates and homoplasy making

microsatellite markers less informative for ancient divergences (Beaumont and Bruford 1999, Zink 2010), they have frequently proven to be useful for supporting phylogeographic patterns that reflect more recent (e.g., late Pleistocene) divergences (e.g., Koskinen et al. 2002, Hänfling et al. 2002).

An increasingly favored alternative to microsatellite markers is the use of single nucleotide polymorphisms (SNPs), which are DNA sequence variations at a single nucleotide. SNPs have relatively low mutation rates (10^{-8} to 10^{-9}, Brumfield et al. 2003), and are therefore less susceptible to homoplasy. Other advantages of SNPs include availability of large numbers (often thousands) of nuclear loci being more representative of the genome, low scoring error rates, simple mutation models (e.g., infinite sites model), the possibility to detect SNPs in candidate genes under selection, and being highly informative for population genetic analyses if at least two to six times more SNPs than microsatellite loci are available (Morin et al. 2004, Helyar et al. 2011). On the other hand, SNPs have only two alleles per locus and may be less powerful to detect genetic bottlenecks than microsatellites (Morin et al. 2004). Another possible drawback can be ascertainment bias introduced by preferentially scoring heterozygotes and avoiding rare SNPs, which may bias population genetic parameters such as estimates of genetic diversity, population size, or population structure (Morin et al. 2004, Helyar et al. 2011). Methods for "next-generation" SNP discovery are diverse (e.g., Morin et al. 2004, Baird et al. 2008, Davey et al. 2011, Seeb et al. 2011), and often rely on sequencing of a reduced portion of the genome such as transcriptome sequencing or use of restriction site enzymes (e.g., restriction site associated DNA [RAD] markers, Miller et al. 2007). While the development of thousands of SNP markers across the genome can be labor intensive

and introduce ascertainment bias (e.g., Garvin et al. 2010), it is now becoming increasingly affordable and rapidly transforming research areas (e.g., Luikart et al. 2003, Tautz et al. 2010).

1.4 Prickly sculpin as a study system

This thesis addresses patterns of historical and contemporary genetic structuring in prickly sculpin (*Cottus asper*), a common, small-bodied fish with a wide distribution range in the Pacific Northwest. Prickly sculpin (*Cottus asper*) is one of the largest freshwater sculpins (up to 22.5 cm SL), and characterized by strong palatine teeth, a single median chin pore, and 15-19 (usually 16-18) anal fin rays (McPhail 2007). During spawning season (April to June, in northern regions until late July), adults of both sexes develop an orange band on the edge of the first dorsal fin. Coastal river populations perform spawning migrations into the estuaries. Males excavate caves under rocks and wood debris, where females deposit eggs that are guarded by the males. Female fecundity typically ranges between 280 and 7410 eggs per female (93-176 mm SL) (Patten 1971), and males are thought to spawn with up to ten females, resulting in up to 30,000 eggs in one nest (e.g., Ringstadt 1974). Males use their pectoral fins to aerate the eggs until they hatch (15-16 days at 12° C). Larvae are 5-6 mm in size, transparent, and remain planktonic for about 30-35 days, allowing for along-shore drift and dispersal to neighbouring rivers. After a pelagic larval stage of about 30 days, juveniles settle to a benthic life style and start migrating back up the rivers. Sexual maturity is reached in about two years (e.g., Northcote 1954, Patten 1971), and the maximum age of prickly sculpin is 8-9 years (McPhail 2007). Prickly sculpin are generalist omnivores. Planktonic

postlarval sculpins feed on zooplankton (e.g., calanoid copepods, *Daphnia*), while benthic juvenile sculpins (~15-19 mm SL) switch to a diet being largely composed of Chironomidae larvae, Gammarus amphipods, Hydropsychid larvae (*Trichoptera*), mussels and snails (e.g., Broadway and Moyle 1978, Bolnick et al. 2010). Fish larvae (e.g., Sacramento sucker, cyprinids, cenrarchids, Pacific lamprey) appear to be common prey items when they are most abundant during May and June (Merz 2002). As they grow, sculpins become increasingly piscivore, with a wide range of prey fishes being reported (including other sculpins, mosquitofish, stickleback, speckled dace, larval sacramento suckers, juvenile sockeye salmon, cyprinids, centrarchids, pacific lamprey, redside shiner, squawfish fry, steelhead trout, pumkinseeds, longfin smelt). Known predators of prickly sculpin are Largemouth bass (Broadway and Mole 1978), Bull trout (Cannings and Ptolemy 1998), Pikeminnow (White and Harvey 2001), Cutthroat trout, Brown trout, Dolly varden, White sturgeon, Northern pike and Cod (McPhail 2007).

Previous research has revealed a disjunct phenotype distribution between coastal and inland populations of *C. asper* (Krejsa 1965). Coastal populations usually follow an amphidromous life cycle, which includes a planktonic, salt-tolerant larval stage, whereas inland populations employ a purely freshwater life cycle. These populations differ in morphological, behavioral and physiological traits (i.e., lateral prickling, spawning migration behavior, salinity tolerance; McPhail 2007), which presumably have diverged recently (< 18,000 years BP), given the young glacial history of this region. High gene flow among populations is facilitated through an extended planktonic larval stage and upstream migration of juveniles, which could require strong selection to achieve genetic divergence among locally adapted life history ecotypes occurring in sympatry.

Therefore, prickly sculpin has emerged as an interesting study organism to explore effects of demographic history on population divergence, and to investigate local adaptation in the presence of gene flow. The overall aim of this thesis was to provide a baseline framework for future research on *C. asper* in local adaptation, the early stages of population divergence with gene flow, and parallel evolution across multiple glacial lineages. The individual data chapters (Chapters II - IV) are briefly outlined below, and examine phylogeography, conservation genetics at range margins, and local adaptation between potential life history ecotypes inferred from a population genetics study. Chapters that have been published or are under review are presented in the layout style of the corresponding journal. A conclusion chapter (Chapter V) briefly summarizes the main findings of the individual chapters.

1.5 Phylogeography and local adaptation in Cottus asper

The first research chapter (Chapter II) investigates the phylogeographic pattern of *C. asper*, and tests alternative biogeographic hypotheses for the distribution of "prickling phenotypes" in British Columbia, Canada. Previous studies based on morphology suggested that prickly sculpin survived the Last Glacial Maximum (LGM) in southern coastal and inland refugia, favoring allopatric divergence and giving rise to coastal and inland "prickling" phenotypes, which vary in the degree to which spine-like scales cover the body of the fish. Herein, I used data from mitochondrial haplotypes and microsatellite loci to identify distinct glacial lineages as a basis to interpret patterns of phenotypic evolution. The occurrence of three major mtDNA lineages suggests that prickly sculpin survived the LGM in three glacial refugia: a southern coastal, a southern inland and a

previously unrecognized northern coastal refugium. This pattern was supported by the analysis of microsatellite allele frequencies. Prickling can be polymorphic within populations, but populations differ in this trait both within and between lineages. Each glacial lineage has independently contributed to the occurrence of a highly prickled phenotype, which predominates in genetically differentiated inland populations. Together, these results suggest that these phenotypes did not evolve allopatrically in separate refugia, but in parallel across multiple refugia, and most likely from standing genetic variation from highly differentiated ancestral coastal populations.

The next chapter (Chapter III) continues at the intersection of phylogeography and conservation genetics. Here, I addressed the consequences of postglacial range expansion and a possible ongoing invasion into the Peace River in Alberta for genetic diversity and conservation management of a recently discovered peripheral population of *C. asper*. Populations at the edge of their range often invoke taxonomic confusion and are increasingly considered to harbour cryptic genetic diversity of significant adaptive potential. In the Peace River region of northwestern Canada, three sculpin species have been reported: spoonhead (*Cottus ricei*), slimy (*C. cognatus*) and prickly sculpin (*C. asper*). Prickly sculpin occurrence in this region represents the most eastern edge of its distribution, but its status has remained uncertain following its initial discovery in 1989. These populations may represent an independently evolving lineage of special conservation concern, or be the consequence of an ongoing range expansion, possibly accompanied by interspecific hybridization with local species. Using a combination of mtDNA sequencing and microsatellite analyses, neither peripheral population differentiation nor interspecific hybridization was detected, suggesting that the Albertan

Peace River population belongs to the same genetic group as its western counterparts. Future studies will benefit from a greater understanding of whether demographically independent prickly sculpin populations established in Alberta without the typical genetic signatures of expansion at the periphery of their range.

Chapter IV continues to explore population genetic structure on a smaller geographic scale in southwestern British Columbia. While the previous chapters provided evidence for high genetic connectivity across larger geographic scales, here I examined whether sympatric life history ecotypes are genetically differentiated despite high gene flow. Life history transitions from amphidromous to freshwater life cycles have evolved repeatedly in various aquatic taxa, but the ecological and evolutionary conditions favoring such a transition within a species are poorly understood. Amphidromy is usually characterized by high genetic connectivity among populations due to wide dispersal of an extended planktonic larval stage and subsequent upstream migrations of juveniles. This may require strong selection to allow for life history divergence in the presence of high gene flow. I used population genetics methods to investigate genetic structuring in 14 microsatellite loci, to test for genetic differentiation and isolation-by-distance among coastal rivers around the Strait of Georgia, and among putative freshwater and amphidromous groups occurring in sympatry within the Lower Fraser River, British Columbia (Canada). Consistent with other amphidromous species, high genetic connectivity among coastal rivers was found. Within the main channel of Fraser River, no genetic differentiation was found across a distance of ~80 km, whereas tributaries to the Fraser River even close to the estuary were genetically differentiated and formed a distinguishable genetic cluster. Genetic differentiation between tributaries and main

channel was independent from geographic distance, suggesting a possible role for natural selection favoring life history divergence in tributary freshwater habitats. I propose that isolation by environment to discrete freshwater habitats may play a larger role for the early stage of amphidromy-freshwater transitions than subsequent macro-evolutionary changes in life history characteristics such as increased egg size and abbreviated larval development. The evolution of divergent freshwater life histories is likely most promoted in complex aquatic systems like the Fraser River, where large tributary streams and lakes allow for large effective population sizes.

A follow-up study that builds up on the finding of habitat-associated genetic differentiation in chapter III has been initiated, which aims to better understand local adaptation and genetic divergence of life history ecotypes in the presence of gene flow. A genomics approach is used that targets *a priori* identified teleost candidate genes underlying salinity tolerance divergence among brackish-water and freshwater groups of *C. asper*. For this purpose, two differentiated population pairs in the Lower Fraser River are used for whole genome sequencing of pooled DNA ("Pool-Seq"), where the pooling of individuals for each population provides a relatively cost-effective method to explore genome wide divergence among groups. Outlier loci indicative of divergent selection are then compared against the threespine stickleback (*Gasterosteus aculeatus*) and European sculpin (*Cottus gobio)* genomes to indentify candidate genes underlying salinity tolerance and osmoregulation. This will allow testing for parallel adaptation to freshwater environments and teasing apart parallel from non-parallel signatures of selection.

Chapter 2: Phylogeography of prickly sculpin (*Cottus asper*) in the Pacific Northwest reveals parallel phenotypic evolution across multiple glacial lineages

2.1 Abstract

Aim Glacial cycles during the Pleistocene may have frequently contributed to parallel evolution of phenotypes across independently evolving genetic lineages associated with separate glacial refugia. Previous studies based on morphology suggested that prickly sculpin survived the Last Glacial Maximum (LGM) in southern coastal and inland refugia, favoring allopatric divergence and giving rise to coastal and inland "prickling" phenotypes, which vary in the degree to which spine-like scales cover the body of the fish. Herein, we aimed to test for the alternative of parallel evolution across multiple glacial lineages as an explanation for the biogeographic distribution of prickling phenotypes.

Location The Pacific Northwest, SE-Alaska and Canada (British Columbia).

Methods We used data from mitochondrial haplotypes and 19 microsatellite loci to identify distinct glacial lineages as a basis to interpret patterns of phenotypic evolution.

Results The occurrence of three major mtDNA clusters suggests that prickly sculpin survived the LGM in three glacial refugia: a southern coastal, a southern inland and a previously unrecognized northern coastal refugium. This pattern was supported by the microsatellite data. Prickling can be polymorphic within populations, but populations

differ in this trait both within and between lineages. We show that more than one glacial lineage has independently contributed to the occurrence of a highly prickled phenotype, which predominates in genetically differentiated inland populations.

Main conclusions Together, these results suggest that highly prickled phenotypes evolved repeatedly from more than one glacial refugium, presumably from standing genetic variation of ancestral coastal populations. Prickly sculpin in the Pacific Northwest therefore provide an example of rapid (postglacial) parallel evolution potentially facilitated by high allelic frequencies already present in the ancestral populations.

Keywords Pleistocene, Pacific Northwest, phylogeography, parallel evolution, morphometrics, *Cottus*

2.2 Introduction

Understanding how phylogeographic patterns of neutral genetic variation reveal the signature of a species' demographic past remains the first step in efforts to elucidate how geological events, environmental influences, and geographical factors interact with aspects of a species ecology and natural history in shaping its evolution (Avise *et al.* 1987, Knowles 2009). Recent studies have reinforced that when such approaches are integrated with phenotypic data, phylogeography provides a backdrop for an array of evolutionary and ecological questions that could not be addressed without an understanding of phylogeographic history (Knowles 2009). Such approaches are

particularly important in species occupying ranges associated with potentially complex histories and repeated climatic changes (Dinca *et al.* 2011).

The Pacific Northwest of North America is characterized by such a complex geomorphological history and often reveals multifaceted phylogeographic patterns, which were largely shaped by repeated glaciations during the Pleistocene (~2.5 Myr – 12,000 years BP; Shafer *et al.* 2010). The increasing knowledge of the postglacial history of numerous taxa in this region creates unique opportunities to investigate evolutionary consequences of allopatric separation in glacial refugia, rapid range expansions into recently deglaciated areas, and mixing of genetic lineages in zones of secondary contact (Shafer *et al.* 2010). Such investigations contribute to a better understanding of the relative importance of historical and ecological effects on intraspecific geographic variation (Shikano *et al.* 2010), and therefore are necessary to make inferences about adaptive divergence of local populations (Knowles 2009). Ecological adaptation through natural selection is usually assumed when the repeated colonization of a new habitat across independently evolving genetic lineages results in parallel evolution of the same phenotype (Schluter 2000). However, recent studies have made it clear that the genetic basis of parallel phenotypes may vary among populations, ranging from identical or different mutations in the same gene to mutations in different genes (Elmer & Meyer 2011). For example, historical differentiation in standing genetic variation may lead to different genetic pathways causing parallel evolution across a species distribution range (Prunier *et al.* 2012). Therefore, it is increasingly important to consider the historical context of parallel evolution and its underlying genetic basis, in order to better understand targets and mechanisms of natural selection. As mitochondrial DNA presents a biased

view of demographic history and may not be selective neutral, phylogeographic studies that additionally include nuclear markers are required (e.g., Dowling *et al.* 2008). Genetic introgression among divergent lineages should increase geographic variation of genomic architectures, potentially favoring different genetic pathways during the parallel evolution of a similar phenotype. Hence, more phylogeographic studies that use both mitochondrial and nuclear DNA markers for investigating the historical background of parallel phenotypes across a species range are needed.

The prickly sculpin (*Cottus asper* Richardson) is a euryhaline fish species characterized by both amphidromous and purely freshwater populations. Consistent phenotypic variants have been observed across a wide distribution range between southern Alaska and California, representing potentially diverse historical backgrounds (Krejsa 1965; McPhail 2007). Postglacial colonization of inland regions has resulted in the predominance of a highly prickled phenotype, which is characterized by a dense lateral coverage with spiny, scale-like epidermal structures (Krejsa 1965). A heritable genetic basis of prickling likely exists, since a major QTL for prickling has been found in European sculpin *C. gobio* (Cheng & Nolte 2013). Geographically disjunct distributions in prickling intensity have been found in various *Cottus* species (e.g., *C. gobio*, Kontula & Väinölä 2004; *C. rhotheus*, McPhail 2007), but we are not aware of any apparent phenotype-environment associations that would indicate a common adaptive function of prickling.

It has been suggested that *C. asper* survived the Last Glacial Maximum (LGM) in two glacial refugia south of the ice sheets: a coastal refugium located in the Chehalis River region (south of Puget Sound, Washington State, USA), and an inland refugium in the

Columbia River system (British Columbia, Canada, and Washington State, USA) (McAllister and Lindsey 1961; Krejsa 1965). Accordingly, two northward post-glacial colonization routes have been proposed: (1) coastal along-shore dispersal through salt-tolerant planktonic larvae, and (2) inland through proglacial lakes creating ephemeral connections between river systems (Krejsa 1965). Phenotypic divergence between highly prickled inland sculpins and weakly prickled coastal sculpins has therefore been interpreted as a consequence of allopatric divergence in separate coastal and inland glacial refugia (Krejsa 1965, McPhail 2007). However, assumptions about post-glacial dispersal routes and phenotypic divergence of *C. asper* are based on the traditional view that species persisted in glacial refugia only south of the ice sheets (Bennett *et al.* 1991; Hewitt 2004). Meanwhile, a large number of phylogeographic studies have revealed the existence of more northern, cryptic glacial refugia (Shafer *et al.* 2010), providing additional sources for allopatric divergence and postglacial range expansions. Thus, highly prickled inland populations may have evolved in parallel across multiple glacial lineages originating from both southern and northern refugia, and secondary contact among lineages could have diversified the range-wide standing genetic variation available for selection during parallel evolution. As has recently been pointed out, gaining knowledge about phylogeographic pattern is an essential prerequisite to correctly characterize adaptive genetic polymorphisms (Prunier *et al.* 2012). Here, we present multi-locus data (mitochondrial DNA and microsatellites) as well as phenotypic data (prickling categories, morphometrics) to provide a phylogeographic context for the evolution of a parallel phenotype in prickly sculpin. Specifically, we test for the presence of northern refugia, secondary contact zones and postglacial colonization routes, and aim

to clarify whether the disjunct geographic distribution of prickling phenotypes is the result of allopatric divergence or parallel evolution.

2.3 Materials and Methods

2.3.1 *Sample collection and DNA sequence amplification*

Sculpins were sampled with baited minnow traps during spring and summer 2009 and 2010 from 29 locations in British Columbia and one location in Alberta (Fig. 2.1). A total of 1053 sculpins were collected, euthanized with an overdose of clove oil, and preserved in 95% ETOH. Additionally, 14 fin clips from Auke Creek (Juneau, SE- Alaska) were generously provided by Dr. David Tallmon (University of Alaska Southeast). Extractions of genomic DNA were obtained from either abdominal muscle or fin-clip tissue using a standard phenol-chloroform technique. Polymerase chain reactions (PCRs) were used to amplify a total of 1585 base pairs (bp) of mitochondrial DNA sequence for 141 sculpins from 12 sites: Alaska (n = 14), Meziadin Lake (n = 11), Mosquito Lake (n =10), Bella Coola River (n =11), Nimpo Lake (n =12), McLeod Lake (n =13), Peace River in British Columbia (n =11), Peace River in Alberta (n =10), Falls Creek (n =10), Little Campbell River (n =16), Harrison Lake (n =10), and Okanagan Lake (n =13) (Fig. 2.1; for primer sequences and PCR protocols, see Appendix S1 in the Supporting Information). Sequence proofreading was conducted with 4PEAKS 1.7.2 (Griekspoor & Groothuis 2006), and alignments were carried out with ClustalX 2.1 (Larkin *et al.* 2007). Sequences of mtDNA haplotypes are listed in Appendix 5.

2.3.2 *Phylogenetic analysis of sequence data*

Phylogenetic trees were built for concatenated mtDNA sequences using an maximum-likelihood (ML) approach implemented in RAxML 7.3.2 (Silvestro & Michalak 2011) with 1000 bootstrap runs, and a Bayesian Inference (BI) approach in MRBAYES 3.2 (Ronquist *et al.* 2012). The best-fit nucleotide substitution model was determined in JMODELTEST (Posada 2008), based on the corrected Akaike information criterion (AICc). A Bayes Factor test implemented in TRACER 1.5 (Rambaut & Drummond 2009) indicated a better fit of a codon-partitioned model (by gene and 3rd codon), opposed to an unpartitioned model. Analyses in MrBayes consisted of four Monte Carlo Markov chains running for 30 million generations (average standard deviation of split frequencies < 0.01). Trees were sampled every 1000th generation with a default burn-in of 25% of samples. A 50% majority-rule consensus tree was built from the remaining trees and used to calculate posterior probabilities of tree branches. Convergence and stationarity of likelihood scores was confirmed in TRACER. A haplotype network under statistical parsimony was constructed with TCS 1.13 (Clement *et al.* 2000).

2.3.3 *Genetic diversity and divergence time estimates*

We used ARLEQUIN 3.5.1.2 (Excoffier *et al.* 2005) for calculating population pairwise F_{ST} values for mtDNA sequences (θ, Weir & Cockerham 1984), genetic diversity estimates, and analyses of molecular variance (AMOVAs) in order to test for variation within and among geographic groups as well as major mtDNA haplotype clusters. Genetic diversity estimates were calculated for each putative population and geographic mtDNA group (Alaska, Bella Coola, North, South coast, South inland), which were

derived from the AMOVA analyses (Table 2.1). Average uncorrected pairwise genetic distances (p-distances) within and between major mtDNA tree groups were calculated in MEGA 5.05 (Tamura *et al.* 2011).

To obtain estimates of divergence times between main mitochondrial tree clusters, we employed a relaxed molecular clock analysis in MrBayes using an independent gamma rate model, which is a continuous uncorrelated model of rate variation across lineages and allows the variance of effective branch lengths to increase over time. Sequences were partioned for gene and codon position, in order to allow for different substitution rates. A HKY+I substitution model was chosen for all partitions, but were allowed to vary independently from each other (among genes and codon positions) using an automatic Dirichlet prior. Because no external calibration points were available, we assumed a conventional mtDNA mutation rate of 2% (0.01 substitutions/site/million years), which has been widely used for numerous vertebrate taxa, including freshwater sculpins (Brown *et al.* 1979; Engelbrecht *et al.* 2000; Yokohama & Goto 2002). Using a normally distributed 2% clock rate prior, we allowed for both slower and faster clock rates by applying a 0.3% standard deviation. The follwing MrBayes settings were used: set partition = codongene; lset applyto=(all) nst=2 rates=invgamma; unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all); prset applyto=(all) ratepr=variable; prset brlenspr=clock:uniform; prset clockratepr = normal(0.01, 0.0015); prset treeagepr = exponential(0.01); prset clockvarpr=IGR; prset ratepr=variable. Analyses were run in four Monte Carlo Markov chains for 30 million generations, with trees being sampled every 1000th generation, and we controlled for convergence and stationarity of likelihood scores in TRACER.

2.3.4 *Microsatellite amplification and analyses*

We amplified a total of 19 microsatellite loci, including 18 microsatellite loci from Nolte et al. (2005a) and one newly designed marker "*EDA1*". This locus is linked to the *Ectodysplasin* (EDA) region in *C. gobio* and was suspected to be related to prickling in *C. asper*, but showed no deviations from neutrality in a F_{ST} outlier test or gametic phase disequilibrium with the prickling phenotype (unpublished data). We genotyped 273 individuals from the 12 populations used for the sequence analyses (including the same individuals), and 132 individuals from four additional locations (Fig. 2.1): Lakelse Lake (n=30), Martins Lake on Campbell Island (n=51), Martins Lake outlet (n=31), and Tlell River (n=20). Allelic sizes (in base pairs) were determined by reference to the internal sizing standard in the software GENEMAPPER version 4.1 (Applied Biosystems). For primer sequences and PCR conditions, see Appendix S1 in Supporting Information.

We used an extended version of Fisher's exact test for testing microsatellite loci for Hardy-Weinberg equilibrium (HWE, 10^5 Markov chain steps, 10^4 dememorization steps) and linkage disequilibrium (LDE, 10^5 Markov chain steps, 10^4 dememorization steps) implemented in the software ARLEQUIN v. 3.5.1.2 (Excoffier *et al.* 2005). Sequential Bonferroni correction was applied to the P values to correct for multiple comparisons in the same dataset. Pairwise F_{ST} values, as well as observed (H_O) and expected (H_E) heterozygosities were calculated in ARLEQUIN. Genetic diversity and allelic richness (mean number of alleles, corrected for smallest sample number) was calculated in FSTAT 2.9.3.2 (Goudet 2001). We used a permutation test (15000 permutations) in FSTAT to compare allelic richness and genetic diversity between coastal and inland populations.

The possibility of null alleles, large allele drop-outs and scoring errors was evaluated using MICROCHECKER v. 2.2.3 (Van Oosterhout *et al.* 2004).

In order to assess genetic variability based on the three major mtDNA groups, an analysis of molecular variance (AMOVA) was conducted, excluding the high admixture populations in Auke Creek (Alaska) and Bella Cool River. Individual-based chord distance measures (D_C, Cavalli-Sforza & Edwards 1967) were calculated and used for the construction of a 1000 times bootstrapped neighbor-joining tree in POPULATIONS 1.2.32 (Langella 1999). An individual-based assignment approach implemented in STRUCTURE 2.3.3 (Pritchard *et al.* 2000) was used to infer genetic population structure. We used an admixture model to test for number of populations (K) from 4 to 16. We applied a 100,000-iteration burn-in followed by 900,000 iterations, and repeated each K four times to ensure stability. The most likely number of genetic clusters was determined by using the ΔK method (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER (Earl & von Holdt 2012). For microsatellite data, see Appendix 6.

2.3.5 *Morphology*

Sculpins were bleached for 2 -10 days (3 parts of 0.5% potassium hydroxide to 1 part glycerol, containing 50 µl of 50% hydrogen peroxide per 10 ml KOH:glycerol solution), and stained for 48 h in a 1% KOH and Alzarin-red solution. We assessed prickling pattern and body shape variation among coastal and inland populations. Prickling intensity was characterized as “high”, “medium” or “low” for each individual, which corresponds to prickling categories 2-4 as described by Nolte *et al.* (2005b) and reflects the coverage of the lateral body side with prickle-shaped, scale-like bony structures. A

Pearson's chi-square test was conducted to test whether coastal and inland sculpin populations differ in frequencies of prickling categories.

Body shape was analyzed using geometric morphometrics, employing twenty-three homologous landmarks digitized on the left lateral body side of photographed sculpins using tpsDIG 2.14 and a ruler in the image for scaling (Rohlf 2009a) (Appendix S2). Because preservation caused pronounced arching of the fish bodies, we included only samples showing a low degree of bending in analyses (coastal: n = 548; inland: n = 183). To remove remaining effects of bending from the landmark configuration, we used the "unbend" function implemented in tpsUTIL 1.44 (Rohlf 2009b). For this purpose, landmarks 8 and 21-23 were chosen to represent a straight line (Appendix S2). A quadratic curve was then fitted along these landmarks and used to correct for the arch in the complete landmark set. After removing landmarks 21-23, we used MorphoJ 1.05d (Klingenberg 2011) to conduct a generalized least squares Procrustes superimposition to remove the effects of rotation, scale and non-allometric effects of size. The resulting partial warp scores and uniform components were used as shape variables in subsequent analyses. To test for allometric effects within coastal and inland groups, we used a multivariate analysis of covariance (MANCOVA) with (log) centroid size as a covariate, and tested for equal slopes among coastal and inland groups using a MANCOVA as implemented in tpsRegr 1.38 (Rohlf 2011). We obtained size-corrected shape variables by performing a MANCOVA of Procrustes coordinates on (log) centroid size using a permutation test for each subgroup (10,000 permutations), and used the regression residuals for a size-corrected analysis (Berner 2011). We used a jackknifed discriminant function analysis (DFA) on both size-corrected and uncorrected data to determine how

well *a priori* defined coastal and inland populations can be distinguished by body shape variables. A principal component analysis (PCA) on the variance-covariance matrix of shape coordinates was conducted to visualize the major pattern of shape variability.

2.4 Results

2.4.1 Mitochondrial DNA

We detected 20 mitochondrial haplotypes (20 variable positions, with five being parsimony informative) in 141 individuals from 12 populations. For the mtDNA data set, the substitution model HKY + I was chosen as the most appropriate model, as determined by the lowest AIC in jMODELTEST. The statistical parsimony haplotype network inferred three main groups of mtDNA haplotypes (Fig. 2.2a), which are denoted according to their main geographic distribution as North (N), South-Coast (SC) and South-Inland (SI) clusters. These three major genetic clusters were also apparent in the phylogenetic trees, which show identical topologies and similar posterior probability support for both maximum likelihood (ML) and Bayesian Interference (BI) constructions (Appendix S3). Uncorrected average pairwise distances within and between major tree clusters were low compared to reports from other freshwater sculpins (e.g., Engelbrecht *et al.* 2000), ranging from 0.09% to 0.21% between clusters, and from 0.02% to 0.03% within each cluster.

In contrast to other study populations, Auke Creek (SE-Alaska) and Bella Coola River revealed a high mixture of haplotypes originating from different mtDNA haplotype clusters (Fig. 2.2b). Accordingly, mtDNA haplotype and nucleotide diversity was higher in Alaska and Bella Coola River compared to other groups (Table 2.1). The AMOVA

supported five distinct geographic groups: Alaska, Bella Coola, North, South coast, and South inland (F_{CT} = 0.638, P < 0.001), with 63.88 % of the total variance being explained by differences among geographic regions (Table 2.2). Assuming a mutation rate of 2% (± 0.3%), age interval estimates (95 % highest posterior density, HPD) were 2,400-32,300 years before present (BP) for the basal node of the N-cluster, 2,200-29,300 years BP for the SI-cluster, and 2,400-30,200 years BP for the SC-cluster.

2.4.2 Microsatellite loci

In 405 individuals, the degree of polymorphism in the 19 microsatellite loci was highly variable, with the total number of alleles ranging from 2 to 31. Allelic richness (standardized to a minimum sample size of seven) of polymorphic loci ranged from 1.8 to 6.3, and gene diversity ranged from 0.15 to 0.77 (Table 2.1). Significant (sequential Bonferroni-corrected) departures from HWE were found for locus *Cott153* in Okanagan Lake and Martins Lake, and for locus *CottE30* in Martins Lake outlet. Evidence for linkage disequilibrium (after sequential Bonferroni-correction) was found in 38 (out of 2,736) comparisons, and potential null alleles occurred in nine locus-population pairs. Coastal populations showed overall higher genetic diversity estimates than inland populations, with an average allelic richness of 5.04 compared to 3.23 (Fstat permutation test, two-sided, P = 0.0036), and a gene diversity of 0.665 compared to 0.422 (P = 0.0011), respectively. Significant pairwise F_{ST} values ranged from 0.038 (Falls Creek – Martins Lake outlet) to 0.57 (McLeod Lake – Okanagan Lake). The AMOVA results showed that most of the variation existed within populations (61.3 %), while 20.25 % of the variation was explained by the mtDNA groups (Table 2.2). Clustering results in

STRUCTURE (Fig. 2.3a) and a highest Δk value (Evanno et al. 2005) supported k=11 clusters by grouping Peace River, McLeod Lake and Nimpo Lake in one cluster, Martins Lake and outlet in another cluster, and forming a third cluster with Little Campbell River and Falls Creek. These clusters are consistent with a NJ- tree on D_C distances of microsatellites (Fig. 2.3b).

2.4.3 Morphology

Prickling intensity differed significantly between inland and coastal sculpins (χ^2 = 208.1902, df = 2, $p < 0.001$), with inland populations being dominated by highly prickled sculpins, whereas coastal sculpins exhibited high frequencies of medium and low prickling categories (Fig. 2.2c, Appendix S2). Allometric effects of centroid size on body shape variation were significant, but explained only 3.91 % (coastal: MANCOVA, 10,000 permutations, $P < 0.001$) and 3.88 % (inland: MANCOVA, 10,000 permutations, $P < 0.001$) of shape variation, respectively. A discriminant function analysis (DFA) on both size-corrected and uncorrected data gave similar results and indicated a small but significant difference between coastal and inland groups (Procrustes distance: 0.0088, $P < 0.001$). Classification accuracy in the cross-validated DFA was 81.9% for coastal and 77% for inland sculpins for size-corrected data. Inland sculpins showed a subtle tendency towards a shorter and higher caudal peduncle, a smaller eye and a slightly stouter snout compared to coastal sculpins (Appendix S2). These differences among coastal and inland groups were also evident when performing a DFA on highly prickled (category 4) sculpins only (not shown). All landmark data can be found under Appendix 8.

2.5 Discussion

In this study, we present phenotypic and multi-locus genetic data to evaluate the phylogeographic history of prickly sculpin (*Cottus asper*) and the evolutionary context of a geographic discontinuity in prickling intensity. Our finding that highly prickled inland phenotypes are distributed across three mitochondrial lineages suggests parallel evolution, rather than an allopatric origin from an inland refugium, most likely contributes to the repeated phenotypic dichotomy observed. The presence of multiple glacial refugia as sources for repeated colonization of inland regions was also supported by patterns of microsatellite differentiation. Future studies will need to reveal whether the genetic basis of parallel phenotypes is identical across lineages, or whether a high standing genetic variation of the putative ancestral coastal populations has favored different, lineage-specific genetic variants.

2.5.1 Glacial refugia and post-glacial colonization

We first aimed to distinguish between the presence of only southern glacial refugia, and the alternative of both southern and northern glacial refugia. Assuming postglacial colonization from only southern refugia, the expectation is that genetic variation should decrease towards more northern latitudes as a consequence of sequential founder effects (Hewitt 2004). In contrast, no association between latitude and genetic diversity is expected when northern refugia have contributed to postglacial re-colonization (e.g., Petit *et al.* 2003). High genetic diversities may then appear in northern latitudes, and geographic patterns of genetic divergence should be consistent with the location of putative glacial refugia (e.g., Shafer *et al.* 2010). Our results show a pattern of

mitochondrial haplotypes forming three genetic clusters, albeit weakly differentiated but consistent with the divergence among three glacial lineages originating from two southern refugia and one northern coastal refugium. Additionally, the previous assumption of glacial refugia only south of the ice sheets appears unlikely given our data, because genetic diversity estimates did not decrease from south to north as would have been expected from a northwards re-colonization from southern refugia (Hewitt 2004). Instead, high diversity estimates were found in more northern sites , suggestive of a northern coastal refugium. While our limited sampling scheme and postglacial admixture does not allow pinpointing the exact location of a northern coastal refugium, both mtDNA and microsatellite data are consistent with this hypothesis, and has been suggested for numerous other species in the Queen Charlotte Islands and the Alexander Archipelago area (Shafer *et al.* 2010). Some northern temperate fish species such as threespine stickleback, rainbow trout and at least two salmon species may have survived the LGM in this region (O'Reilly et al. 1993, Wood et al. 1994, McCusker et al. 2000, Smith et al. 2001). The current northern range limit of *C. asper* in SE-Alaska appears to make glacial survival in areas of a more northern Beringian refugium, such as coastal regions of the Chukchi Sea, Bering Sea or Aleutian Islands, unlikely. Given the high structure found in the microsatellite loci among northern coastal populations, we could however speculate about the presence of a mosaic of separate, partly disconnected coastal refugia forming what has been termed "refugia within refugia" (e.g., Shafer et al. 2010). Regarding southern refugia, the strong phylogeographic break between southern coastal and inland populations supports the previous assumption of coastal and inland southern refugia (Krejsa 1965, McPhail 2007), which has commonly been reported for other taxa

within this region (Shafer *et al.* 2010). For aquatic taxa, this coastal-inland break has often been attributed to well-established refugial areas located in the Chehalis River and lower Columbia River valleys (McPhail & Carveth 1993; Taylor *et al.* 1999; McPhail 2007; Ardren *et al.* 2011). For example, the crayfish *Pacifastacus leniusculus* exhibits divergent coastal and inland lineages in this region, which includes an "Okanagan group" that likely has colonized the Okanagan Valley via river connections from a sub-Okanagan Lobe glacial refugium in the Columbia River (Larson *et al.* 2012). This connection to the inland Columbia River refugium may also explain our finding of a distinct south-inland mtDNA lineage in Okanagan Lake for *C. asper*.

Postglacial colonization of *C. asper* likely followed two major pathways, one along the coast and one inland through ephemeral connections between river systems. Inland dispersal was facilitated during deglaciation, when drainage of large proglacial lakes allowed crossing watersheds through temporal connections between rivers (McPhail & Carveth 1993). Similar to other species (e.g., pygmy whitefish, Witt *et al.* 2011), prickly sculpin apparently accessed the Peace River watershed via the Fraser and Skeena Rivers (McPhail 2007). Founder effects during inland colonization events originating from coastal regions may have contributed to the lower genetic diversities in inland populations. Elevated genetic diversities in coastal compared to landlocked freshwater fish populations has been frequently described, and typically explained by larger effective population sizes and more gene flow among coastal populations (Teacher *et al.* 2011). Gene flow among coastal populations of *C. asper* is facilitated by an amphidromous life cycle, with a high dispersal capability of salt-tolerant, planktonic larval stages (Krejsa 1965; McPhail 2007). Hence, secondary contact of multiple glacial lineages along the

coast has likely contributed to the occurrence of high genetic diversities. The presence of an admixture zone along the central-northern coast of British Columbia and SE-Alaska is supported by the finding of haplotypes from all three mitochondrial clades, and elevated genetic diversities, in Auke Creek (SE-Alaska) and Bella Coola River (central BC), which furthermore occupy an intermediate position between northern and southern-coastal groups in the microsatellite NJ-tree.

2.5.2 Comparison of mitochondrial and nuclear genetic data

Analyses of microsatellite loci revealed pronounced population structuring, which is overall consistent with the characterization of three mtDNA tree clusters. This shows that genetic divergence in microsatellite markers among glacial lineages has not been eroded by high migration rates during more recent times, as has been suggested for other species (e.g., Atlantic herring, Gaggiotti *et al.* 2009). Moreover, the high structure in the microsatellite data indicates that male-biased dispersal or female-biased philopatry are not major drivers of mtDNA structuring. The finding of additional, more fine-scaled genetic structure within mtDNA lineages in the microsatellite markers is expected in the absence of high migration rates, given the higher mutation rates of microsatellite loci (e.g., Koskinen *et al.* 2002). While high mutation rates and homoplasy make microsatellite markers less informative for more ancient divergences and have frequently failed to find structure in the presence of mitochondrial genetic structuring (Zink 2010), they still may be useful to support phylogeographic patterns that reflect more recent (late Pleistocene) divergences in some taxa (Koskinen *et al.* 2002; Hänfling *et al.* 2002).

An unexpected discordance between nuclear and mitochondrial DNA was found in a pronounced genetic break between northern coastal and inland populations in the microsatellite loci but not the mtDNA, which is dominated by the most common northern haplotype. This could suggest mitochondrial introgression following secondary contact between glacial lineages, as has been reported in other studies (e.g., Nolte *et al.* 2005b; Toews & Brelsford 2012). A possible double-colonization scenario allowing mitochondrial introgression upon secondary contact could be postglacial dispersal of the southern-coastal lineage into the Peace River watershed via the Fraser River, followed by the introgressive invasion of the northern mtDNA lineage via the Skeena River. While a recent spread of the northern mtDNA haplotype is supported by a star-like pattern in the haplotype-network, a negative Tajima's D and a unimodal mismatch distribution (Appendix 2.S4), we recognize the speculative nature of such a double-colonization scenario, and propose that future studies should test for introgression of a northern mitochondrial haplotype that is better adapted to cold temperatures and has therefore replaced a southern haplotype (e.g., Mee & Taylor 2012). In general, selection on the mitochondrial genome may be common (Dowling *et al.* 2008), and selective sweeps could play an important yet frequently overlooked role in shaping phylogeographic patterns (Rato *et al.* 2011).

2.5.3 Parallel evolution

Our results suggest that multiple glacial lineages have independently contributed to the occurrence of highly prickled inland populations, which indicates parallel phenotypic evolution (Elmer & Meyer 2011). We found only weak differentiation in body shape

between coastal and inland groups, which does not support deep allopatric divergence either and emphasizes a recent evolutionary origin of highly prickled inland phenotypes. The parallel fixation of a highly prickled phenotype across independent glacial lineages suggests a potential role of natural selection (Schluter 2000), which assumes a heritable genetic basis of prickling. This seems plausible given the finding of a major prickling QTL in *C. gobio* (Cheng & Nolte 2013). Regardless of whether prickling is adaptive, the high variability in prickling intensity in the presumed ancestral coastal populations suggests a more likely role of standing genetic variation rather than new mutations as a source of parallel evolution. Accordingly, the availability of standing genetic variation allows faster fixation of alleles compared to low-frequency new mutations, which may enhance the probability of finding parallel phenotypes in recently deglaciated, postglacial environments such as those found in the Pacific Northwest (e.g., threespine stickleback, Colosimo *et al.* 2004). Selection on standing genetic variation may result in different genetic pathways underlying parallel phenotypes across different genetic lineages in the presence of historical differentiation of gene pools (e.g., black spruce *Picea mariana*, Prunier *et al.* 2012). Alternatively, standing genetic variation may provide just one mutation in the same gene that is repeatedly selected for during parallel evolution (e.g., oldfield mice *Peromyscus polionotus*, Domingues *et al.* 2012). Conte *et al.* (2012) predicted a high probability for the repeated use of the same genes during parallel evolution, because usually only a small number of genes carrying beneficial mutations are available. This appears plausible for highly conserved genetic pathways such as the EDA pathway (Conte *et al.* 2012), which is also supported by a recent study of Leinonen et al. (2012) reporting limited standing genetic variation in EDA, leading to an

evolutionary alternative of reduction of lateral plate size rather than plate numbers in freshwater populations of threespine stickleback. Hence, further studies are needed to reveal whether parallel evolution of prickling phenotypes in *C. asper* is based on a single mutation in the EDA pathway as suggested by Cheng & Nolte (2013) for European *Cottus* or elsewhere, or if historical differentiation has diversified genetic variation underlying highly prickled phenotypes. The latter case might raise the question of whether selection of available genetic variants is non-random with respect to differentiated genetic lineages, and indirectly affected by an optimized integration with the respective genomic architecture of each lineage.

2.6 Conclusions

In summary, we provide an example of a parallel phenotype without known adaptive function that has originated from multiple glacial lineages, and repeatedly became the predominant phenotype after independent colonization of inland regions. Future studies should test whether mitochondrial introgression among two lineages may have facilitated colonization of northern inland regions, and if standing genetic variation of the highly admixed ancestral coastal populations could have resulted in lineage-specific genetic polymorphisms underlying highly prickled phenotypes. While the adaptive function of parallel phenotypes often remains to be experimentally tested, the possibility of alternative parallel evolutionary pathways (e.g., genetic constraints, ancient selective regimes) deserves more attention.

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2.9 Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Primer sequences and PCR protocols.

Appendix S2 Prickling and body shape differences between inland and coastal sculpins.

Appendix S3 Fifty percent majority-rule consensus tree of mtDNA sequences.

2.10 Biosketch

Stefan Dennenmoser is broadly interested in the intersection of biogeography, natural history and local adaptation and its implications for the genetic basis of adaptive change. This study was part of his PhD research on phylogeography of *Cottus asper* in the Pacific Northwest.

Author contributions: S.D., S.M.V., A.W.N., and S.M.R. designed the study. S.D. performed the research and analyzed the data, and all authors contributed to writing the paper.

2.11 Figures and Tables

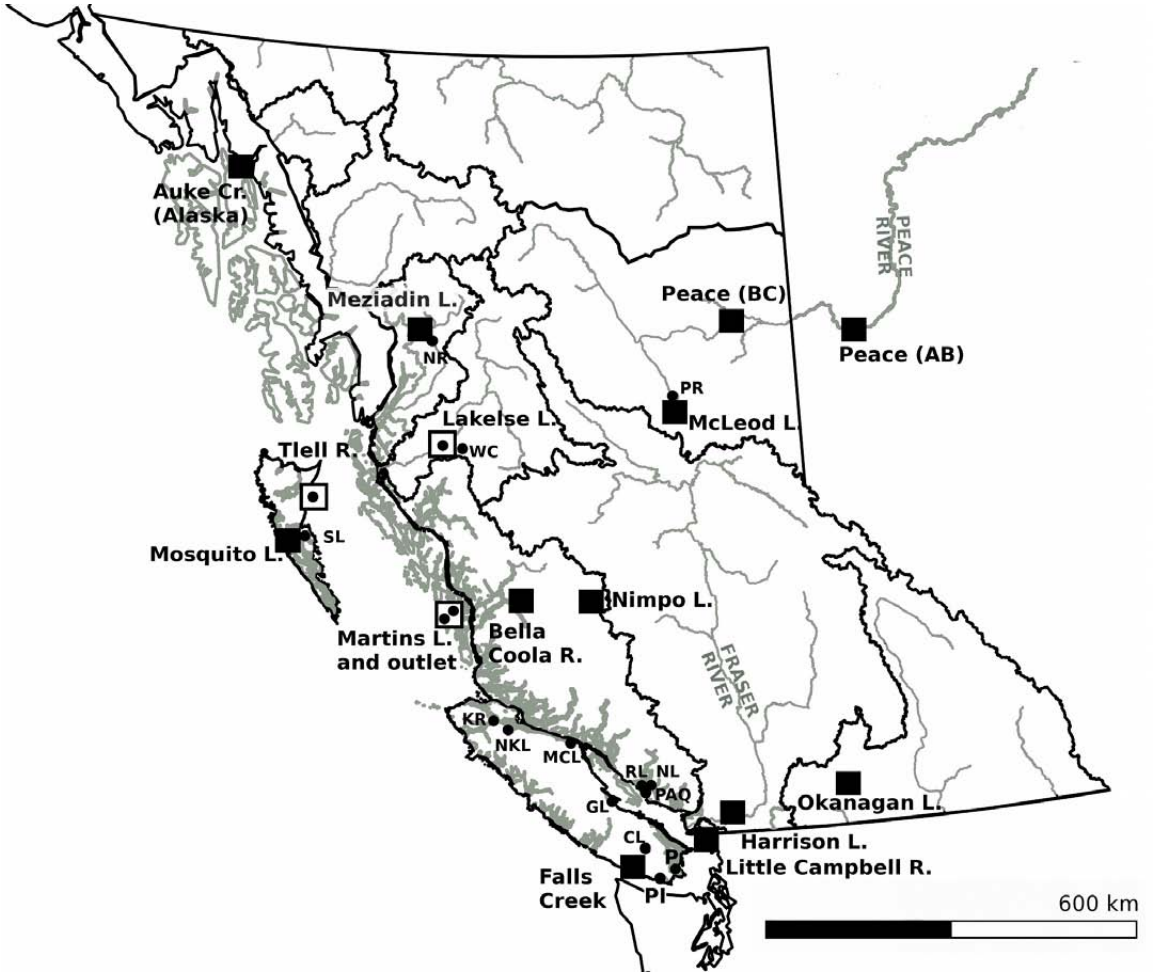


Figure 2.1 Map showing sampling locations in the study area. Filled squares represent locations with both sequence and microsatellite data available, open squares locations with microsatellite data only, and black circles additional sampling sites where morphometric data were collected. Abbreviations read as follows: CL = Cowichan Lake, GL = Graham Lake, KR = Keogh River, MCL = McCreight Lake, NKL = Nimpkish Lake, NL = North Lake, NR = Nass River, P = Prospect lake, PAQ = Paq Lake, PI = Poirier lake, PR = Parsnip River, RL = Ruby Lake, SL = Skidegate Lake, WC = Williams Creek. Major watershed boundaries are outlined in black.

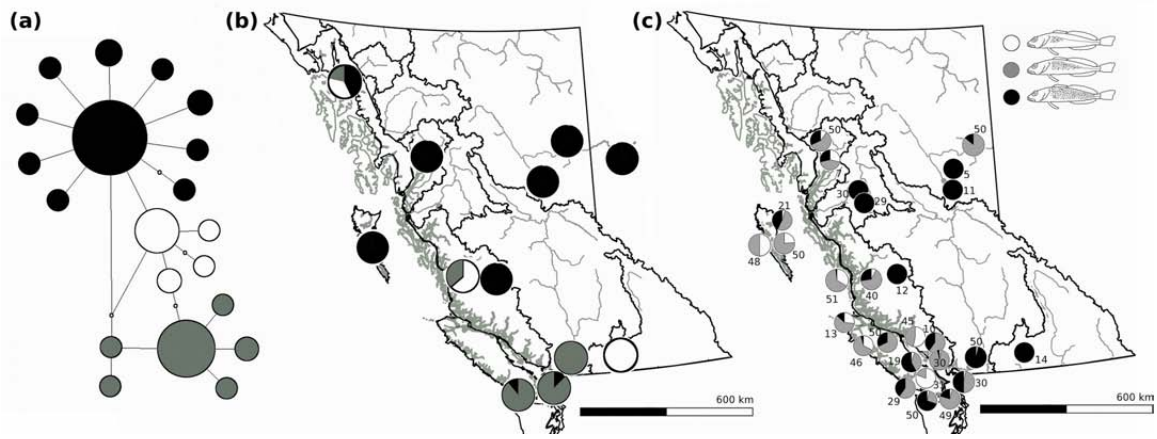


Figure 2.2 (a) Haplotype network (mtDNA). (b) Map showing geographic distribution of major groups of mtDNA haplotypes. Black represents the North cluster, grey the South coast cluster, and white the South inland cluster. (c) Geographic distribution of prickling categories: high (black), medium (grey) and low (white). Numbers indicate sample sizes.

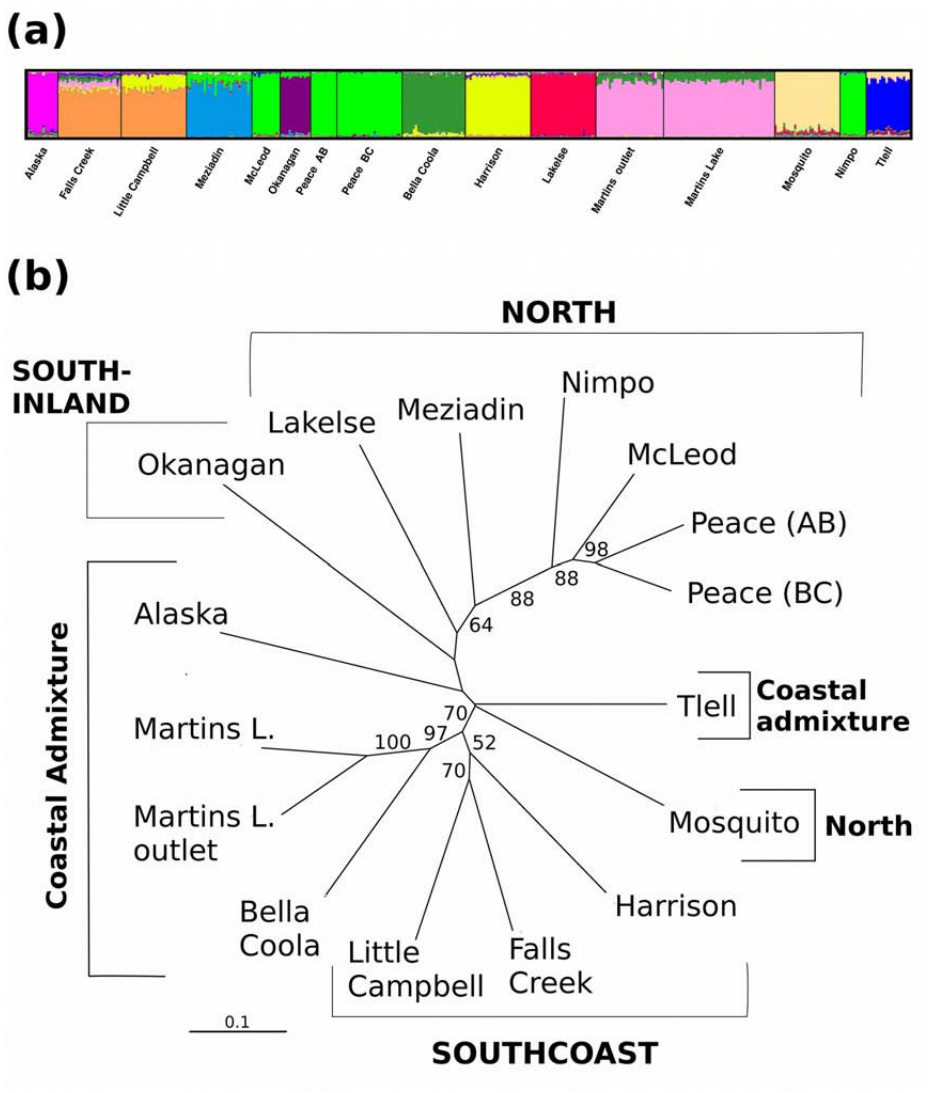


Figure 2.3 (a) STRUCTURE plot showing assignment of 405 individuals from 16 sampling locations into 11 clusters. (b) Neighbor-joining tree based on D_C distances among populations based on 19 microsatellite loci (numbers indicating 1000x bootstrap support).

Table 2.1: Sampling locations and genetic diversity estimates for microsatellite loci and mitochondrial DNA of *Cottus asper* (N = number of samples, H_E = expected heterozygosity, H_O = observed heterozygosity, A_R = allelic richness, s = number of polymorphic sites, h = haplotype diversity, π = nucleotide diversity). Summary statistics for the three major mitochondrial DNA clusters "North", "South coast" and "South inland" (excluding admixture populations Alaska and Bella Coola River) are given at the end of the table.

		Microsatellites				mtDNA			
Location	**Lat./Long.**	**N**	**H_E**	**H_O**	**A_R**	**N**	**s**	**h**	**π**
Coastal									
Alaska (Auke Creek, A)	58° 22.96'N 134° 38.13'W	14	0.60	0.57	3.54	14	5	0.85 (+/- 0.07)	0.0012 (+/- 0.0008)
Bella Coola River (BC)	52° 22.02'N 126° 44.15'W	29	0.68	0.64	5.34	11	5	0.76 (+/- 0.1)	0.0012 (+/- 0.0008)
Little Campbell River (LC)	49° 0.9' N, 122° 46.7' W	30	0.73	0.70	5.66	16	3	0.44 (+/- 0.14)	0.0004 (+/- 0.0004)
Falls Creek (FC)	48° 34.02'N 124° 21.25'W	29	0.77	0.75	6.30	10	4	0.78 (+/- 0.13)	0.0008 (+/- 0.0006)
Mosquito Lake (MOL)	53° 4.2' N, 132° 4.2' W	30	0.55	0.57	4.00	10	2	0.53 (+/- 0.18)	0.0003 (+/- 0.0003)
Martins Lake (MAL)	52° 10.33'N 128° 8.83'W	51	0.69	0.66	5.24				
Martins Lake outlet (MALO)	52° 10.31'N 128° 8.74'W	31	0.70	0.71	5.18				
Tlell River (TR)	53° 34.88'N 131° 56.61'W	20	0.66	0.64	4.9				
Inland									
Meziadin Lake (MEL)	56° 5.14'N 129° 18.31'W	30	0.45	0.44	3.45	11	1	0.67 (+/- 0.12)	0.0003 (+/- 0.0003)
Nimpo Lake (NIL)	52° 20.02'N 125° 9.22'W	12	0.15	0.13	1.80	12	1	0.45 (+/- 0.17)	0.0001 (+/- 0.0002)
McLeod Lake (ML)	54° 59.4' N, 123° 2.1' W	13	0.34	0.33	3.10	13	3	0.54 (+/- 0.16)	0.0003 (+/- 0.0003)
Peace River, AB (PA)	55° 55.1' N, 118° 36.4' W	12	0.35	0.36	2.99	10	2	0.2 (+/- 0.15)	0.0003 (+/- 0.0003)
Peace River, BC (PB)	56° 3.9' N, 121°50.5' W	30	0.33	0.34	2.89	11	0	0.18 (+/- 0.14)	0
Harrison Lake	49° 32.19'N	30	0.63	0.63	5.02	10	3	0.84	0.0005

(HL)	121° 52.45'W							(+/- 0.1)	(+/- 0.0005)
Okanagan Lake (OL)	50° 12.5' N, 119° 27.9' W	14	0.46	0.45	3.66	13	3	0.53 (+/- 0.15)	0.0004 (+/- 0.0004)
Lakelse Lake (LAL)	54° 22.6' N, 128° 33 ' W	30	0.41	0.41	3.30				
mtDNA groups									
NORTH	(MOL, MEL, NIL, ML, PA, PB)					67	8	0.44 (+/- 0.08)	0.0002 (+/- 0.0002)
SOUTHCOAST	(FC, LC, HL)					36	6	0.66 (+/- 0.09)	0.0006 (+/- 0.0005)
SOUTHINLAND	(OL)					13	3	0.53 (+/- 0.15)	0.0004 (+/- 0.0004)

Table 2.2: Analyses of molecular variance (AMOVAs) for mitochondrial sequence and microsatellite data of *Cottus asper* (* P < 0.001, 1023 permutations).

	Source of variation	**df**	**Sum of squares**	**Variance component**	**Percentage of total variation**	**Fixation indices**
mtDNA, 5 groups	Among groups	4	67.825	0.688 *	63.88	F_{CT} = 0.638
	Among populations Within groups	7	2.51	0	0	F_{SC} = 0
	Within populations	129	50.58	0.392 *	36.4	F_{ST} = 0.636
	Total	140	120.91	1.08		
mtDNA, 3 groups	Among groups	2	90.64	1.07 *	82.55	F_{CT} = 0.826
	Among populations Within groups	14	6.8	0.037 *	2.86	F_{SC} = 0.164
	Within populations	124	23.49	0.189 *	14.59	F_{ST} = 0.854
	Total	140	120.9			
Microsatellites, 3 mtDNA groups	Among groups	2	326.2	0.762 *	20.25	F_{CT} = 0.2
	Among populations, Within groups	10	387.4	0.742 *	19.71	F_{SC} = 0.247
	Among individuals, Within populations	302	667.9	0	0	F_{IS} = 0
	Within individuals	315	726	2.31 *	61.3	F_{IT} = 0.387

Chapter 3: Conservation genetics of prickly sculpin (*Cottus asper*) at the periphery of its distribution range in Peace River, Canada

3.1 Abstract

Populations at the edge of their range often invoke taxonomic confusion and are increasingly considered to harbour cryptic genetic diversity of significant adaptive potential. In the Peace River region of northwestern Canada, three sculpin species have been reported: spoonhead (*Cottus ricei*), slimy (*C. cognatus*) and prickly (*C. asper*) sculpin. Prickly sculpin occurrence in this region represents the most eastern edge of its distribution, but its status has remained uncertain following its initial discovery in 1989. These populations may represent an independently evolving lineage of special conservation concern, or be the consequence of an ongoing range expansion, possibly accompanied by interspecific hybridization with local species. Using a combination of mtDNA sequencing and microsatellite analyses, we did not find peripheral population differentiation or interspecific hybridization, suggesting that the Albertan Peace River population belongs to the same genetic group as its western counterparts. Future studies will benefit from a greater understanding of whether demographically independent prickly sculpin populations established in Alberta without the typical genetic signatures of expansion at the periphery of their range.

3.2 Introduction

The maintenance of genetic variation and evolutionary processes that generate diversity are of major interest in conservation biology, with the aim of enhancing the adaptive

potential of threatened species and, ultimately, their chances of long-term survival (Ficetola and Bonin 2011). Populations at the periphery of their distribution range can be small and experience increased genetic drift compared to core populations (Lesica and Allendorf 1995). This may support the emergence of population differentiation and cryptic genetic diversity critical in the face of rapid environmental change (Moritz et al. 2012). However, peripheral populations may not only be sources of adaptive diversity, but can also represent immigrant-dominated sinks where adaptation is hampered by small population sizes and reduced or maladaptive gene flow from central populations (Sexton et al. 2009). Moreover, postglacial colonization may contribute to low genetic diversity and weak divergence of peripheral populations (e.g., Cassel and Tammaru 2003), but surprisingly few studies have examined genetic structure at the leading edge of range expansions (Short and Petren 2011, Nullmeier and Hallatschek 2013). Range expansions may also increase chances of interspecific hybridization with neighboring species (Allendorf et al. 2001), which under some circumstances can facilitate expansion of the invading species (Ellstrand and Schierenbeck 2000). Hence, it is becoming increasingly important to understand the stability and demographic independence of peripheral populations.

Freshwater sculpins are cold-tolerant fishes of northern-temperate regions, and are of conservation interest because they often occur in highly structured populations over small geographic areas (e.g., Nolte et al. 2005a, Junker et al. 2012). Prickly sculpin (*Cottus asper*) occurrence in the Peace River region of Alberta is thought to represent the most eastern edge of its distribution range, but its status since its initial discovery in 1989 has remained uncertain (Roberts 1990). Albertan prickly sculpin populations are presumably

of low abundance, isolated and may have diverged from distant source populations thereby representing independently evolving lineages of special conservation interest. Alternatively, their presence could be the consequence of an ongoing range expansion and be reflected by high immigration rates and weak population differentiation. Hybridization with local *Cottus* species appears possible given numerous reports of hybridization among even distantly related clades of *Cottus* species (Nolte et al. 2005a, McPhail 2007). Herein, we used microsatellites and mitochondrial DNA to test for peripheral population differentiation and interspecific hybridization of *C. asper*.

3.3 Methods

3.3.1 Sampling and DNA analysis

We sampled sculpins non-invasively from the Peace River in British Columbia in 2009 near Hudson's Hope, and in 2010 in Alberta near Dunvegan and Many Islands Park with minnow traps or backpack-electrofishing (Figure 3.1, Table 3.1). Samples from British Columbia were exclusively *Cottus asper*, whereas Albertan samples also included *C. cognatus* and *C. ricei*. Additionally, we included *C. asper* samples from the southwestern periphery of the Peace River watershed (McLeod Lake), and provide genetic diversity indices from two coastal (Little Campbell River, Mosquito Lake) and two inland (Okanagan Lake, Lakelse Lake) populations across the distribution range of *C. asper* in British Columbia (Figure 3.1, Table 3.1). Tissues from the second dorsal fin were used for genetic analyses and stored in 95% Ethanol. DNA was extracted using a standard phenol-chloroform technique. Using the general fish primers FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC -3') and FishR1 (5'-

TAGACTTCTGGGTGGCCAAAGAATCA- 3') (Ward et al. 2005), 583 base pairs of mitochondrial DNA (Cox1) was PCR-amplified and sequenced for species verification and a comparison of *C. asper* populations from the Peace River in British Columbia (n = 10) and Alberta (n = 12). PCR reactions were performed in 25-µl volumes containing 1x PCR-Buffer, 2mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer, 0.625 units of TAQ-polymerase, and ~200 ng genomic DNA. Cycling conditions consisted of 95°C for 2 minutes, followed by 35 cycles of 94°C [30s], 52°C [40s], 72°C [60s], and a final elongation of 72°C for 10 minutes. A total of 19 microsatellite loci from Nolte et al. (2005b) were used to estimate population differentiation between *C. asper* populations (*Cott170, Cott207, Cott255, Cott54, CottE30, LCE54, Cott214, Cott224, Cott78, Cott348, CottE11, CottE20, Cott153, Cott213, CottE13, LCE81, Cott50, Cott686, CottE23*). Interspecific cross amplification of the following subsets of loci was experimentally validated and permitted assessing hybridization among species: *C.asper-C.cognatus*: 16 loci (*Cott170, Cott207, Cott255, Cott54, LCE54, Cott214, Cott224, Cott78, CottE11, CottE20, Cott153, Cott213, CottE13, LCE81, Cott686, CottE23*), *C.asper-C.ricei*: 8 loci (*Cott255, CottE30, Cott224, CottE20, CottE13, LCE81, Cott50, Cott686*), *C.ricei-C.cognatus*: 6 loci (*Cott207, Cott255, Cott224, CottE20, CottE13, LCE81*). Microsatellites were multiplexed with up to six fluorescently-labeled primers using QIAGEN Multiplex Kits, and amplified in 10 µl-reactions (5 µl Qiagen-Multiplex-Kit, 0.3-0.6 µM primer, 40 ng DNA-template). PCR protocols consisted of an initial denaturation step of 95°C for 15 minutes, followed by 38 cycles of 94°C [30s], 60°C [90s], 72°C [60s], and a final elongation of 72°C for 30 minutes. PCR products were run on an ABI 3730xl sequencer, and allelic sizes (in base pairs) were determined by

reference to the internal sizing standard in the software GENEMAPPER version 4.1 (Applied Biosystems). Microsatellite data files are deposited under Dryad (DOI: 10.5061/dryad.9061f).

3.3.2 Statistical Analyses

Mitochondrial DNA sequences were aligned and visually inspected using ClustalX 2.1 (Larkin et al. 2007). We used an extended version of Fisher's exact test for testing microsatellite loci for Hardy-Weinberg equilibrium (HWE, 10^5 Markov chain steps, 10^4 dememorization steps) and linkage disequilibrium (LDE, 10^5 Markov chain steps, 10^4 dememorization steps) implemented in the software ARLEQUIN v. 3.5.1.2 (Excoffier et al. 2005). Sequential Bonferroni correction was applied to P values to correct for multiple comparisons in the same dataset. Pairwise F_{st} values were calculated in ARLEQUIN to test for differentiation between *C. asper* populations. Allelic richness (mean number of alleles, corrected for smallest sample number of ten) was calculated in FSTAT 2.9.3.2 (Goudet 2001). We used a permutation test (15000 permutations) in FSTAT to compare allelic richness and genetic diversity between 1) coastal and inland populations, and 2) Peace River and inland populations. The possibility of null alleles, large allele drop-outs and scoring errors was evaluated using MICROCHECKER v. 2.2.3 (Van Oosterhout et al. 2004). No evidence for scoring errors or large allele drop-outs was found, and only three loci with potential null alleles were identified: *Cott54* and *CottE13* in *C. cognatus*, and *Cott686* in *C. ricei*. Significant (Bonferroni-corrected) departures from HWE were found in four comparisons (three in *C. cognatus*, one in *C. ricei*). Two pairs of loci showed evidence for linkage disequilibrium (*Cott255-CottE30* in *C. asper*, and *CottE20-*

CottE13 in *C. cognatus*). We calculated individual-based chord distance measures (D_C, Cavalli-Sforza and Edwards 1967) for all three species in MSA 4.05 (Dieringer and Schlötterer 2003). Distance measures were used for construction of a neighbor-joining tree in POPULATIONS 1.2.32 (Langella 1999), which was bootstrapped 1000 times and visualized in TREEVIEW v. 1.6.6 (Page 1996). An individual-based assignment approach implemented in STRUCTURE 2.2.3 (Pritchard et al. 2000) was used to test for hybridization among species-pairs. Assuming an admixture model and independent allele frequencies, we set the number of clusters to K = 2 (two parental species), applied $2x10^4$ burn-in steps followed by 10^5 Markov-Chain Monte-Carlo (MCMC) steps, and replicated each analysis five times. Individuals were assigned to parental species for $q > 0.9$, while hybrids were expected to fall within a threshold of $0.1 < q < 0.9$ (Vähä and Primmer 2006).

3.4 Results and Discussion

Freshwater sculpins have typically been found to exhibit strong population genetic structure on small geographic scales (< 50 km), reaching extremes between headwaters of small streams separated by only a few kilometers (Nolte et al. 2005a, Junker et al. 2012). In contrast, we found only weak within-watershed genetic structure over a larger geographic scale (> 200 km) between Peace River and McLeod Lake ($F_{st} = 0.013$, P = 0.015; Figure 3.1). This is particularly surprising given the presence of two dams separating Peace River populations from Williston Lake and adjacent populations such as McLeod Lake. No genetic differentiation was found between Peace River populations of *C. asper* in British Columbia and Alberta, corresponding to the finding of just one

mitochondrial DNA haplotype, and a pairwise F_{st} value for microsatellites of zero. Consistently, genetic diversity indices were highly similar (Table 3.1). Albertan populations of *C. asper* are apparently not demographically independent from distant (>250 km) putative source populations in British Columbia, which could reflect an ongoing range expansion into Alberta with high immigration rates. An extended planktonic larval stage of 30-35 days and subsequent juvenile upstream migration has been described for coastal populations (Krejsa 1967) and may contribute to the absence of genetic structure, but this remains speculative because we are unaware of any studies that have examined life history characteristics of inland populations. Given the possibility of high migration rates or high numbers of founders overriding effects of genetic drift (e.g., Nullmeier and Hallatschek 2013), more data are needed to make inferences about the timing of range expansion (postglacial or very recent) into Alberta. In contrast to conservation concerns attributed to recent and ongoing expansions into neighboring species ranges (Allendorf et al. 2001), we found no indications of hybridization among species in the STRUCTURE analyses (Figure 3.2). This may suggest that local sculpin species in Alberta are not currently threatened by hybridization with *C. asper*, and that hybridization has not facilitated the expansion of *C. asper* into Alberta.

Genetic diversity indices of *C. asper* were lower in inland populations with an average allelic richness of 3.9 compared to 6.1 in coastal populations (Fstat permutation test, two-sided, P = 0.044), and a genetic diversity of 0.39 compared to 0.65, respectively (P = 0.049). Peace River did not show a significantly lower allelic richness (P = 0.81) or genetic diversity (P = 0.83) than other inland populations. Hence, low genetic diversity is more likely a consequence of postglacial (< 15000 y BP) colonization from coastal

populations via temporal connections between the Peace and Fraser/Skeena River systems (McPhail and Lindsey 1970). Similarly, other studies have suggested that postglacial colonization, often in concert with founder effects, may contribute more to low genetic diversity than the peripheral location of a population (e.g., Cassel and Tammaru 2003). While we found no evidence for a marginal population of *C. asper* in the Peace River that might qualify as a conservation designation unit (Dalton1991, COSEWIC 2005), the lack of genetic differentiation at neutral genetic markers does not necessarily inhibit the adaptive potential of peripheral populations (Hendry and Taylor 2004). Source-sink dynamics have repeatedly been suggested to favor local maladaptation in peripheral populations (Anderson and Geber 2009), but the ecological and demographic conditions that allow peripheral sink populations to establish and to expand without diverging from source populations need further research.

3.5 Acknowledgements

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3.7 Figures and Tables

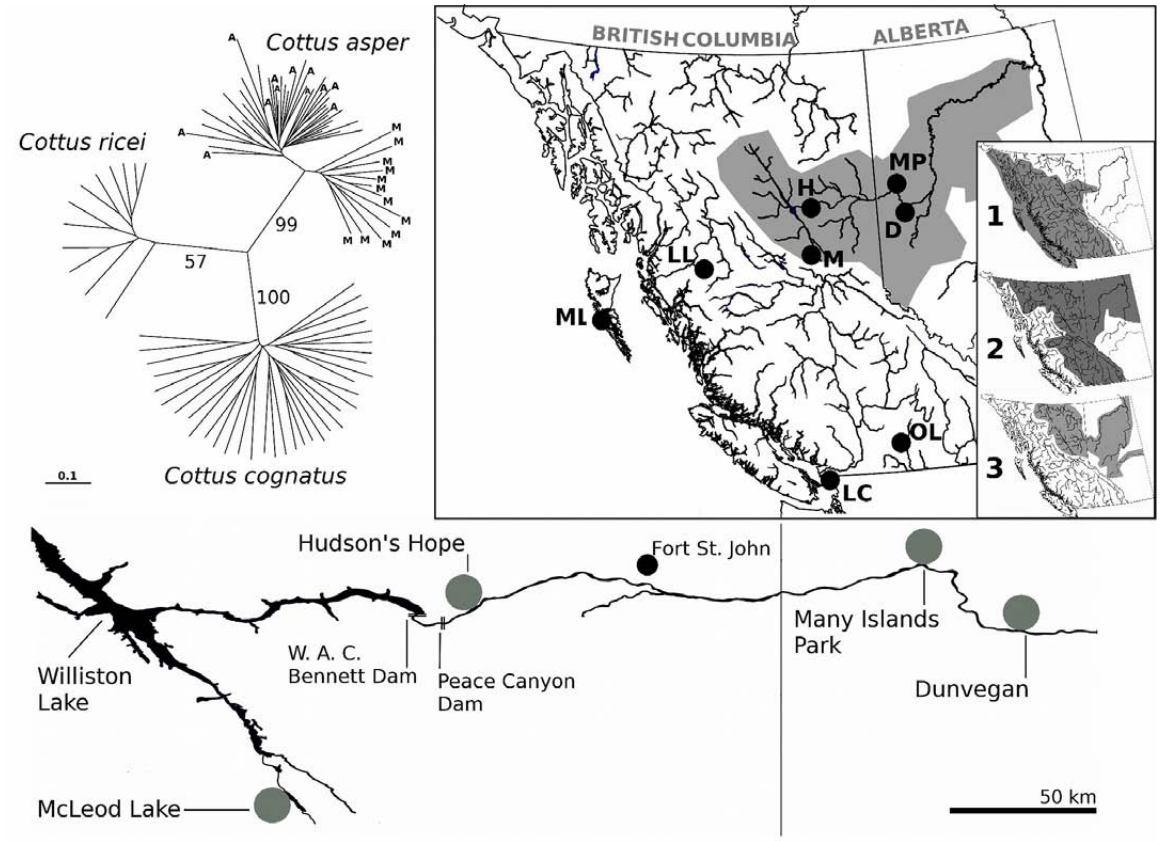


Figure 3.1 Map showing sampling sites and study area (Peace River watershed shaded in grey). Abbreviations represent Mosquito Lake (ML), Lakelse Lake (LL), Little Campbell River (LC), Okanagan Lake (OL), McLeod Lake (M), Hudson's Hope (H), Many Islands Park (MP) and Dunvegan (D). Inlet picture shows distribution ranges of *C. asper* (1), *C. cognatus* (2) and *C. ricei* (3) in Alberta and British Columbia. A bootstrapped Neighbor-Joining tree based on D_C distances of microsatellites is presented on the upper left (for *C. asper*, Albertan samples are marked as "A", and McLeod Lake samples as "M" at branch tips)

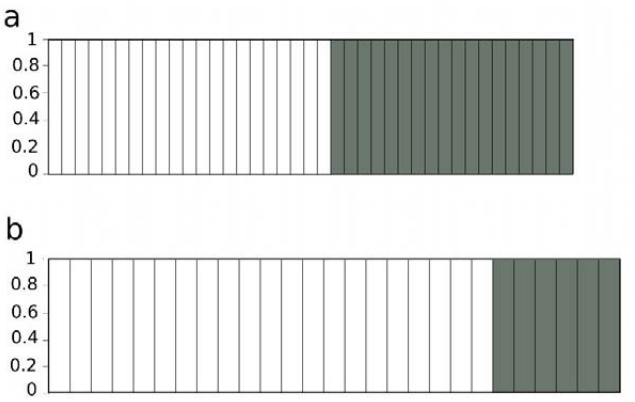


Figure 3.2 STRUCTURE analyses. A: Comparison of *C. asper* (white) and *C. cognatus* (grey). B: Comparison of *C. asper* (white) and *C. ricei* (grey)

Table 3.1: Sampling sites for *Cottus asper, C. cognatus and C. ricei* with respective geographic location (lat./long.), number of samples (N), allelic richness (A_R) and average expected heterozygosity (H_E).

Location	Lat., Long.	N	A_R	H_E
Cottus asper				
Dunvegan (D)	55° 55.1' N, 118° 36.4' W	12	3.66	0.37
Hudson's Hope (H)	56° 3.9' N, 121°50.5' W	30	3.68	0.36
McLeod Lake (M)	54° 59.4' N, 123° 2.1' W	12	3.89	0.37
Lakelse Lake (LL)	54° 22.6' N, 128° 33 ' W	30	4.01	0.43
Okanagan Lake (OL)	50° 12.5' N, 119° 27.9' W	14	4.37	0.47
Mosquito Lake (ML)	53° 4.2' N, 132° 4.2' W	30	5.15	0.59
Little Campbell River (LC)	49° 0.9' N, 122° 46.7' W	30	7.11	0.76
Cottus cognatus				
Dunvegan (D)	55° 55.1' N, 118° 36.4' W	10	4.5	0.49
Many Islands Park (MP)	56° 18.9' N, 119° 8.9' W	26	4.9	0.52
Cottus ricei				
Dunvegan (D)	55° 55.1' N, 118° 36.4' W	12	3.1	0.38

Chapter 4: Genetic population structure reflects isolation by environment between amphidromous and freshwater populations in prickly sculpin (*Cottus asper*)

4.1 Abstract

Aim Life history transitions from amphidromous to freshwater life cycles have evolved repeatedly in various aquatic taxa, but the ecological and evolutionary conditions favoring such a transition within a species in the presence of gene flow remain poorly understood. Herein, we aimed to identify sympatric amphidromous and freshwater groups of *Cottus asper* in a high gene flow setting, and to test for effects of habitat and geographic distance on the distribution of life history ecotypes.

Location Southwestern British Columbia, Canada (Strait of Georgia, Lower Fraser River)

Methods We used 14 microsatellite loci to test for genetic differentiation and isolation-by-distance among coastal rivers, isolated lakes, and between Fraser River main channel and tributary sites. Distance scaled migration rates and a partial mantel test were used to evaluate the relative contribution of habitat and geographic distance on genetic differentiation among freshwater and amphidromous groups in the Lower Fraser River.

Results Consistent with other amphidromous species, high genetic connectivity among coastal rivers was found. Within the main channel of Fraser River, no genetic differentiation was found across a distance of ~80 km, whereas tributaries to the Fraser

River even close to the estuary were genetically differentiated and formed a distinguishable genetic cluster. Genetic differentiation between river tributaries and main channel was independent from geographic distance, suggesting a possible role for natural selection favoring life history divergence in tributary freshwater habitats.

Main Conclusions We propose that isolation by environment to discrete freshwater habitats may play a larger role for the early stage of amphidromy-freshwater transitions than subsequent macro-evolutionary changes in life history characteristics such as increased egg size and abbreviated larval development. The evolution of divergent freshwater life histories is most likely promoted in complex aquatic systems like the Fraser River, where large tributary streams and lakes allow for large effective population sizes.

Keywords Amphidromy, Isolation-by-ecology, Life-history transition, *Cottus*, Population genetics, asymmetric migration

4.2 Introduction

Evolutionary transitions of life histories along environmental gradients are often accompanied by major changes in fitness traits related to survival and reproduction (Braendle *et al.* 2011), and can directly affect reproductive isolation between locally adapted life history ecotypes (e.g., Lowry & Willis 2010), or indirectly reduce gene flow by selection against maladapted hybrids or immigrants (e.g., Nosil *et al.* 2005). Consequently, life history divergence may evolve rapidly and has been suggested to

promote speciation on recent time-scales (e.g., Puritz *et al.* 2012). Among the most fascinating life history changes are marine to freshwater transitions, which have frequently contributed to the remarkable diversification of freshwater taxa (e.g., Taylor *et al.* 1996; Cook *et al.* 2006, Davis *et al.* 2012). However, the ecological and evolutionary conditions that favor such transitions remain often unknown, particularly in the case of amphidromous to freshwater life history transitions (McDowall 2010).

Amphidromy, a sub category of diadromy (Myers 1949), is found in many species of shrimp, gastropods and fishes, and characterized by reproduction in freshwater where large numbers of small, planktonic larvae are transported to the sea, pass through an extended planktonic larval stage and growth period in marine or brackish waters, and migrate back to freshwater as juveniles where they complete their adult life cycle (McDowall 2007; 2010). The ability to disperse through marine habitats and colonize distant river systems has often been suggested to be a key advantage of amphidromy, allowing for the colonization of new habitats as they become available (McDowall 2010). This appears particularly critical on small oceanic islands, which represent the major geographic setting where amphidromy is found (McDowall 2010). Here, large distances among islands and short and steep river systems prevail, with populations suffering from stochastic perturbations like volcanic eruptions or extreme changes in water flow, potentially favoring high dispersal and re-colonization abilities through numerous planktonic larvae (McDowall 2007; 2010, Crandall *et al.* 2010). In contrast, amphidromy is less common in continental river systems (e.g., Dennenmoser *et al.* 2010; Cook *et al.* 2012), which has been attributed to reduced benefits of wide dispersal given the higher

persistence of large rivers, and to increased costs of larval dispersal (McDowall 2010; Crandall *et al.* 2010; Cook *et al.* 2012).

Besides potential advantages, costs associated with amphidromy include energetic costs of migration, larval loss to the ocean, osmoregulatory challenges, as well as increased predation risk and larval starvation during prolonged downstream drift passages (McDowall 2010). In particular, the large distance between headwaters and estuaries has been suggested to impose high migration costs and larval mortalities, thus favoring a transition to purely freshwater life cycles (McDowall 2004; Cook *et al.* 2006, 2012). Transitions from amphidromous to purely freshwater life cycles are therefore mostly expected along large, continental rivers (Cook *et al.* 2006; Page & Hughes 2007; Cook *et al.* 2012). However, the ecological circumstances under which an amphidromous life cycle should be abandoned remain unclear, particularly if risks associated with amphidromy are compensated through behavioral mechanisms. For example, the risk of larval starvation during prolonged downstream passages can be reduced by adult spawning migrations towards the estuaries (e.g., *C. hangiongensis*, Goto 1986), and larval retention behaviors in estuaries may reduce larval loss to the ocean (Cook *et al.* 2012).

Previous work has mainly focused on egg-size vs. fecundity trade-offs to explain the co-occurrence of closely related amphidromous and freshwater species (Closs *et al.* 2013). While such a trade-off likely contributes to the sympatric occurrence of long diverged species, it may not explain the initial transition towards a freshwater life cycle within a species, well before larger egg sizes have evolved. Herein, we propose that early divergence between amphidromous and freshwater populations in the same river system

can be best understood through a reduction of gene flow among locally adapted sub-groups occupying discrete freshwater habitats such as tributary streams or lakes (i.e., "isolation-by-adaptation", or "isolation-by-environment", Nosil *et al.* 2005, 2008; Shafer & Wolf 2013). Initial genetic differentiation among those groups presumably arises from a combination of isolation-by-distance (IBD) and isolation-by-environment (IBE). Their relative contributions may vary both among and within species, and disentangling the effects of IBD from IBE on population genetic structure and life history divergence presents a major challenge for ecological and evolutionary studies (Lee & Mitchell-Olds 2011; Wang *et al.* 2013). Hence, more studies on a wider range of taxa and geographic settings are warranted for a more complete understanding of the ecological conditions that favor the abandonment of amphidromy in favor of a purely freshwater life cycle.

Freshwater sculpins (*Cottidae*) are small-bodied, northern-temperate fishes, and occur as both amphidromous and freshwater species (Kinziger *et al.* 2005). Diversification of freshwater sculpins is thought to have followed transitions from marine ancestors towards freshwater species, which underwent a radiation resulting in at least 68 species (Kinziger & Wood 2010). To date, most studies on amphidromous sculpins come from Japanese species (*Cottus hangiongensis, C. amblystomopsis, C. pollux* small and medium egg type; e.g., Goto & Andoh 1990; Goto & Arai 2003, 2006), while the two amphidromous North American species – *C. aleuticus* and *C. asper* – have attracted far less attention, despite their recent postglacial history that potentially allows investigations of life history transitions in the early stages of divergence. Prickly sculpin (*C. asper*) occupy a wide geographic range between SE-Alaska and California, including far inland areas (McPhail 2007, Dennenmoser *et al.* 2013). Populations adopting purely freshwater life cycles occur

in landlocked inland regions, but have also been reported from at least one tributary lake to the Lower Fraser River in southwestern British Columbia, Canada (McAllister & Lindsey 1961). Thus, purely freshwater populations of recent (postglacial) origin may occur in many other tributaries along the Lower Fraser River, which has been colonized after the last glacial maximum (~14,000 years BP). Egg sizes of *C. asper* are small as expected from amphidromous species (1.2 – 1.9 mm, e.g. Cartwright 1956; Ringstadt 1974; Wang 1986), and to the best of our knowledge, neither Fraser tributary lake nor far inland populations have evolved larger egg sizes (1.1 – 1.3 mm, pers. observations). Hence, *C. asper* provides a valuable model organism to investigate amphidromous to freshwater transitions in the early stage of divergence, before major evolutionary changes in egg number and size have been manifested. In this study, we aimed to test the following predictions: (1) Genetic connectivity is high among coastal rivers due to wide dispersal of planktonic larvae, low among isolated lake populations, and reduced among Fraser tributary compared to Fraser main channel sites. (2) Amphidromous groups within the Fraser River main channel are more connected with each other than putative freshwater populations in the Fraser tributaries. (3) Isolation by environment is reflected by genetic differentiation of tributaries, and reduced migration rates between Fraser River main channel and tributary sites, which we expect to be independent of geographic distance.

4.3 Methods

Sculpins were sampled using baited minnow traps during spring 2009 and 2012 from 31 locations in the Lower Fraser River valley and around the Strait of Georgia in southern British Columbia, Canada (Fig. 4.1). Extractions of genomic DNA were obtained from fin-clip tissue using a standard phenol-chloroform technique (Sambrook *et al.* 2001). In 750 individuals, we amplified the following 14 microsatellite loci derived from European sculpin, *C. gobio* (Nolte *et al.* 2005a): *Cott54, CottE30, Cott207, Cott170, LCE54, Cott50, Cott686, CottE23, CottE11, Cott348, LCE81, Cott213, CottE13, Cott153.* Microsatellites were multiplexed with four to five fluorescently-labeled primers using QIAGEN Multiplex Kits, and amplified in 5 μl-reactions (1.8 μl Qiagen-Multiplex-Kit, 0.1-0.3 μM primer, 40 ng DNA-template). PCR-protocols consisted of an initial denaturation step of 95°C for 15 minutes, followed by 34 cycles of 94°C [30s], 60°C [90s], 72°C [60s], and a final elongation of 72°C for 30 minutes. Allelic sizes (in base pairs) were determined by reference to the internal sizing standard in the software GENEMAPPER version 4.1 (Applied Biosystems).

We used an extended version of Fisher's exact test for testing microsatellite loci for Hardy-Weinberg equilibrium (HWE, 10^5 Markov chain steps, 10^4 dememorization steps) and linkage disequilibrium (LDE, 10^5 Markov chain steps, 10^4 dememorization steps) implemented in the software ARLEQUIN v. 3.5.1.2 (Excoffier *et al.* 2005). Sequential Bonferroni correction (Rice 1989) was applied to the P values to correct for multiple comparisons in the same dataset. Pairwise F_{ST} values, as well as observed (H_O) and expected (H_E) heterozygosities were calculated in ARLEQUIN. Genetic diversity and allelic richness (mean number of alleles, corrected for smallest sample number) was

calculated in FSTAT 2.9.3.2 (Goudet 2001). We compared genetic diversity estimates and average F_{ST} values among four groups (landlocked lakes, coastal rivers, Fraser estuary plus main channel, Fraser tributaries) using a permutation test in FSTAT (15,000 permutations). A second permutation test was conducted to compare only Fraser tributaries with Fraser estuary/ main channel. The possibility of null alleles, large allele drop-outs and scoring errors was evaluated using MICROCHECKER v. 2.2.3 (Van Oosterhout *et al.* 2004). We calculated individual-based chord distance measures (D_C, Cavalli-Sforza & Edwards 1967) for construction of a neighbor-joining tree in POPULATIONS 1.2.32 (Langella 1999), which was bootstrapped 1000 times and visualized in FIGTREE 1.3.1 (Rambaut 2011).

An individual-based assignment approach implemented in STRUCTURE 2.3.3 (Pritchard *et al.* 2000) was used to infer genetic population structure and potential differentiation among life history ecotypes. We used an admixture model to test for number of putative populations (K genetic clusters) from 18 to 31. We applied a 100,000-iteration burn-in followed by 900,000 iterations of the Monte Carlo Markov Chain (MCMC), and repeated each K five times to ensure stability. The most likely number of genetic clusters was determined by using the ΔK method (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER (Earl & von Holdt 2012). The five replicates of the best-supported K were summarized in CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007), and results were visualized in DISTRUCT 1.1 (Rosenberg 2004). The STRUCTURE analysis was repeated on a reduced dataset, after excluding isolated lakes. To further assess how well Fraser tributaries are genetically distinguishable from the

Fraser estuary and main channel, we applied a Discriminant Analysis of Principal Components (DAPC), as implemented in the R package *adegenet* (Jombart *et al.* 2010).

Geographic effects on genetic differentiation were evaluated by testing for isolation-by-distance using the online software "IBD Web Service" (IBDWS, Jensen *et al.* 2005) on four datasets: Isolated lakes (AL, CL, DL, GL, JH, P, PS), Coastal rivers (C, CA, CR, FA, FC, FS, LC), Fraser tributaries (AN, AS, CQ, HS, HZ, PI, PK, S, SR), and Fraser estuary and main channel (FA, FE, FN, FP, FS, FT, FW). Additionally, we performed partial Mantel tests in IBDWS on a combined Fraser tributary and estuary/main channel data set. First, we used habitat type (tributary vs. estuary/main channel) as an indicator variable in order to test for a correlation between genetic differentiation and habitat, while controlling for geographic distance. Second, we used geographic distance as an indicator variable in order to test for a correlation between genetic differentiation while controlling for habitat type. Geographic distances (in km) were retrieved from GoogleEarth (v. 5.2.1, Google Inc.) using shortest waterways between sampling sites, and Euclidean distances among isolated lakes.

We used Migrate-n (Beerli 2006) to test for migration rates between putative amphidromous and freshwater groups within the Lower Fraser system, with the expectation of reduced migration between compared to within groups. A total of eight sites were chosen for estimating a full migration model, consisting of four tributary sites (CQ, HZ, PI, SR), and four estuarine/main channel sites (FE, FS, FP, FT). To account for unequal geographic distances and IBD effects, we included a distance matrix file to calculate distance scaled migration rates. Estimates of mutation-scaled effective population size θ ($4N_e\mu$), and mutation-scaled migration rates M (m/μ) were obtained

using a Bayesian search strategy, with one long chain (100,000 steps recorded, with an increment of 100, and a burn in of 20,000 steps) to sample the posterior distribution of the parameters under a static heating scheme (temperatures: 1.0. 1.5, 3.0, 100000). After initial test runs to determine suitable parameter priors, we used broad exponential prior distributions for M (0.0, 10, 10000) and θ (0.0, 1, 100), and conducted five replicates for each run. Convergence was ensured by inspection of posterior density distributions of parameters and ESS values (> 55,000). We converted θ to effective population size (N_e) estimates, assuming a generation time of one year and a conventional mutation rate of 5 x 10^{-4} that has been widely used for teleost fishes (e.g., Estoup & Angers 1998, Lippe *et al.* 2006). Microsatellite data files are provided under Appendix 7.

4.4 Results

In 750 individuals, the 14 microsatellite loci recovered 2 to 29 alleles (mean: 8.3). Allelic richness (standardized to a minimum sample size of 14) of polymorphic loci ranged from 2.23 to 8.28, and gene diversity of polymorphic loci ranged from 0.32 to 0.75 (Table 4.1). Significant (sequential Bonferroni-corrected) deviations from HWE were found for 28 (out of 438) locus-population pairs. Evidence for linkage disequilibrium (after sequential Bonferroni-correction) was found in 47 (out of 2821) comparisons. Null alleles, as indicated by Microchecker, occurred in 46 locus-population pairs. We did not exclude any loci from the analyses, because no consistent deviations across the majority of sites were found.

Significant pairwise F_{ST} values ranged from 0.011 (Alouette R. South – Salmon R.) to 0.560 (Graham Lake – Paq Lake), and non-significant F_{ST} values ranging between zero

and 0.011 were frequently found among sites within the Lower Fraser system. The STRUCTURE analysis supported k=23 clusters for the complete dataset, and k= 15 for the reduced dataset without isolated lakes, as indicated by a highest Δk value (Evanno *et al.* 2005), and revealed weak yet consistent allele frequency differences between Fraser tributaries and Fraser main channel / estuarine sites, albeit with pronounced genetic clusters formed by isolated lakes and a hydroelectric complex consisting of Alouette Lake, Stave Lake, Hayward Lake and Stave River (Fig. 4.2a). Consistently, the NJ- tree on D_C distances showed strong genetic clustering of Fraser tributary sites, which were separate from the Fraser estuary and main channel sites, while isolated lakes and the hydroelectric complex sites were highly differentiated (Fig. 4.2b). A discriminant analysis of principal components (DAPC) confirmed the genetic differentiation between Fraser tributaries and main channel / estuary, and showed high assignment probabilities of 0.9 (tributary group) and 0.86 (estuary/main channel group) (Fig. 4.3).

Landlocked lakes, coastal rivers, Fraser tributaries and Fraser estuary/main channel groups differed significantly from each other in allelic richness ($P < 0.001$), expected heterozygosity ($P < 0001$) and F_{ST} ($P < 0.0001$) in the FSTAT permutation test (Table 4.2). When comparing only the two groups Fraser tributaries vs. Fraser estuary / main channel in a FSTAT permutation test, no significant difference was found for either genetic diversity estimates or F_{ST} value ($P > 0.5$). Mantel tests performed in IBDWS on these four groups revealed significant isolation-by-distance pattern among coastal rivers ($Z = 198.2013$, $r = 0.7186$, $P = 0.019$, $R^2 = 0.516$) and Fraser tributaries ($Z = 15.0118$, $r = 0.5139P = 0.005$, $R^2 = 0.264$), while no significant correlation was found among isolated lakes ($Z = 1005.8351$, $r = -0.113$, $P = 0.675$, $R^2 = 0.013$), or among Fraser estuary/main

channel sites ($Z = 5.5581$, $r = 0.3392$, $P = 0.05$, $R^2 = 0.115$) (Fig. 4.3). For the latter, only two significant pairwise F_{ST} values were detected (FW-FA: $F_{ST} = 0.017$; FW-FE: $F_{ST} = 0.014$). Accordingly, main channel sites in the Fraser River located up to 80 km away from the estuary showed non-significant, low F_{ST} values (e.g., FA-FT: $F_{ST} = 0$), whereas tributaries close to the estuary showed weak but significant differentiation from estuarine sites (e.g., CQ-FA: $F_{ST} = 0.037$). A partial Mantel test using habitat type as indicator variable on the combined tributary and estuary/main channel data showed a significant correlation between genetic differentiation and habitat type, when controlling for geographic distance ($r = 0.6055$, $P < 0.001$). A second partial Mantel test found a weak but significant correlation between genetic differentiation and geographic distance, when controlling for habitat type ($r = 0.3073$, $P = 0.004$).

Estimates of effective population sizes in Migrate-n were highly similar among four tributary and four main channel sites and ranged from 6350 to 8400 (Table 4.3). These values likely present overestimates, because they rely on the assumptions of a generation time of one year, and a mutation rate of $\mu = 5 \times 10^{-4}$. A more realistic generation time of two years for *C. asper* (Patten 1971) would result lower effective population size estimates.

Migration rates were generally high and varied widely between 201 and 4765 immigrants per generation. We were interested in four migration rate patterns: (1) among tributaries, (2) among main channel/estuary sites, (3) from tributary to main channel/estuary sites, and (4) from main channel/estuary to tributary sites. As expected, highest migration rates were found among main channel/estuary sites (mean: 1743.4; 95% CI: 1571.2 - 1909), followed by tributary to main channel/estuary sites (mean:

1447.7; 95% CI: 1274.1 - 1614.3). Migration rates were lower among tributaries (mean: 992.8; 95% CI: 821.5 - 1154.8), and lowest from main channel/estuary to tributary sites (mean: 782.3; 95% CI: 611.5 - 945.4).

4.5 Discussion

Our finding of a clear signal for isolation by environment between Fraser tributaries and Fraser estuary/main channel suggests strong selection, which might cause reduced success of immigrants or hybrids. This interpretation is supported by a strong effect of habitat on genetic differentiation when accounting for IBD, and by estimates of distance-scaled migration rates, showing lowest migration rates from the main channel into the tributaries. We propose that life history transitions from amphidromous to freshwater life cycles can be initiated by reducing gene flow among locally adapted groups occupying discrete freshwater habitat types, without the prerequisite of large distances to the estuary, or the evolution of increased egg sizes.

4.5.1 Isolation by distance and environment under high gene flow

High gene flow has traditionally been thought to impede local adaptation, unless strong selection pressures exist to overcome the homogenizing effects of gene flow (e.g., Lenormand 2002; Kawecki & Ebert 2004; Thibert-Plante & Hendry 2010). Because underlying selective mechanisms are often unknown, the frequent finding of adaptive differentiation under high gene flow remains a major challenge in evolutionary ecology. Similarly, amphidromy is characterized by pronounced gene flow among populations, which makes the evolutionary transition to freshwater life cycles puzzling. In contrast to

purely freshwater sculpins, which often show high genetic structuring and strong IBD pattern (e.g., Nolte *et al.* 2005b; Junker *et al.* 2012; Lamphere & Blum 2012), amphidromous sculpins are generally expected to exhibit comparably weaker genetic structure due to a high dispersal capacity of an extended planktonic larval stage, allowing for wide dispersal among coastal estuaries (Goto & Andoh 1990). This is consistent with our observation of strong genetic structuring among isolated lakes, and weak differentiation among coastal rivers around the Strait of Georgia. Isolated lakes did not show a pattern of isolation by distance, indicating that strong genetic differentiation is largely shaped by genetic drift.

Because wide dispersal through planktonic larval stages is typical for amphidromous taxa, absent or only weak IBD over large geographic scales has frequently been observed (e.g., Berrebi *et al.* 2005; Cook *et al.* 2006; Crandall *et al.* 2010; Tsukagoshi *et al.* 2011; Page *et al.* 2013). Here, we found significant IBD among coastal rivers around the Strait of Georgia, which could indicate some limitations to dispersal. Cook *et al.* (2012) speculated that larval retention behavior in amphidromous species might be selected for in continental rivers to reduce larval loss to the ocean, which may promote IBD patterns usually not being found in island archipelago settings. However, these and other studies did not find much support for IBD among continental rivers (Dennenmoser *et al.* 2010; Cook *et al.* 2012), and larval loss to the ocean may not present a strong enough selective force in the semi-enclosed Strait of Georgia to favor larval retention behavior in *C. asper*. While we cannot rule out larval retention in estuaries for *C. asper*, environmental barriers such as ocean currents presumably play a larger role in limiting genetic connectivity among coastal rivers (e.g., Tsukagoshi *et al.* 2011; Alberto *et al.* 2011).

Within the Lower Fraser River system, we found genetic differentiation and IBD among tributaries, whereas Fraser estuary and main channel sites were largely unstructured. The absence of IBD in the Fraser main channel over a distance of ~ 80 km, together with the presence of genetically differentiated tributaries even close to the estuary, suggests that isolation by environment (IBE), rather than by distance, is the predominant driver of genetic differentiation. Despite an overall high genetic admixture, Fraser tributaries clustered together and were genetically distinguishable from the estuary/main channel. A strong correlation between genetic differentiation and habitat type independent of geographic distance was confirmed in a partial Mantel test, and isolation by environment was further supported by reduced, distance-scaled migration rates into tributaries from estuarine/main channel sites.

A strong signal of habitat dependent, ecological divergence in neutral genetic markers is expected when divergent selection is strong and migration intermediate, and generally easier to detect when population sizes are large (Thibert-Plante & Hendry 2010). Selective forces promoting genetic differentiation among tributaries and nearby estuarine/main channel sites could include selection against migrants and hybrids, differences in reproductive timing, and assortative mating (e.g., Räsänen & Hendry 2008), as well as larval retention behavior within the tributaries. While we currently do not know the degree of spawning habitat overlap among ecotypes or the exact selective regimes favoring divergence among habitat types, differences in salinity tolerance and osmoregulation abilities among estuarine and freshwater tributary populations of *C. asper* have been shown before (Bohn & Hoar 1965), suggesting a potential selective role in adaptive divergence that could be addressed in future studies. While the present patterns

of IBE suggest a role of natural selection, genetic differentiation among main channel and tributary sites could have been initiated through neutral founder effects associated with postglacial colonization events of newly available tributary sites. If first colonizers were able to rapidly establish large population sizes, while undergoing fast local adaptation, a priority effect might have preserved neutral genetic differentiation resulting from founder effects, and subsequently reduced the relative impact of immigration. Such a scenario has recently been termed "isolation-by-colonization" (IBC) (Orsini et al. 2013), and emphasizes the persistence of demographic founder effects promoted by priority effects, large population sizes and local adaptation, which has previously been outlined as "monopolization hypothesis" (DeMeester et al. 2002). In *C. asper*, the rapid formation of large population sizes following colonization of tributary habitats might have been aided by the high fecundity associated with an amphidromous life history, together with the availability of large tributary habitats. Rapid local adaptation to tributary freshwater habitats could have been facilitated by standing genetic variation, by taking advantage of relatively high frequencies of already-present adaptive alleles (Barrett & Schluter 2008). Whether IBE in *C. asper* has been initiated through IBC remains to be shown. Disentangling the relative importance of IBA and IBC, while controlling for IBD, would be highly facilitated by including both neutral and adaptive genetic markers (Orsini et al. 2013). Overall, the possible presence of IBC needs to be considered in studies addressing patterns of genomic differentiation under similar demographic scenarios, where elevated neutral genetic differentiation could be misinterpreted as genomic hitchhiking of numerous neutral loci being linked to loci under selection.

4.5.2 Evolution of amphidromous to freshwater life history transitions

Current hypotheses for explaining amphidromous to freshwater transitions are largely based on observations that freshwater species produce fewer and larger eggs than closely related amphidromous species across numerous aquatic taxa, indicating selection for increased larval survival in the often harsher and less productive freshwater environments, at the cost of reduced fecundity (e.g., Diesel *et al.* 2000; Goto & Arai 2006; Closs *et al.* 2013). For example, Closs et al. (2013) compared egg size and fecundity characteristics among closely related amphidromous and freshwater species in the fish families Cottidae, Galaxiidae, Eleotridae and Gobiidae, showing a trend towards the production of fewer, larger eggs in freshwater compared to amphidromous life cycles, and the same trend was also evident among populations within species such as *Galaxias truttaceus*. Accordingly, a trade-off between egg number/size and fecundity was proposed to explain the co-occurrence of amphidromous and freshwater life histories (Closs *et al.* 2013). The reasoning is that amphidromy has a fecundity advantage over a purely freshwater life cycle when being close to a pelagic habitat, while becoming recruitment limited with increasing distance to a pelagic habitat due to costs of migration, such as larval starvation during downstream drift, giving an advantage to freshwater life cycles (Closs *et al.* 2013). This argumentation assumes that freshwater residents already have evolved towards fewer and larger eggs, and further implies increased mortality of planktonic larvae during prolonged downstream drift. However, in the early stages of divergence freshwater life cycles may not have evolved larger egg sizes yet, and therefore not experience a fecundity disadvantage. Furthermore, amphidromous larvae may not necessarily suffer from increased mortalities at large distances from the estuary if

behavioral compensation mechanisms exist or larvae are released during periods of high river flows, allowing a rapid passage to the sea. While the evolution of larger eggs and abbreviated larval development in purely freshwater life histories is a recurrent pattern in freshwater adaptation (e.g., Vogt 2013), this may be a long-term evolutionary process being completed long after genetic divergence between amphidromous and freshwater groups has been established.

Divergence among amphidromous and freshwater life histories in *C. asper* has occurred relatively recently, following colonization of the Fraser River after the last glacial maximum around 14,000 years ago. Amphidromy is presumably ancestral to purely freshwater spawning, because salt-tolerance appears to be retained in freshwater spawning populations (Krejsa 1965, Bohn and Hoar 1965). Egg sizes reported for coastal populations are typical for amphidromous species, and in the range of 1.1 – 1.9 mm diameter (Cartwright 1956; Ringstadt 1974; Wang 1986). Our own observations revealed egg sizes of ~ 1.1-1.3 mm for sculpins from both an inland (Kalamalka Lake, Okanagan Valley) and a Fraser tributary (Hayward Lake) egg clutch. Hence, freshwater adaptation in *C. asper* may not have led to larger egg sizes yet, because not enough time has passed to allow for major evolutionary changes in life history characteristics. We speculate that physiological freshwater adaptations related to osmoregulation and larval growth might be more likely to evolve within a short evolutionary time frame, and be detectable during the early stages of divergence. Salinity tolerance has repeatedly been shown to underlie adaptive divergence among aquatic taxa that are distributed across salinity gradients (e.g., Whitehead *et al.* 2011, 2013; Purcell *et al.* 2012).

The likelihood for transitions toward freshwater life histories has also been suggested to increase with the distance to the estuary, due to increased larval mortality during long downstream passages, and a fecundity advantage of amphidromy at close distances to the estuary (Closs *et al.* 2013). In *C. asper*, larvae are released during spring melt water runoff, and sculpins conduct spawning migrations towards the estuary (Krejsa 1965). Therefore distance to the estuary presumably does not predict the distribution of amphidromous and freshwater life histories at the geographic scale we have considered in this study. Behavioral adjustments of larval dispersal and migration timing to seasonal stream flow regimes may be common among amphidromous species (e.g., Bauer 2011), which in some cases might allow long-distance dispersal and prolonged downstream drift of planktonic larvae (e.g., > 1500 km in *Macrobrachium ohione,* Bauer 2011). Large distances from the estuary can promote genetic differentiation because of smaller effective population sizes in headwaters, and decreased immigration from downstream sites. For example, in the amphidromous shrimp *Paratya australiensis* genetically differentiated clusters occur in upstream locations at large distances from the estuary, which could have favored a transition to freshwater life histories (Cook *et al.* 2006). While large distances to the estuary can favor transitions to divergent life histories, our study shows that short distances to the estuary do not necessarily prohibit the establishment of freshwater life cycles, particularly if habitat characteristics allow the persistence of large effective population sizes. This is likely the case for *C. asper* in the Lower Fraser River, where the presence of large tributary lakes allows large population sizes for groups adopting purely freshwater life cycles. Hence, immigration rates from the estuary and the main channel into tributaries might be relatively low compared to the

number of freshwater residents, which could reduce the contribution of adaptive divergence on gene flow, while promoting genetic differentiation and local adaptation (Räsänen & Hendry 2008).

4.6 Conclusions

Evolutionary transitions from marine to freshwater environments have played a major role in the diversification of freshwater taxa, but environmental and demographic conditions that could initiate such a transition are often not well understood. We suggest that physiological and behavioral adaptations to discrete freshwater habitats may play a larger role for initiating marine-freshwater transitions than subsequent macro-evolutionary changes in major life history characteristics such as increased egg size and abbreviated larval development. We predict that heterogeneous aquatic landscapes providing discrete habitat types are more likely to favor transitions towards freshwater life cycles, particularly if they allow the establishment of large effective population sizes.

4.7 Acknowledgements

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4.9 Supporting Information

Additional Supporting Information:

Appendix S1 Table with pairwise F_{ST} comparisons among sampling sites.

4.10 Biosketch

Stefan Dennenmoser is currently a PhD candidate broadly interested in the intersection of biogeography, natural history and local adaptation and its implications for the genetic basis of adaptive change.

Steven M. Vamosi is an Associate Professor of Population Biology interested in factors associated with the origin, maintenance and loss of biodiversity at several levels of organization.

Sean M. Rogers is interested in the ecological and evolutionary processes that influence genetic variation within and among natural populations of fishes.

Author contributions: S.D., S.M.V., and S.M.R. designed the study. S.D. performed the research and analyzed the data, and all authors contributed to writing the paper.

4.11 Figures and Tables

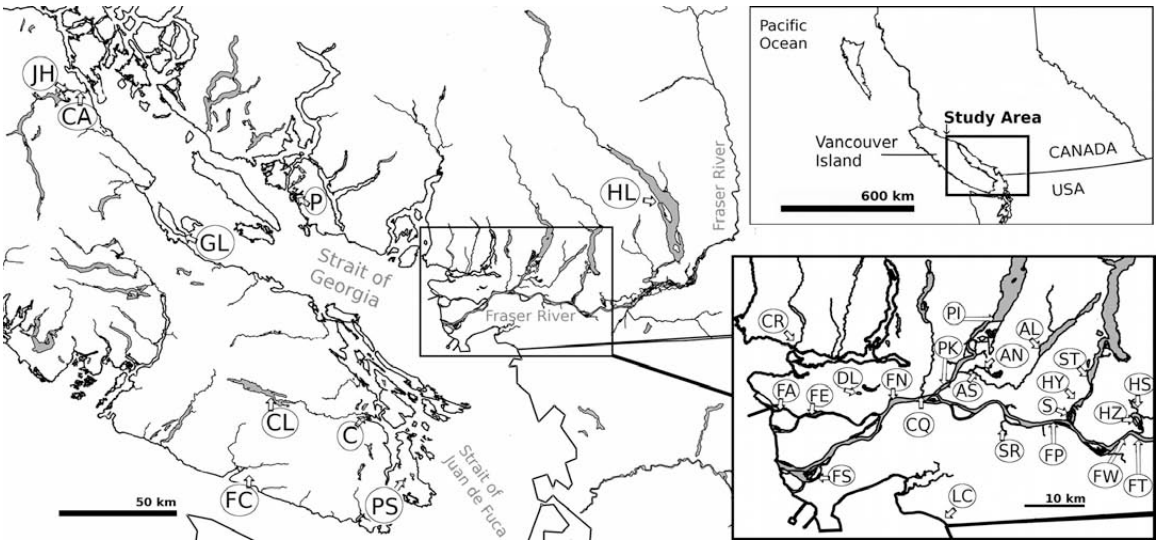


Figure 4.1 Maps showing location of study area and sampling locations around Strait of Georgia and Lower Fraser River in southern British Columbia, Canada. Abbreviations for sampling locations read as follows: AL = Alouette Lake, AN = Alouette River North, AS = Alouette River South, C = Cowichan River, CA = Campbell River, CL = Cowichan Lake, CQ = Coquitlam River, CR = Capilano River, DL = Deer Lake, FA = Fraser "Airport", FC = Falls Creek, FE = Fraser "East Kennedy", FN = Fraser "Sapperton", FP = Fraser "Poplar Bar", FS = Fraser "Deas Slough", FT = Fraser "Train", FW = Fraser "Walters Rd.", GL = Graham Lake, HL = Harrison Lake, HS = Hatzic Slough, HY = Hayward Lake, HZ = Hatzic Lake, JH = John Hart Lake, LC = Little Campbell River, PI = Pitt Lake, PK = Pitt River "Kennedy Rd.", P = Paq Lake, PS = Prospect Lake, S = Stave River, SR = Salmon River, ST = Stave Lake.

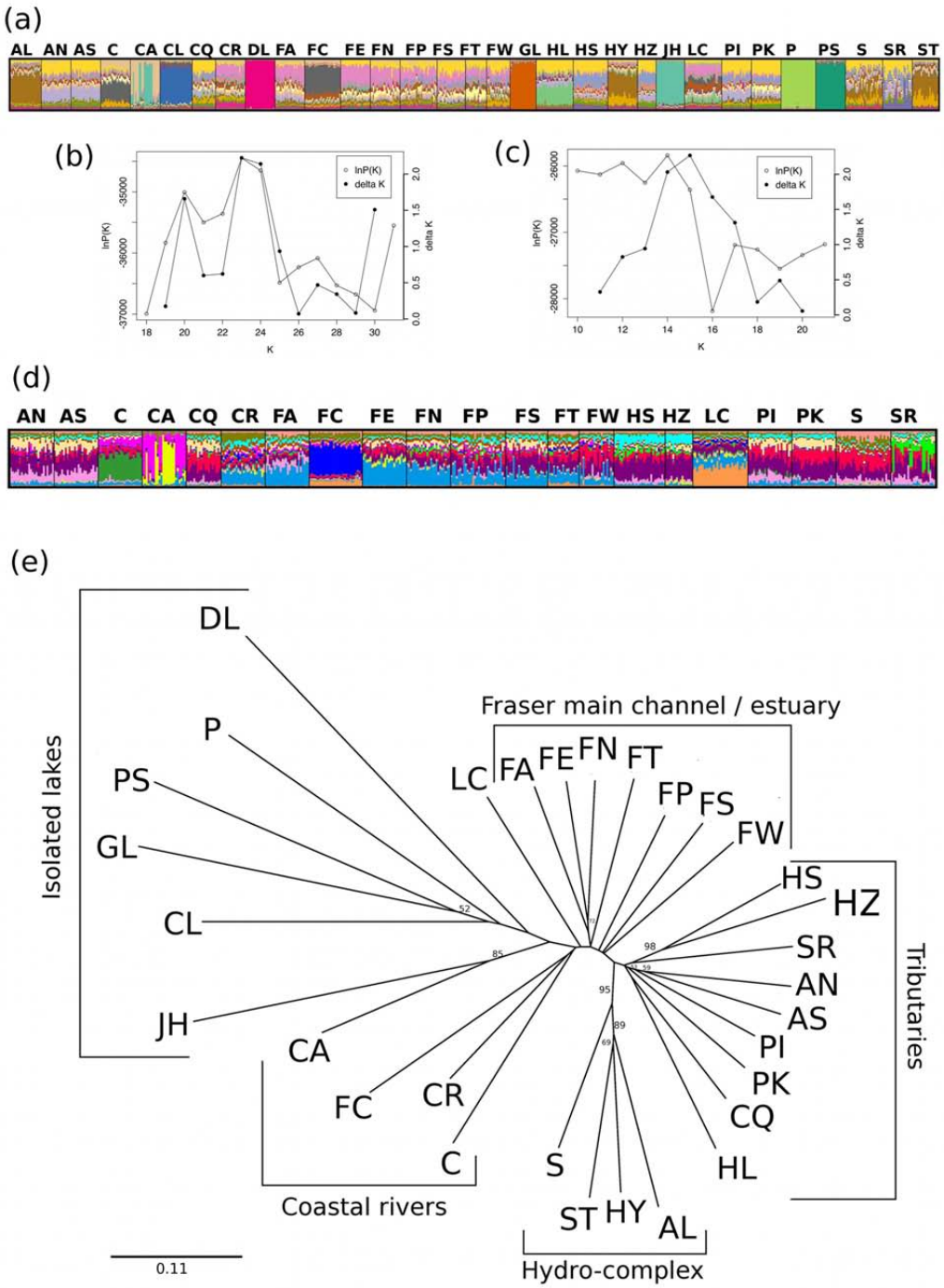


Figure 4.2 (a) STRUCTURE diagram (K = 23); (b) Log-likelihood plot supporting K = 23; (c) Log-likelihood for reduced dataset, supporting K = 15; (d) STRUCTURE diagram for reduced dataset (K = 15) (e) Neighbor-Joining tree on D_C distances of 14 microsatellite loci (numbers indicate bootstrap values).

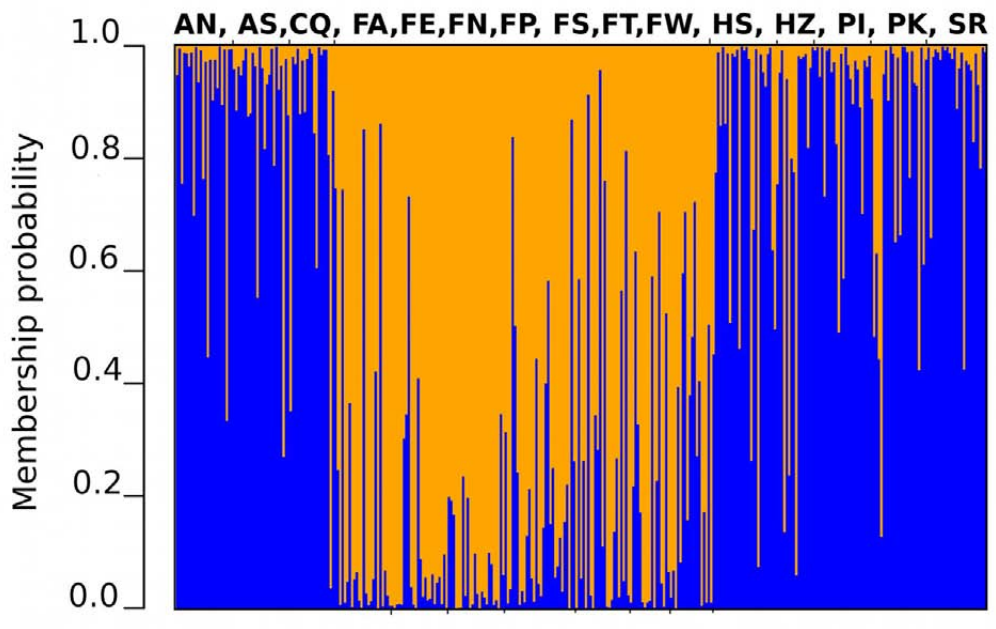


Figure 4.3 Discriminant Analysis of Principal Components (DAPC) barplot showing membership probabilities for Fraser tributaries (blue) and Fraser estuary/main channel (orange).

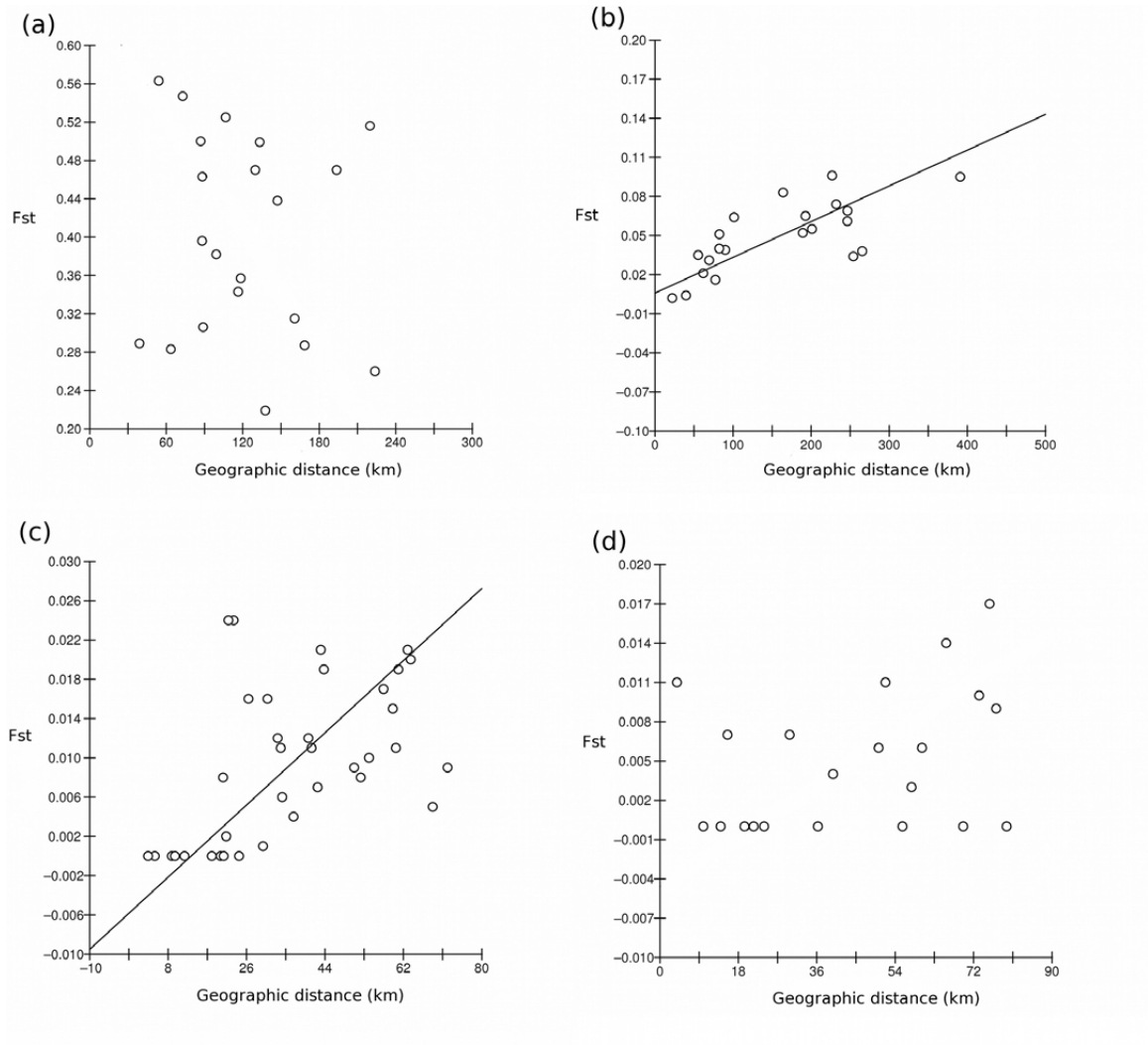


Figure 4.4 Isolation-by-distance among (a) Isolated lakes, (b) Coastal Rivers, (c) Fraser tributaries, (d) Fraser Estuary and main channel sites.

Table 4.1: Sampling locations and genetic diversity estimates for 14 microsatellite loci of *Cottus asper* (N = number of samples, H_E = expected heterozygosity, H_O = observed heterozygosity, A_R = allelic richness).

ID, Location	Lat., Long.	N	H_E	H_O	A_R
Landlocked Lakes					
AL, Alouette Lake	49° 19.475'N, 122° 26.992'W	25	0.62	0.56	6.20
CL, Cowichan Lake	48° 49.085'N, 124° 10.190'W	26	0.59	0.58	5.92
DL, Deer Lake	49° 14.241'N, 122° 58.081'W	24	0.42	0.40	2.23
GL, Graham Lake	49° 30.710'N, 124° 45.309'W	21	0.36	0.32	3.13
HY, Hayward Lake	49° 11.893'N, 122° 24.390'W	24	0.65	0.64	6.37
JH, John Hart Lake	50° 2.640'N, 125° 20.540'W	23	0.42	0.40	4.19
P, Paq Lake	49° 36.687'N, 124° 1.366'W	28	0.35	0.31	2.58
PS, Prospect Lake	48° 30.448'N, 123° 26.683'W	24	0.44	0.43	3.87
ST, Stave Lake	49° 14.244'N, 122° 21.701'W	21	0.64	0.61	6.41
Coastal Rivers					
C, Cowichan R.	48° 45.967'N, 123° 38.651'W	24	0.67	0.65	6.39
CA, Campbell R.	50° 2.256'N, 125° 17.624'W	24	0.65	0.51	5.82
CR, Capilano R.	49° 19.416'N, 123° 8.163'W	24	0.73	0.71	7.75
FA, Fraser "Airport"	49° 12.831'N, 123° 10.160'W	24	0.70	0.66	7.54
FC, Falls Creek	48° 34.018'N, 124° 21.252'W	29	0.75	0.72	8.28
LC, Little Campbell R.	49° 0.968'N, 122° 46.715'W	30	0.71	0.66	7.69
Fraser Tributaries					
AN, Alouette R. North	49° 16.293'N, 122° 38.843'W	24	0.67	0.56	6.92
AS, Alouette R. South	49° 14.970'N, 122° 38.729'W	24	0.67	0.59	7.00
CQ, Coquitlam R.	49° 15.817'N, 122° 47.078'W	19	0.65	0.62	6.94
HL, Harrison Lake	49° 32.192'N, 121° 52.446'W	30	0.60	0.60	6.25
HS, Hatzic Slough	49° 11.352'N, 122° 14.852'W	28	0.65	0.60	6.75
HZ, Hatzic Lake	49° 9.935'N, 122° 13.641'W	15	0.68	0.68	6.74
PI, Pitt Lake	49° 20.973'N, 122° 36.522'W	24	0.68	0.66	6.62
PK, Pitt River	49° 14.490'N, 122° 44.038'W	24	0.66	0.66	6.20
S, Stave R.	49° 11.601'N, 122° 24.532'W	30	0.66	0.58	7.18
SR, Salmon R.	49° 9.570'N, 122° 35.351'W	24	0.66	0.63	6.77
Fraser estuary / main channel					
FE, Fraser "East Kennedy"	49° 12.317'N, 123° 2.679'W	24	0.70	0.67	7.31
FN, Fraser "Sapperton"	49° 13.043'N, 122° 53.556'W	24	0.72	0.67	7.47
FP, Fraser "Poplar Bar"	49° 10.005'N, 122° 27.043'W	30	0.71	0.63	7.78
FS, Fraser "Deas Slough"	49° 7.487'N, 123° 3.240'W	23	0.70	0.64	7.30
FT, Fraser "Train"	49° 7.696'N, 122° 13.341'W	17	0.71	0.64	7.25
FW, Fraser "Walters Rd."	49° 8.372'N, 122° 16.050'W	19	0.68	0.60	6.93

Table 4.2 FSTAT comparisons of genetic diversity estimates (N = sample size, A_R = allelic richness, Hs = expected heterozygosity) and average F_{ST} values among four groups of *C. asper*.

	N	A_R	Hs	F_{ST}
Landlocked Lakes (AL, CL, DL, GL, JH, P, PS)	171	4.02	0.433	0.403
Coastal rivers (C, CA, CR, FA, FC, LC)	155	7.25	0.695	0.052
Fraser tributaries (AN, AS, CQ, HS, HZ, PI, PK, S, SR)	212	6.8	0.646	0.009
Fraser estuary/main channel (FE, FN, FP, FS, FT, FW)	137	7.34	0.688	0.003

Table 4.3 Migration rates and effective population size estimates for *C. asper* for 8 sites within the Lower Fraser River, as estimated in Migrate-n. Migration rates are scaled by mutation rate and by geographic distance. Donating and receiving sites are above and below the diagonal, respectively (95% CI given in parentheses). On the diagonal, numbers in bold font indicate mutation-scaled effective population sizes θ ($4N_e\mu$), with estimates of N_e provided in parentheses (assuming $\mu = 5 \times 10^{-4}$).

	CQ	**PI**	**SR**	**HZ**	**FE**	**FS**	**FP**	**FT**
CQ	***14.3***	1545	1545	1545	1545	1545	1545	1545
	(7150)	[1373,1706]	[1373,1706]	[1373,1706]	[1373,1706]	[1373,1706]	[1373,1706]	[1373,1706]
PI	845	***13.3***	1018	845	305	845	303	845
	[673,1007]	(6650)	[847,1180]	[673,1007]	[133,467]	[673,1007]	[133,467]	[673,1007]
SR	1018	831	***12.7***	831	201	1018	1018	831
	[847,1180]	[660,993]	(6350)	[660,993]	[33,367]	[847,1180]	[847,1180]	[660,993]
HZ	201	845	845	***14.8***	239	320	211	201
	[33,366]	[673,1007]	[673,1007]	(7400)	[67,407]	[153,487]	[40,373]	[33,367]
FE	1545	217	210	217	***13.9***	219	297	208
	[1373,1706]	[47,387]	[40,380]	[47,387]	(6950)	[47,380]	[127,460]	[40,373]
FS	4765	213	213	218	4765	***13.5***	4765	213
	[4593,4927]	[40,380]	[40,373]	[47,387]	[4593,4927]	(6750)	[4593,4927]	[40,380]
FP	365	4765	4765	4765	217	4765	***13.4***	4765
	[193,527]	[4593,4927]	[4593,4927]	[4593,4927]	[47,380]	[4593,4927]	(6700)	[4593,4927]
FT	210	209	233	253	221	220	266	***16.8***
	[40,373]	[40,373]	[33,407]	[73,440]	[47,387]	[47,387]	[87,453]	(8400)

Chapter 5: Conclusions and future directions

The aim of this thesis was to provide a baseline framework for future research on historical effects on population divergence, and mechanisms of local adaptation in the presence of gene flow in *C. asper*. The main findings were: (1) parallel evolution of a prickling phenotype across multiple glacial lineages, (2) high genetic connectivity over large geographic distances, and (3) genetic evidence for the sympatric occurrence of two differentiated life history ecotypes in the Lower Fraser River. Implications of these results are discussed in the individual chapters. In this chapter, the main results of this thesis are briefly synthesized, and potential future research directions are outlined.

5.1 Synthesis

The phylogeographic study (Chapter II), in contrast to previous assumptions (Krejsa 1965, McPhail 2007), showed that the occurrence of highly prickled inland populations in British Columbia is not a result of allopatric divergence in coastal and inland glacial refugia, but an example of parallel evolution. Presumably three glacial lineages have independently contributed to the occurrence of a highly prickled phenotype, which most likely evolved from standing genetic variation from highly differentiated ancestral coastal populations. Postglacial colonization from coastal towards inland regions and associated founder effects resulted in decreased genetic diversities, which was also evident in a subsequent study on conservation genetics of a recently discovered peripheral population in the Peace River in Alberta (Chapter III). While no divergent "conservation designation unit" (*sensu* Dalton 1991) was found in the Peace River, the lack of genetic differentiation at neutral genetic markers does not necessarily inhibit the adaptive

potential of peripheral populations (Hendry and Taylor 2004). Consistently, range expansions have repeatedly been shown to be associated with adaptive changes of allele frequencies, despite an overall loss of neutral genetic diversity (e.g., White et al. 2013). This becomes particularly interesting when independent genetic lineages undergo range expansions into similar environments, such as the repeated colonization of freshwater habitats by threespine stickleback around the globe (e.g., Jones et al. 2012), or multiple postglacial range expansions from coastal into inland regions by prickly sculpin in British Columbia, as reported herein (Chapter II). Such cases provide unique opportunities to investigate parallel patterns of freshwater adaptation, and allow a better understanding of mechanisms underlying local adaptation and natural selection by disentangling parallel from non-parallel changes in allele frequencies and phenotypes (e.g., Kaeuffer et al. 2011). Accordingly, the parallel fixation of a highly prickled phenotype in inland populations of *C. asper* is suggestive of an adaptive function favored by natural selection. Unfortunately, the question whether prickling has an adaptive function remains unsolved. Krejsa (1965) suggested a possible adaptive function of prickling as a barrier to ion loss in freshwater to reduce osmotic stress of non-migratory freshwater spawners. However, this does not seem a plausible explanation given that sculpins in coastal estuaries are often highly prickled, whereas sculpins in some landlocked coastal lakes can be almost "naked" (pers. observations, Chapters II and IV). In contrast to marine fish, which often use their skin for active sodium chloride excretion, the role of skin for ion homeostasis appears to be less important in freshwater settings (Glover et al. 2013). Another possible prickle function could include a defense mechanisms against predators (and against cannibalistic conspecifics), which might be especially important for the more vulnerable

juvenile stages. Based on the observation of extensive slime production in sculpins, I speculate that prickles could serve to improve adhesiveness of a thick slime coat to the abdomen, which may provide increased protection against parasites and might help to escape chemo-sensory detection by some predatory fish.

Overall, a possible role of strong skin prickling for freshwater adaptation is currently not well supported, and less conspicuous changes in many other characteristics (e.g., physiological, or behavioral traits) that could be related to freshwater adaptation deserve more attention. Once adaptive traits with known selective agents are identified, investigations of parallel-evolved traits become more meaningful because the genetic basis of adaptive change under similar selective regimes can be uncovered. Subsequently, more general insights into freshwater adaptations during inland colonization, and evolutionary transitions from marine towards freshwater life histories are possible. As a first step towards a better understanding of life history transitions from amphidromous to freshwater life cycles in *C. asper*, a population genetics study was conducted in the Lower Fraser River system in order to identify sympatric life history ecotypes (Chapter IV). In accordance with previous chapters, high genetic connectivity over large geographic distances was found. Furthermore, significant genetic differentiation between tributaries and main channel groups independent from geographic distance was found, suggesting a possible role for natural selection. These results indicate that isolation by environment to discrete freshwater habitats could play a role for the early stage of amphidromy-freshwater transitions, which may be further favored by the presence of large tributary streams and lakes allowing for large effective population sizes. Life history divergence in the presence of gene flow is also relevant in the context of

"speciation with gene flow" models (e.g., Nosil and Feder 2012), which can be tested using population genomic approaches. A research project is underway that will explore patterns of genomic divergence among tributary and main channel groups of *C. asper*, and reveal whether candidate genes for teleost salinity tolerance and osmoregulation are differentiated among these putative life history ecotypes.

5.2 Future research

Numerous future research avenues could be explored in order to further investigate effects of demographic history, parallel evolution and local adaptation on phenotypic and genetic change in the early stages of population divergence in *C. asper*.

An outstanding question in evolutionary biology is how frequently genomic divergence and speciation occurs in the presence of gene flow (e.g., Smadja and Butlin 2011). A study in progress that was started during the final stage of my PhD addresses the question whether genomic population divergence among sympatric life history ecotypes in the Lower Fraser River could reflect adaptation to different salinity regimes in either estuarine (brackish-water) or tributary (freshwater) habitats. The availability of a *Cottus* genome from European sculpin (*Cottus gobio*), which shows a high similarity to the Threespine stickleback (*Gasterosteus aculeatus*) genome (Cheng and Nolte 2013), allows a targeted search for differentiated genomic regions containing known teleost candidate genes for salinity tolerance and osmoregulation. The use of two differentiated population pairs may allow teasing apart parallel from non-parallel pattern of genomic divergence, and thereby identify candidate genes under divergent selection underlying differentiation during the early stages of amphidromy-freshwater transitions.

The role of ecology in mediating adaptive divergence through natural selection is widely recognized (e.g., Schluter 2000, Shafer and Wolf 2013), but often remains poorly understood. In order to better understand divergence among amphidromous and freshwater life history ecotypes in *C. asper*, future studies could investigate ecological differences in more detail, and relate them to environmental characteristics of tributary and main channel habitats of the Lower Fraser River. Ecotype characterizations could include life history traits (e.g., egg size, developmental time, larval growth rates), morphology (e.g., lateral line architecture, head pore numbers, larval body shape), behavioral traits (e.g., habitat preferences, migration behavior), or salinity tolerance experiments, amongst others. Adaptations in larval ecology might be crucial in determining survival in contrasting aquatic habitats, and could be investigated in the field and in the laboratory. Ideally, such investigations would be replicated using multiple river systems across two or more genetic lineages, in order to test for signs of parallel evolution of adaptive traits.

Life history divergence among coastal and inland populations is commonly found in freshwater fishes, and may provide valuable study systems to investigate patterns of parallel evolution (e.g., Taylor et al 1996, Palkovacs et al. 2008). Information about life history characteristics and ecology of inland populations of *C. asper* is largely lacking, and it remains unclear to what extent inland sculpins are adapted to purely freshwater life cycles. Future studies could explore life history characteristics of inland (landlocked) populations in more detail, and thereby reveal parallel patterns of evolutionary change.

Understanding mechanisms favoring reproductive isolation during the early stages of speciation is a central goal in evolutionary biology (e.g., Langerhans and Riesch 2013).

Recently diverged genetic lineages originating from separate glacial refugia provide suitable study systems to address the evolution of pre- or postzygotic reproductive barriers. In *C. asper*, crossbreeding experiments among highly divergent southern coastal and inland sculpins could be conducted, in order to generate insights into mechanisms involved in the early stage of speciation.

The contribution of adaptive introgression to adaptive divergence, speciation and diversification appears to be largely underestimated in animals (Hedrick et al. 2013), although selective sweeps of mitochondrial DNA has been frequently suggested to explain mitochondrial DNA capture between species, or discordances of biogeographic pattern among nuclear and mitochondrial genes (e.g., Irwin et al 2009, Toews and Brelsford 2012). Hence, the hypothesis that potential adaptive mitochondrial introgression in northern inland regions of British Columbia occurred upon secondary contact of two genetic lineages should be further tested. The widespread dominance of a single mitochondrial haplotype across northern coastal and inland regions, despite strong genetic differentiation in nuclear microsatellite loci (Chapter II) and nuclear intron sequences (unpublished data), could suggest mitochondrial introgression as a consequence of a selective sweep in favor of a northern mitochondrial haplotype.

The genetic basis of parallel-evolved traits is of interest for a better understanding of targets and mechanisms of selection (e.g., Elmer and Meyer 2011). Uncovering a candidate gene for prickling might help to elucidate the evolutionary history of highly prickled phenotypes of *C. asper* across British Columbia. Breeding experiments coupled with QTL-mapping could be used to find out whether prickling is a heritable trait, and if a major QTL in the *Ectodysplasin* (EDA) pathway can be attributed to prickling similar

to what has been found in European sculpin, *C. gobio* (Cheng and Nolte 2013). Given the parallel evolution of highly prickled inland populations across multiple glacial lineages, further investigations could address the question whether different glacial lineages have evolved the same or a different genetic basis underlying highly prickled phenotypes.

In summary, this work provided support for rapid (postglacial) evolution of a parallel phenotype which is presumably facilitated by the high standing genetic variation of coastal populations, demonstrated the high gene flow abilities of *C. asper* likely associated with an extended planktonic larval stage of an amphidromous life cycle, and found genetic evidence for sympatric life history ecotypes occupying discrete habitats in a high gene flow setting in the Lower Fraser River system. These results provide a baseline framework for future research activities, which could include investigations on ecological and genomic divergence of life history ecotypes with gene flow, reproductive isolation among glacial lineages, the contribution of adaptive introgression to the postglacial colonization of northern inland areas of British Columbia, or the genetic basis of a parallel evolved prickling phenotype, as suggested above. Such studies could implement NGS approaches, which would be facilitated by the availability of a reference genome from European sculpin (*Cottus gobio*), as well as by genomic data for *C. asper* becoming available soon through a follow-up project initiated during the final stage of my PhD. Overall, the rapid progress and increasing feasibility of NGS techniques may allow to address such questions in much more detail, and has the potential to produce highly informative data sets that might render outdated the results of this thesis soon.

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Appendix S1: List of primer sequences for mitochondrial and microsatellite markers used, and PCR protocols (see below).

Locus	Primer	Primer sequence (5' – 3')
D-loop, and	CotL1	CCGGAGGTTAAAATCCTCCC
partial tRNA	HN20	GTGTTATGCTTTAGTTAAGC
Cytochrome b	L14724	CGAAGCTTGATATGAAAAACCATCGTTG
	H15149	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA
Cox1	FishF1	TCAACCAACCACAAAGACATTGGCAC
	FishR1	TAGACTTCTGGGTGGCCAAAGAATCA
Microsatellite	Cott170	ACATGGTGCATAATGTTGCCC
		TCAGGGCCTACGCACAGAGC
Microsatellite	Cott207	AGTCCTTGTCGGGAGCCTCG
		ATTGGGCGTTGCTCACCAGC
Microsatellite	Cott255	TCACTACAGCCAGGTGTCTG
		GCATGTGCATGCCTCCACAG
Microsatellite	Cott54	CACGTACATGTGAAACGAACCC
		AACAGAGGAAACGCCATGAC
Microsatellite	CottE30	AAGCGGACAGACGCAAAGAG
		ACTCGCCCAAGAAGACCAGC
Microsatellite	LCE54	ATGTGTGAGGGCCTCAATGC
		CTGTGTGGTGCGTCCATTGG
Microsatellite	Cott214	AGTAGTGCCGCTGCACCCAC
		TCTCCCTCGTGGTGCAAACC

Microsatellite	Cott78	TCTGCTCCGCAGGGTCGCTG
		TGACACATGGTGAACCCGTG
Microsatellite	Cott348	TGGACCACTGAGTTGCTGTG
		TGGTGTGTCATACCTGCAGC
Microsatellite	CottE11	TCAAGAGGTGAGAGGCCAAGAG
		CATCCTGGTGTTACCATTGCTG
Microsatellite	CottE20	CGTCTGCACAGTTGAGCCTG
		AGCACGCCGTCGCTAACAGC
Microsatellite	Cott153	GGGTTCAGGTGCGTTTGGTC
		ACCTTCAGCTCCGACATCAG
Microsatellite	Cott213	TTGCCATGGATTTGAGGCAG
		AGCATTGCTATTATCAGGCTGC
Microsatellite	CottE13	AGTTACGCAATGCAACACGCCT
		TCGAACACGAAGCTCCCGTCAC
Microsatellite	LCE81	GCTTGTGAACATTTCGGCCTC
		AATGTACAAAGCTCCCACGTCC
Microsatellite	Cott50	TCCTGTGCTAATGCTGTGGA
		AAACCTCACCTCGGTGAGTC
Microsatellite	Cott686	TGGGAGTGCGATCGGGTTGG
		GCGGGCCTTGACACTGTTGG
Microsatellite	CottE23	TGAGCAGCTTTACAGTGTTGG
		TCGTGCTCTTCTGGCATCTG
Microsatellite	EDA1	GGACAGCACACTCCTTCCAT

AGAGGCCCTTAAAAGGCAGA

PCR protocols used in this study:

For obtaining mitochondrial DNA sequences, we applied the following primer combinations: CotL1 and HN20 (partial t-RNA, D-loop, Šlechtová et al. 2004), L14724 *(*Meyer et al. 1990*)* and H15149 (Kocher et al. 1989, cytochrome b), FishF1 and FishR1 (Cox1, Ward et al. 2005). Amplification of D-loop sequences (CotL1/HN20, 583 bp) was performed in 30-µl volumes containing the following final reaction concentrations: 1x PCR-Buffer, 2mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer, 0.625 units of TAQ-polymerase, and ~200 ng genomic DNA. The PCR protocol consisted of 2 minutes at 95°C, followed by five cycles of [94°C -45s, 48°C -45s, 72°C -90s], 29 cycles of [94°C -45s, 52°C -45s, 72°C -90s], and 5 minutes at 72°C final elongation. For Cox1 sequences (FishF1/R1, 583 bp), PCR reactions were performed in 25-µl volumes containing 1x PCR-Buffer, 2mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer, 0.625 units of TAQ-polymerase, and ~200 ng genomic DNA. Cycling conditions consisted of 95°C for 2 minutes, followed by 35 cycles of [94°C -30s, 52°C -40s, 72°C -60s], and a final elongation of 72°C for10 minutes. A cytochrome b fragment (419 bp) was amplified with the L14724/H15149 primer pair in 30-µl volumes containing 1x PCR-Buffer, 2mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer, 0.625 units of *Taq*-polymerase, and ~200 ng genomic DNA. Cycling conditions consisted of 95°C for 2 minutes, followed by 35 cycles of [95°C -50s, 50°C -15s, 72°C -80s], and a final elongation of 72°C for 7 minutes.

Microsatellites were multiplexed with up to six fluorescently-labeled primers using QIAGEN Multiplex Kits (Qiagen, Inc.), and amplified in 10 μl-reactions (5 μl Qiagen-Multiplex-Kit, 0.3-0.6 μM primer, 40 ng DNA-template). PCR-protocols consisted of an initial denaturation step of 95°C for 15 minutes, followed by 38 cycles of [94°C -30s, 60°C -90s, 72°C -60s], and a final elongation of 72°C for 30 minutes.

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Šlechtová V, Bohlen J, Freyhof J, Persat H, Delmastro GB (2004) The Alps as Barrier to Dispersal in Cold-Adapted Freshwater Fishes? Phylogeographic History and Taxonomic State of the Bullhead in the Adriatic Freshwater Drainage. *Molecular Phylogenetics and Evolution*, **33**, 225–239.

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Appendix S2 Prickling and body shape differences between inland and coastal sculpins.

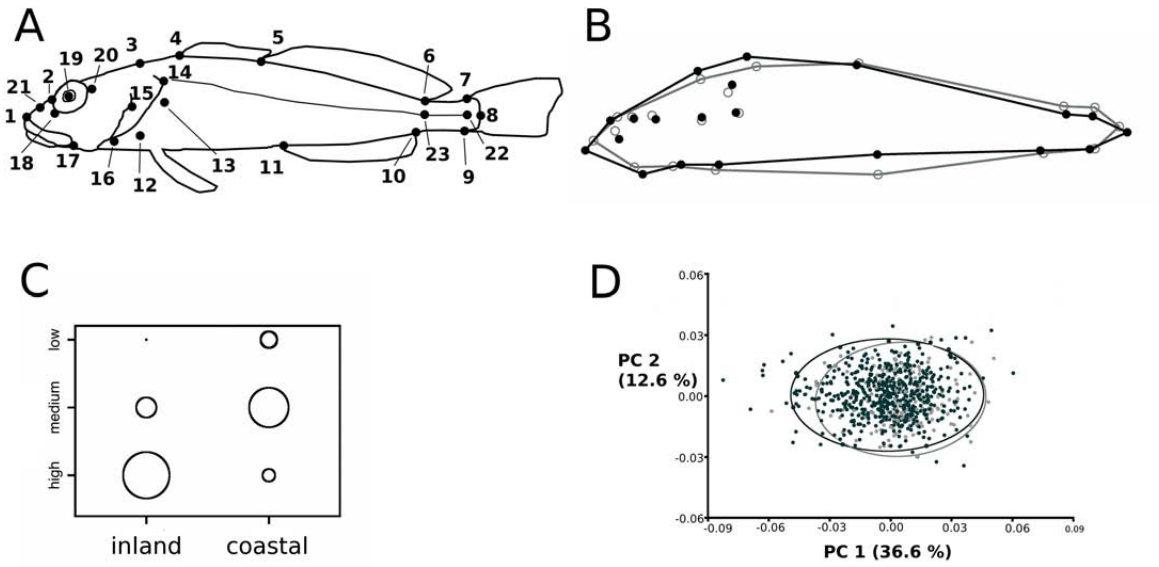


Figure S2 A) Landmark configuration (1 = tip of nasal, 2 = interorbital pore, 3 = end of head, 4 = first dorsal fin origin, 5 = second dorsal fin origin, 6 = end of second dorsal fin, 7 = upper caudal fin origin, 8 = midpoint caudal, 9 = lower caudal fin origin, 10 = anal fin end, 11 = anal fin origin, 12 = lower pectoral fin origin, 13 = upper pectoral fin origin, 14 = upper origin of gill opening, 15 = opercular spine, 16 = 3rd pore of the preopercular-mandibular canal, 17 posterior end of maxilla, 18 = posterior end of eye, 19 = midpoint of eye, 20 = distal end of eye, 21 = nasal nostril, 22 midpoint between 7 and 9, 23 = mid of caudal peduncle). B) Wireframe graph showing shape differences between coastal (black line) and inland (grey line) groups (scale factor x 6). C) Frequencies (circle sizes represent percentages) of prickling categories (high, medium, low) in coastal and inland populations. D) PCA plot visualizing shape variation among coastal (black) and inland (grey) individuals along the first two principal components (with 95% confidence ellipses).

Appendix S3 Fifty percent majority-rule consensus tree of mtDNA sequences.

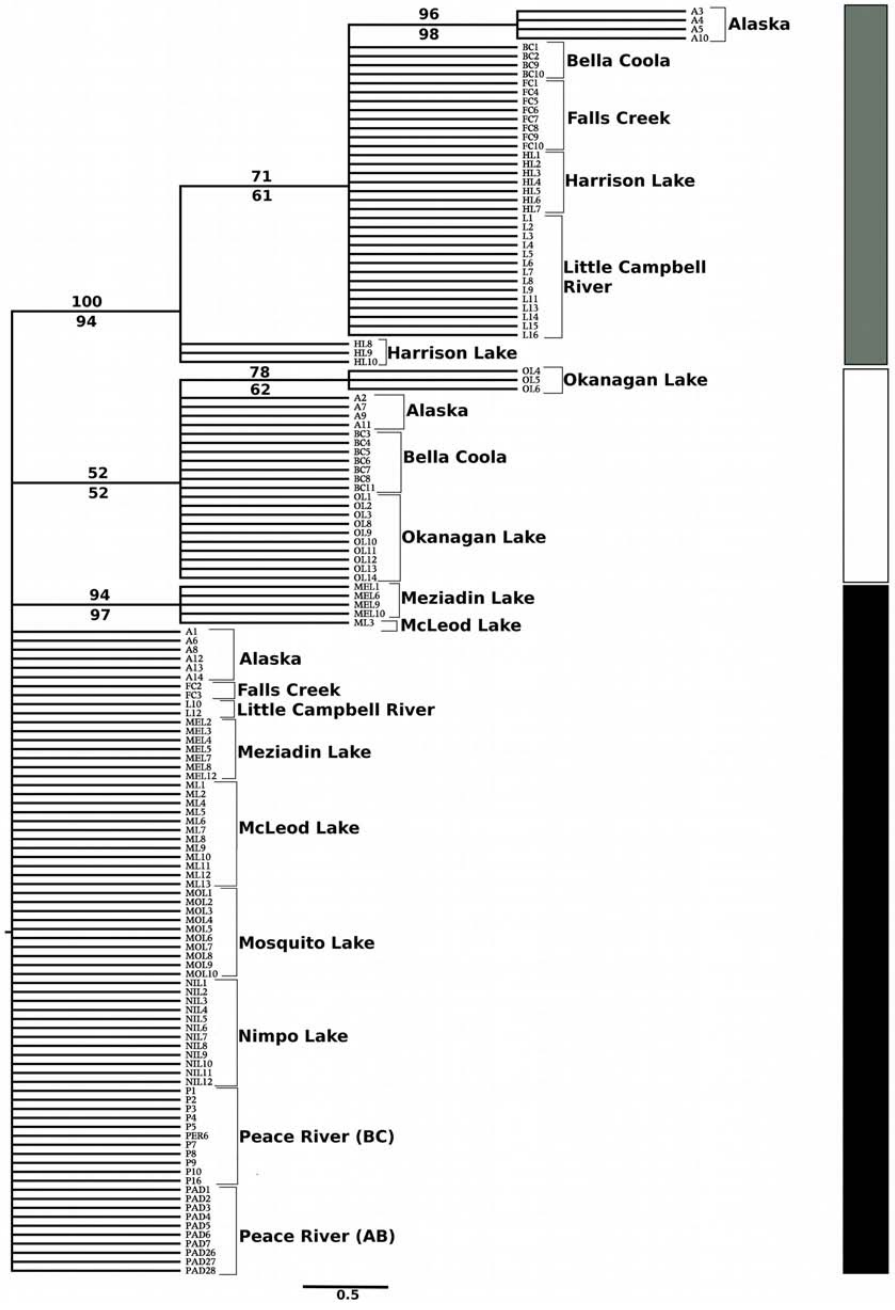


Fig. S3 Fifty percent majority-rule consensus tree of mtDNA sequences. Numbers above branches indicate BI posterior probabilities, and numbers below branches ML bootstrap proportions. Major tree clades are indicated by vertical bars (black = North, grey = South coast, white = South inland).

Appendix 2.S4: Mismatch distributions of mtDNA haplotypes

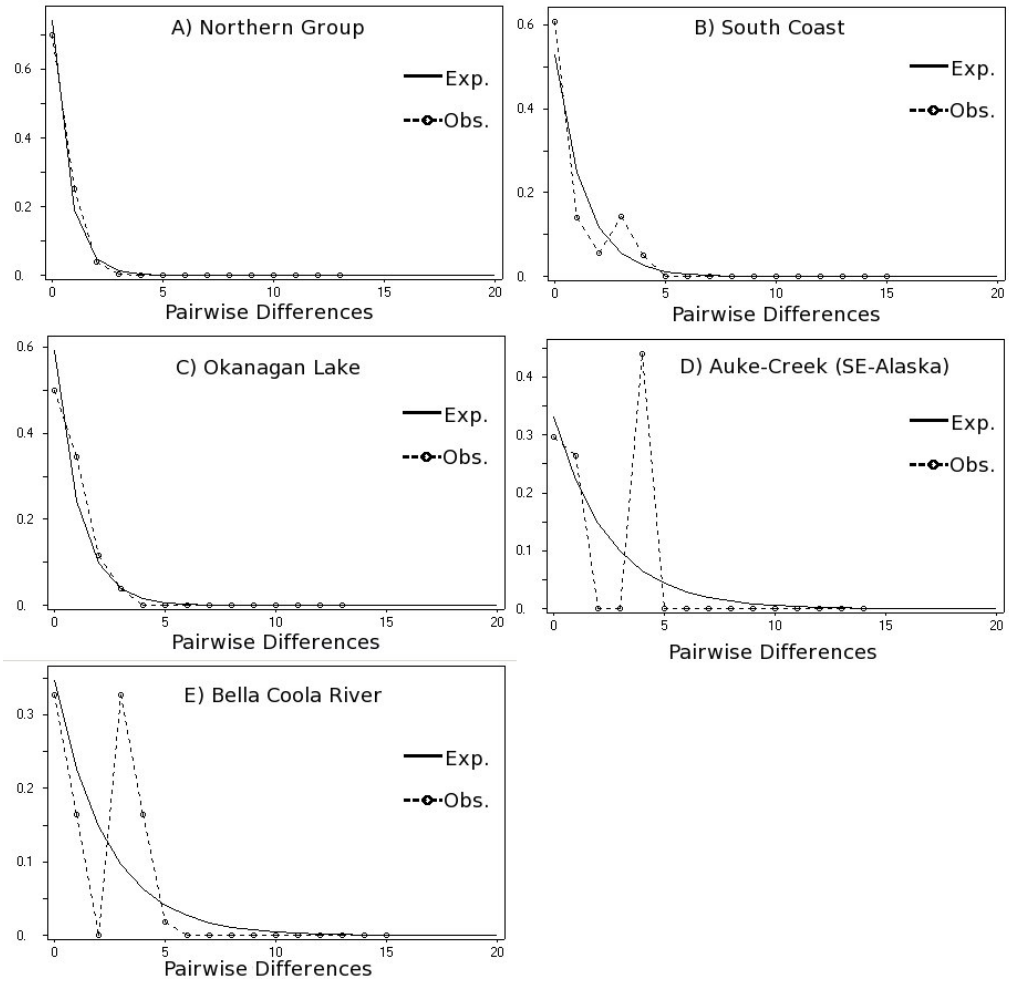


Figure 2.S4 Mismatch distributions of A) Northern mtDNA group, B), South Coast, C) Okanagan Lake, D) Auke-Creek (SE-Alaska), E) Bella Coola River.

Demographic expansion is indicated by significant negative Fu's F (Fu 1997) and Tajima's D (Tajima 1989) values, which we calculated in ARLEQUIN. Mismatch distributions of pairwise nucleotide differences between mtDNA haplotypes were generated for each geographic mtDNA group, with the expectation that a recent demographic expansion would be reflected by a unimodal distribution (Rogers and Harpending 1992). Significant differences between observed and simulated distributions expected under a sudden expansion model (Excoffier et al. 2005) was evaluated using the

sum of square deviations (SSD, 1000x bootstrapped) and Harpending's raggedness index r (Harpeding 1994). Tajima's D and Fu's F estimates were significantly negative only in the northern mtDNA group (D = -2.05, P = 0.002; Fs = -7.7, P < 0.001), indicating a recent demographic population expansion in this region, which is also supported by a unimodal mismatch distribution (Figure 2.S4_A) with non-significant values for both raggedness index (r = 0.247; P = 0.55) and sum of squared deviation (SSD = 0.0025; P = 0.57). No significant signature of population expansion was found for either the South-Coast (D = -1.04, P = 0.17; Fs = -1.75, P = 0.123) or South-Inland group (D = -0.89, P = 0.24; Fs = 0.049, P = 0.42).

Literature:

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47-50.

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Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

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Appendix 4.S1 Occurrence of null alleles for locus-population pairs, with null allele frequency estimates obtained from MICROCHECKER.

	Cott 170	*Cott 207*	*Cott 54*	*Cott E30*	*LCE 54*	*Cott 153*	*Cott 213*	*Cott E13*	*LCE81*	*Cott 348*	*Cott 50*	*Cott 686*	*Cott E11*	*Cott E23*
AL				0.19										
AN		0.11		0.12			0.14		0.09					
AS		0.14		0.08	0.15									
C														
CA	0.36			0.07	0.2									
CL														
CQ				0.07										
CR														
DL				0.17	0.07									
FA				0.12	0.16		0.12							
FC														
FE														
FN				0.22		0.13								
FP	0.1						0.11							
FS				0.07						0.08				
FT														
FW				0.12		0.11								
GL	0.15		0.1		0.16									
HL						0.09								
HS				0.11		0.08				0.09				
HY	0.11					0.07								
HZ													0.1	
JH				0.12		0.09								
LC														
PI														
PK							0.15							
P						0.11								
PS	0.12					0.09								
S	0.11			0.23		0.06								0.08
SR														
ST				0.14										

Appendix 4.S2: Pairwise F_{ST} comparisons among 31 sampling sites (Lower Fraser River system, and around Strait of Georgia, southern British Columbia).

Table 4.S2: Pairwise FST comparisons from 14 microsatellite loci for *Cottus asper*. Significant (P < 0.05) vales are indicated by bold font.

	AL	AN	AS	C	CA	CL	CQ	CR	DL	FA	FC	FE	FN	FP	FS	FT	FW	GL	HL	HS	HY	HZ	JH	LC	PI	PK	P	PS	S	SR	ST
AL	0																														
AN	**0.059**	0.000																													
AS	**0.056**	-0.004	0.000																												
C	**0.119**	**0.068**	**0.072**	0.000																											
CA	**0.111**	**0.083**	**0.080**	**0.096**	0.000																										
CL	**0.219**	**0.185**	**0.196**	**0.172**	**0.189**	0.000																									
CQ	**0.053**	0.008	0.002	**0.080**	**0.093**	**0.209**	0.000																								
CR	**0.072**	**0.033**	**0.031**	**0.039**	**0.065**	**0.156**	**0.040**	0.000																							
DL	**0.289**	**0.277**	**0.263**	**0.318**	**0.304**	**0.382**	**0.281**	**0.258**	0.000																						
FA	**0.081**	**0.032**	**0.027**	**0.040**	**0.052**	**0.195**	**0.037**	0.002	**0.259**	0.000																					
FC	**0.089**	**0.070**	**0.069**	**0.083**	**0.095**	**0.139**	**0.058**	**0.034**	**0.229**	**0.061**	0.000																				
FE	**0.079**	**0.025**	**0.021**	**0.052**	**0.052**	**0.184**	**0.030**	0.006	**0.258**	-0.006	**0.059**	0.000																			
FN	**0.064**	**0.013**	**0.017**	**0.045**	**0.048**	**0.178**	**0.029**	**0.008**	**0.245**	-0.004	**0.052**	-0.002	0.000																		
FP	**0.046**	**0.014**	0.008	**0.052**	**0.054**	**0.168**	**0.023**	0.004	**0.251**	0.006	**0.054**	0.006	-0.001	0.000																	
FS	**0.068**	0.006	0.011	**0.051**	**0.055**	**0.195**	**0.022**	**0.021**	**0.284**	0.004	**0.069**	0.007	-0.004	0.003	0.000																
FT	**0.055**	0.013	0.011	**0.059**	**0.051**	**0.177**	**0.015**	0.002	**0.252**	0.000	**0.036**	-0.003	-0.001	0.000	0.009	0.000															
FW	**0.048**	**0.015**	**0.017**	**0.057**	**0.077**	**0.199**	0.004	**0.020**	**0.269**	**0.017**	**0.049**	**0.014**	0.011	0.007	0.010	0.011	0.000														
GL	**0.287**	**0.276**	**0.265**	**0.279**	**0.323**	**0.396**	**0.298**	**0.264**	**0.499**	**0.266**	**0.272**	**0.278**	**0.262**	**0.247**	**0.269**	**0.286**	**0.305**	0.000													
HL	**0.096**	**0.043**	**0.037**	**0.129**	**0.127**	**0.221**	**0.028**	**0.089**	**0.321**	**0.086**	**0.084**	**0.077**	**0.072**	**0.059**	**0.053**	**0.062**	**0.043**	**0.309**	0.000												
HS	**0.054**	**0.021**	**0.020**	**0.094**	**0.098**	**0.191**	**0.019**	**0.041**	**0.304**	**0.048**	**0.087**	**0.044**	**0.040**	**0.021**	**0.033**	**0.030**	**0.019**	**0.318**	**0.068**	0.000											
HY	0.008	**0.056**	**0.056**	**0.096**	**0.116**	**0.188**	**0.052**	**0.039**	**0.274**	**0.066**	**0.060**	**0.064**	**0.053**	**0.037**	**0.062**	**0.037**	**0.038**	**0.271**	**0.101**	**0.052**	0.000										
HZ	**0.072**	**0.015**	0.011	**0.081**	**0.098**	**0.180**	**0.017**	**0.036**	**0.318**	**0.040**	**0.080**	**0.034**	**0.033**	**0.019**	**0.023**	**0.028**	**0.023**	**0.320**	**0.061**	0.000	**0.066**	0.000									
JH	**0.260**	**0.229**	**0.217**	**0.302**	**0.147**	**0.315**	**0.224**	**0.229**	**0.470**	**0.229**	**0.243**	**0.198**	**0.220**	**0.207**	**0.225**	**0.209**	**0.230**	**0.547**	**0.243**	**0.217**	**0.265**	**0.224**	0.000								
LC	**0.086**	**0.041**	**0.040**	**0.064**	**0.074**	**0.125**	**0.053**	**0.016**	**0.259**	**0.031**	**0.038**	**0.024**	**0.026**	**0.022**	**0.035**	**0.017**	**0.044**	**0.266**	**0.069**	**0.055**	**0.065**	**0.041**	**0.199**	0.000							
PI	**0.047**	-0.006	-0.007	**0.064**	**0.076**	**0.177**	0.001	**0.021**	**0.259**	**0.026**	**0.063**	**0.018**	**0.013**	0.003	0.009	0.006	0.005	**0.275**	**0.041**	**0.009**	**0.040**	0.005	**0.210**	**0.036**	0.000						
PK	**0.039**	-0.008	-0.008	**0.073**	**0.079**	**0.180**	-0.004	**0.030**	**0.287**	**0.030**	**0.068**	**0.024**	**0.014**	0.005	**0.011**	**0.013**	**0.012**	**0.295**	**0.040**	**0.010**	**0.040**	0.009	**0.227**	**0.040**	-0.012	0.000					
P	**0.357**	**0.291**	**0.299**	**0.280**	**0.374**	**0.306**	**0.349**	**0.282**	**0.500**	**0.316**	**0.259**	**0.312**	**0.294**	**0.282**	**0.323**	**0.314**	**0.335**	**0.563**	**0.309**	**0.340**	**0.324**	**0.340**	**0.525**	**0.242**	**0.286**	**0.321**	0.000				
PS	**0.343**	**0.305**	**0.311**	**0.211**	**0.318**	**0.283**	**0.327**	**0.264**	**0.463**	**0.275**	**0.262**	**0.273**	**0.275**	**0.282**	**0.296**	**0.295**	**0.319**	**0.438**	**0.376**	**0.343**	**0.299**	**0.330**	**0.516**	**0.258**	**0.302**	**0.324**	**0.470**	0.000			
S	**0.020**	**0.021**	**0.019**	**0.086**	**0.103**	**0.177**	0.011	**0.034**	**0.283**	**0.051**	**0.060**	**0.045**	**0.037**	**0.020**	**0.038**	**0.027**	**0.016**	**0.281**	**0.058**	**0.016**	**0.013**	**0.024**	**0.224**	**0.048**	0.008	0.006	**0.316**	**0.317**	0.000		
SR	**0.073**	0.012	**0.011**	**0.078**	**0.083**	**0.186**	**0.016**	**0.040**	**0.305**	**0.035**	**0.093**	**0.034**	**0.023**	**0.016**	**0.014**	**0.026**	**0.019**	**0.309**	**0.054**	**0.012**	**0.071**	0.004	**0.205**	**0.051**	0.007	-0.004	**0.323**	**0.332**	**0.024**	0.000	
ST	**0.011**	**0.045**	**0.044**	**0.092**	**0.115**	**0.189**	**0.038**	**0.045**	**0.295**	**0.071**	**0.058**	**0.064**	**0.058**	**0.036**	**0.062**	**0.042**	**0.036**	**0.284**	**0.081**	**0.050**	0.001	**0.062**	**0.257**	**0.057**	**0.032**	**0.035**	**0.341**	**0.307**	0.007	**0.066**	0.000

Appendix 5 Mitochondrial haplotype sequences, split up by gene region (D-loop, Cytb, Cox1).

>A1_dloop

CTCCTACCCCTAACTCCCAAAGCTAGGGTTCTAAATTAAACTATTCCTTGGCGTATGTACTAATTTCAGTATATGATTTATGTAAATACAT
ATATGTATAATCACCACGAATTTATATGAACCAAATCAATAGCATTTAAGGATGTATATGTAATATCAACACATCTCGGTGTTCCACATTC
ATATATCAACATAATACGAAGATAAACATAAAGCATGTAGAGTTTTATATAACATTTAATTAATTCAAGGATAGGCGAGATTTAAGACCG
AACTAAATCACTCATTAGTTAAGTTATACGTTTCTCCACAACCCGTCAAATATCAGTATACGATGTAGTAAGAACCGACCATCAGTTGATT
TCTTAATGCCAACGGTTATTGAAGGTGAGGGACAAGTATTCGTGGGGGGTTTCACAGAGTGCACTATTCCTGGCATTTGGTTCCTATTTCAG
GGCCATTGATTGATGTTATTCCTCCCACTTTCATCGACGCTTGCATAAGTTAATGTTGGCGTACATCCCCGAGATGACCCAGCATGCCGGG
CGTTCTCTCCAGCGAGCCAGGGGTTCTCTTTTTTTTTT

>A1_cytb

AAAAACCCACCCCCTACTAAAAATCGCAAACAATGCACTAGTTGATCTTCCAGCCCCCTCAAATATTTCGGTATGATGAAACTTTGGCTCC
CTCCTAGGTCTTTGTTTAATTATCCAAATCCTAACCGGGCTCTTCCTCGCCATACACTACACCTCTGACATCGCAACAGCCTTCTCATCAGT
CGGACATATCTGCCGAGATGTTAACTACGGGTGACTCATCCGTAACCTCCACGCCAACGGTGCCTCTTTCTTTTTCATTTGCATCTATATGC
ACATCGGACGGGGCCTTTATTACGGTTCCTACCTCTATAAAGAGACTTGAAATATTGGGGTCGTCCTCCTACTCCTTGTAATAATAACCGC
TTTCGTAGGATACGTCCTCCCCTGAGGACAAATATCATTCTGAGGGGCTGCAG

>A1_Cox1

TTTTGGGGGACGACCAGATTTATAWAGTAATTGTTACGGCCCATGCTTTCGTAATAATTTTCTTTATAGTAATACCAATTATAATCGGGGG
TTTCGGGAACTGACTCATCCCCCTAATGATCGGCGCCCCTGATATGGCCTTTCCTCGAATAAACAATATGAGCTTTTGACTTCTTCCCCCAT
CTTTTTTACTCCTCCTAGCCTCTTCGGGGGTCGAAGCAGGAGCGGGAACCGGATGAACAGTTTACCCACCCCTCGCCGGGAACCTCGCCC
ATGCAGGAGCCTCTGTTGACCTAACGATCTTCTCCCTTCACCTAGCAGGTATCTCTTCTATTCTTGGAGCAATCAACTTTATCACAACTATC
ATTAATATGAAACCCCCTGCTATTTCTCAATACCAGACCCCTCTATTCGTATGGTCTGTTCTTATTACTGCTGTCCTACTGCTTCTTTCCCTC
CCCGTACTTGCCGCCGGCATCACAATGCTCCTAACAGACCGAAACCTTAACACCACCTTCTTTGACCCTGCCGGAGGAGGAGACCCAATC
CTTTACCAACACCTCTTTTGATTCTTTGGCCACCC

>A2_dloop

CTCCTACCCCTAACTCCCAAAGCTAGGGTTCTAAATTAAACTATTCCTTGGCGTATGTACTAATTTCAGTATATGACTTATGTAAATACAT
ATATGTATAATCACCACGAATTTATATGAACCAAATCAATAGCATTTAAGGATGTATATGTAATATCAACACATCTCGGTGTTCCACATTC
ATATATCAACATAATACGAAGATAAACATAAAGCATGTAGAGTTTTATATAACATTTAATTAATTCAAGGATAGGCGAGATTTAAGACCG
AACTAAATCACTCATTAGTTAAGTTATACGTTTCTCCACAACCCGTCAAATATCAGTATACGATGTAGTAAGAACCGACCATCAGTTGATT
TCTTAATGCCAACGGTTATTGAAGGTGAGGGACAAGTATTCGTGGGGGGTTTCACAGAGTGCACTATTCCTGGCATTTGGTTCCTATTTCAG
GGCCATTGATTGATGTTATTCCTCCCACTTTCATCGACGCTTGCATAAGTTAATGTTGGCGTACATCCCCGAGATGACCCAGCATGCCGGG
CGTTCTCTCCAGCGAGCCAGGGGTTCTCTTTTTTTTTT

>A2_cytb

AAAAACCCACCCCCTACTAAAAATCGCAAACAATGCACTAGTTGATCTTCCAGCCCCCTCAAATATTTCGGTATGATGAAACTTTGGCTCC
CTCCTAGGTCTTTGTTTAATTATCCAAATCCTAACCGGGCTCTTCCTCGCCATACACTACACCTCTGACATCGCAACAGCCTTCTCATCAGT
CGGACATATCTGCCGAGATGTTAACTACGGGTGACTCATCCGTAACCTCCACGCCAACGGTGCCTCTTTCTTTTTCATTTGCATCTATATGC
ACATCGGACGGGGCCTTTATTACGGTTCCTACCTCTATAAAGAGACTTGAAATATTGGGGTCGTCCTCCTACTCCTTGTAATAATAACCGC
TTTCGTAGGATACGTCCTCCCCTGAGGACAAATATCATTCTGAGGGGCTGCAG

>A2_Cox1

TTTTGGGGGACGACCAGATTTATAATGTAATTGTTACGGCCCATGCTTTCGTAATAATTTTCTTTATAGTAATACCAATTATAATCGGGGG

TTTCGGGAACTGACTCATCCCCCTAATGATCGGCGCCCCTGATATGGCCTTTCCTCGAATAAACAATATGAGCTTTTGACTTCTTCCCCCAT

CTTTTTTACTCCTCCTAGCCTCTTCGGGGGTCGAAGCAGGAGCGGGAACCGGATGAACAGTTTACCCACCCCTCGCCGGGAACCTCGCCC

ATGCAGGAGCCTCTGTTGACCTAACGATCTTCTCCCTTCACCTAGCAGGTATCTCTTCTATTCTTGGAGCAATCAACTTTATCACAACTATC

ATTAATATGAAACCCCCTGCTATTTCTCAATACCAGACCCCTCTATTCGTATGGTCTGTTCTTATTACTGCTGTCCTACTGCTTCTTTCCCTC

CCCGTACTTGCCGCCGGCATCACAATGCTCCTAACAGACCGAAACCTTAACACCACCTTCTTTGACCCTGCCGGAGGAGGAGACCCAATC

CTTTACCAACACCTCTTTTGATTCTTTGGCCACCC

>A4_dloop

CTCCTACCCCTAACTCCCAAAGCTAGGGTTCTAAATTAAACTATTCCTTGGCGTATGTACTAATTTCAGTATATGAATTATGTAAATACAT

ATATGTATAATCACCACGAATTTATATGAACCAAATCAATAGCATTTAAGGATGTATATGTAATATCAACACATCTCGGTGTTCCACATTC

ATATATCAACATAATACGAAGATAAACATAAAGCATGTAGAGTTTTATATAACATTTAATTAATTCAAGGATAGGCGAGATTTAAGACCG

AACTAAATCACCCATTAGTTAAGTTATACGTTTCTCCACAACCCGTCAAATATCAGTATACGATGTAGTAAGAACCGACCATCAGTTGATT

TCTTAATGCCAACGGTTATTGAAGGTGAGGGACAAGTATTCGTGGGGGTTTCACAGAGTGCACTATTCCTGGCATTTGGTTCCTATTTCAG

GGCCATTGATTGATGTTATTCCTCCCACTTTCATCGACGCTTGCATAAGTTAATGTTGGCGTACATCCCCGAGATGACCCAGCATGCCGGG

CGTTCTCTCCAGCGAGCCAGGGGTTCTCTTTTTTTTTT

>A4_cytb

AAAAACCCACCCCCTACTAAAAATCGCAAACAATGCACTAGTTGATCTTCCAGCCCCCTCAAATATTTCGGTATGATGAAACTTTGGCTCC

CTCCTAGGTCTTTGTTTAATTATCCAAATCCTAACCGGGCTCTTCCTCGCCATACACTACACCTCTGACATCGCAACAGCCTTCTCATCAGT

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Appendix 6 Genotypes of 19 microsatellite loci genotyped for *Cottus asper* in GENEPOP format

MSA-Output from file: combined19MSA.txt 2001,2002,2003 written by Daniel Dieringer
Cott170
Cott207
Cott255
Cott54
CottE30
LCE54
Cott214
Cott78
Cott348
CottE11
CottE20
Cott153
Cott213
CottE13
LCE81
Cott50
Cott686
CottE23
EDA1
POP
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Alaska, 0405 0405 0708 0303 1819 0311 0608 1821 0303 1125 0507 1123 0404 1414 1623 0415 0708 0410 0508
Alaska, 0405 0505 0407 0303 1318 0505 0608 1420 0715 1111 0507 1111 0404 1414 1621 0411 0303 0508 0505
Alaska, 0505 0505 0708 0103 1819 0505 0606 1422 1515 1125 0505 1111 0204 1414 1723 1115 0303 0405 0505
Alaska, 0506 0505 0408 0103 1319 0303 0106 2122 0303 1111 0205 1121 0404 1414 2323 0415 0303 0405 0508
Alaska, 0606 0405 0707 0103 1818 0306 0608 1920 1515 2525 0505 1119 0404 1414 1623 0415 0303 0405 0508
Alaska, 0506 0405 0808 0103 1919 0505 0606 1822 0315 2424 0505 0000 0000 1414 2323 0415 0307 0405 0505
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Alaska, 0405 0404 0408 0101 1319 0311 0608 2122 0315 1111 0507 1119 0204 1419 1623 0513 0303 0404 0808
Alaska, 0505 0405 0408 0303 1319 0311 0606 1922 0303 1111 0507 1111 0404 1414 0916 0404 0707 0508 0508
Alaska, 0606 0405 0808 0303 1919 0303 0606 1321 0317 1125 0205 1111 0404 1414 0423 0415 0708 0909 0808
Alaska, 0405 0404 0707 0103 1818 0511 0606 1919 0715 1325 0207 1117 0406 1414 2323 1515 0303 0404 0505
Alaska, 0406 0405 0909 0103 2020 0311 0606 1922 0315 1125 0505 1521 0406 1414 1616 0415 0307 0404 0508
Alaska, 0405 0405 0708 0103 1820 0305 0606 1922 0315 2525 0205 1921 0204 1214 2323 0415 0708 0808 0508
POP
FallsCreek, 0204 0506 0707 0103 0000 0404 0618 1218 0717 3346 0404 0809 0206 1422 2334 1011 0708 0809 0505
FallsCreek, 0506 0407 0303 0303 1111 0304 0606 1021 1717 0223 0107 0718 0606 0117 2347 1111 0303 0810 0512
FallsCreek, 0306 0304 0713 0102 0000 0404 0617 2023 0515 0911 0507 1222 0206 0114 0112 1111 0307 0710 0514
FallsCreek, 0507 0404 0214 0303 0909 0404 0623 1421 0417 4646 0407 0809 0204 2222 1216 1213 0307 0710 0505
FallsCreek, 0405 0405 0207 0103 0918 0411 0312 2023 0726 0521 0305 1225 0206 2022 0116 1113 0308 0508 0505
FallsCreek, 0405 0405 0203 0303 0911 0414 0512 1010 0717 3640 0712 0909 0606 2222 0409 1114 0307 0810 0513
FallsCreek, 0606 0405 0207 0103 0918 0404 0406 1415 0731 0133 0405 0824 0404 1430 1212 1011 0303 0809 0507
FallsCreek, 0203 0505 0714 0101 1826 0404 0616 0423 0717 0521 0712 0708 0306 0117 0416 1111 0307 0808 0000
FallsCreek, 0506 0405 0407 0103 1318 0304 0519 0813 0407 1521 0407 0309 0202 2329 1617 0911 0303 0407 0707
FallsCreek, 0606 0404 0308 0303 1119 0404 0510 1318 0209 0521 0507 1220 0406 1414 1216 1114 0307 0708 0513
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POP
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LittleCampbell, 0404 0404 0307 0103 1118 0303 0621 1421 0817 0444 0505 0809 0204 1722 2330 1111 0303 0707 0505
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Lakelse, 0606 0404 0213 0303 1721 1111 0606 1414 1137 0505 0404 2228 0202 1414 1717 0411 0303 0808 0508
Lakelse, 0606 0404 1314 0103 1721 1111 0607 1422 1111 0525 0404 1319 0202 1414 1616 0204 0303 0208 0508
Lakelse, 0606 0404 0708 0303 1718 1111 0606 1414 1111 0511 0404 1624 0202 1414 0416 1115 0303 0410 0508
Lakelse, 0606 0404 0714 0303 0719 1111 0606 1414 1137 0505 0404 0418 0202 1414 0417 0000 0000 0000 0000
Lakelse, 0606 0404 0713 0303 1718 0511 0606 1416 1111 0521 0404 1724 0202 1416 0416 0000 0000 0000 0508
Lakelse, 0606 0404 0707 0303 1117 1111 0606 1214 1111 0505 0407 1821 0202 1414 0404 0404 0000 0000 0508
Lakelse, 0606 0404 0707 0303 1521 1111 0608 1422 0911 0505 0404 1717 0202 1414 1616 0411 0303 0808 0508

Lakelse, 0606 0404 0707 0103 1818 1111 0000 0000 1133 0505 0407 1819 0202 1417 0404 0000 0000 0000 0508
Lakelse, 0606 0404 0713 0303 0319 1111 0606 1014 1137 2626 0404 1819 0202 1414 0416 0415 0303 0608 0808
Lakelse, 0606 0404 0213 0103 0723 1111 0000 1014 1137 0621 0404 1219 0202 1414 1417 1515 0303 0610 0808
Lakelse, 0606 0404 0707 0103 1823 0311 0606 1414 1111 2525 0404 1720 0202 1414 0417 0411 0303 0608 0508
Lakelse, 0608 0404 0712 0303 1719 1111 0606 1213 1111 0505 0404 1821 0202 1416 1717 1115 0303 0608 0508
Lakelse, 0606 0404 0707 0103 1718 1111 0607 1414 1111 0205 0404 1820 0206 1414 0817 1115 0303 0608 0808
Lakelse, 0606 0404 0707 0303 1823 1111 0000 0000 1037 0525 0404 1921 0202 1414 0416 0415 0303 0410 0505
Lakelse, 0606 0404 0213 0303 1021 1111 0606 1414 1111 0505 0404 1718 0202 1414 0416 0413 0303 0808 0505
Lakelse, 0606 0404 0707 0303 1923 1111 0606 1414 1313 0511 0404 1819 0202 1416 0404 0411 0203 0808 0808
Lakelse, 0606 0404 0707 0101 1019 1111 0606 1418 1111 0526 0404 1619 0202 1414 0425 0000 0000 0000 0508
Lakelse, 0606 0404 0207 0103 0717 1111 0606 1414 1111 0525 0404 1920 0202 1414 0416 1115 0303 0808 0508
Lakelse, 0606 0404 0207 0103 1719 1111 0606 0914 1111 0505 0404 1821 0206 1414 0416 0415 0103 0808 0508
Lakelse, 0606 0404 0707 0101 1821 0311 0606 1414 1111 0525 0404 2124 0202 1417 1616 1213 0606 0000 0808
Lakelse, 0606 0404 0207 0103 1825 1111 0000 0000 1137 0521 0404 1719 0206 1414 0417 0415 0303 0608 0505
Lakelse, 0606 0404 0707 0101 0417 1111 0608 0914 1111 0505 0404 1620 0202 1414 0404 0415 0303 0610 0505
Lakelse, 0106 0404 0207 0303 1118 1111 0606 1414 1111 0511 0404 1120 0202 1414 1621 0415 0303 0608 0808
Lakelse, 0000 0000 0000 0000 0000 0000 0606 1515 1111 0525 0404 1920 0202 1414 1616 0411 0303 0708 0808
Lakelse, 0606 0404 0707 0103 1117 1111 0000 0000 1111 0505 0404 1617 0202 1414 1625 0711 0303 0606 0808
POP
MALout, 0406 0406 0713 0103 1719 0911 0616 1422 0707 3744 0407 1318 0506 1422 1735 1111 0708 0809 0505
MALout, 0304 0305 0203 0101 1719 2021 0000 0919 2828 3333 0509 0813 0203 1420 1956 1113 0708 0712 0505
MALout, 0404 0304 0507 0101 1836 0411 0609 1018 0707 2632 0710 0812 0202 1414 1619 0111 0307 0707 0505
MALout, 0406 0405 0708 0103 1718 0411 0606 0514 1735 1134 0407 0808 0202 1214 1319 1111 0303 0710 0205
MALout, 0304 0405 0312 0101 1843 1122 1616 0816 0707 3036 0512 0808 0206 1014 2151 1111 0707 0712 0505
MALout, 0304 0406 0212 0101 1818 0404 0616 0818 1725 1132 0412 0808 0204 1414 1730 1111 0707 0710 0505
MALout, 0405 0405 0307 0103 1818 0409 0619 1924 0735 1125 0307 0818 0204 1414 1717 1111 0707 1010 0505
MALout, 0206 0405 0305 0103 1717 0404 0619 1014 2235 2125 0405 0813 0206 1414 1117 1011 0707 0910 0505
MALout, 0506 0406 0307 0101 1942 0411 0506 1414 1734 2532 0407 0813 0202 1214 1419 0105 0707 0709 0205
MALout, 0404 0304 0707 0303 1719 0304 0000 0000 0709 2121 0408 0808 0404 1414 1956 1314 0707 0609 0505
MALout, 0405 0406 0405 0101 1717 0304 0616 1819 0717 2539 0407 0813 0202 1414 1619 0105 0707 0709 0505
MALout, 0306 0406 0307 0101 1718 0316 0606 1415 0707 1525 0712 0813 0204 1414 1717 1113 0707 0710 0505
MALout, 0406 0406 0205 0103 1117 0311 0606 0616 1517 1321 0305 0818 0404 1414 0717 0511 0507 0709 0205
MALout, 0406 0506 0313 0103 0821 0416 0606 1014 1728 1328 0405 1313 0206 1414 2131 0000 0000 0000 0505
MALout, 0406 0405 0307 0103 1717 1116 0406 1516 2535 3242 0307 1320 0204 1414 2328 1113 0307 0909 0000
MALout, 0406 0306 0712 0103 1919 0304 0606 1422 0728 2424 0912 0808 0404 1414 1619 1111 0303 0608 0505
MALout, 0406 0506 0204 0103 1818 0303 0606 0523 2828 2535 0305 0812 0204 1414 1752 0111 0307 0910 0505
MALout, 0405 0405 0407 0103 4143 0412 0606 1820 0707 1337 0507 1429 0202 1416 1626 1113 0708 0712 0505
MALout, 0505 0405 0707 0103 1212 1116 0616 0519 2235 2132 0505 0820 0404 1414 1735 0111 0307 0710 0505
MALout, 0405 0304 0205 0101 0707 0416 0616 1020 0735 3434 0712 1313 0204 1425 1617 1111 0707 0609 0508
MALout, 0506 0404 0713 0101 1719 0304 0606 1923 0717 2232 0407 0808 0206 1414 1756 1112 0506 0709 0205
MALout, 0306 0405 0707 0101 3436 0304 0406 1014 0724 2125 0305 0808 0202 1222 1619 1111 0707 0913 0505
MALout, 0204 0406 0207 0103 1717 0412 0416 1014 0717 1115 0712 1010 0206 1422 0817 0000 0000 0000 0505
MALout, 0406 0304 0707 0101 1218 0404 0406 1521 0735 1130 0507 0808 0205 1422 1319 1113 0808 0707 0508
MALout, 0506 0405 0208 0101 1919 0411 0616 1015 0207 2525 0408 0826 0404 1222 1630 1114 0708 0709 0505
MALout, 0405 0404 0307 0103 1217 0404 0606 0816 0935 1919 0404 0808 0505 1417 1617 1113 0303 0810 0505
MALout, 0404 0405 0712 0103 1719 0303 0616 0816 0915 2124 0507 1318 0406 1414 1638 0404 0000 0000 0505
MALout, 0405 0506 0812 0103 1919 0304 0616 0814 0913 3335 0405 0813 0204 1212 3542 1113 0307 0810 0208
MALout, 0404 0406 0307 0103 1743 0404 0606 0522 0735 2125 0408 1313 0204 1425 1717 1112 0507 0712 0205
MALout, 0305 0406 0312 0103 1719 0304 0406 1415 0707 3235 0507 0814 0202 1425 1919 1114 0307 0610 0306
MALout, 0202 0406 0305 0101 1017 0411 0606 1022 1528 2125 0707 1113 0205 1425 0826 1111 0308 0910 0205
POP
MAL, 0306 0406 0507 0103 1717 0309 0606 1619 0000 0000 0000 0808 0206 1515 1619 0511 0307 1010 0505
MAL, 0404 0404 0714 0103 1717 0404 0406 1415 2435 1139 0707 0813 0202 1414 1616 1213 0307 0610 0505
MAL, 0506 0405 0203 0103 1717 1111 1616 0518 0000 0000 0000 0814 0204 1414 1619 0111 0707 0810 0505
MAL, 0606 0406 0507 0101 1717 0416 0606 1016 0717 1219 0409 1314 0202 1417 1617 0310 0307 0607 0505
MAL, 0405 0405 0708 0101 2143 0000 0616 1516 0735 2525 0505 1010 0205 1414 1216 1113 0708 0609 0205
MAL, 0406 0404 0202 0103 4143 0322 0606 0820 2835 1232 0407 0000 0000 0000 0000 1213 0707 0713 0505
MAL, 0206 0606 0707 0101 1719 0916 0616 1010 0722 1322 0505 0000 0000 0000 0000 1111 0307 0709 0505
MAL, 0404 0304 0312 0308 1718 0404 0606 1014 0707 0815 0708 1320 0406 1414 1619 1111 0303 0710 0505
MAL, 0000 0000 0000 0000 0000 0000 1619 1420 0617 3542 0307 0000 0000 0000 0000 1111 0307 0710 0505
MAL, 0404 0404 0310 0101 3535 0304 0506 1419 0634 1133 0205 0000 0000 0000 0000 1111 0707 0710 0505
MAL, 0306 0404 0712 0303 1719 0404 0616 1415 0617 1233 0407 0813 0406 2226 2835 1013 0707 0910 0205
MAL, 0404 0404 1214 0308 1943 0404 0606 1422 2134 2133 0207 1010 0205 1122 1630 0312 0707 0709 0505
MAL, 0406 0406 0313 0103 1717 0416 0606 1629 0606 2145 0407 0000 0000 0000 0000 1111 0303 0713 0505
MAL, 0404 0405 0707 0101 0618 0316 0416 1414 0617 2539 0304 0000 0000 0000 0000 0511 0708 0909 0505
MAL, 0306 0406 0813 0103 1723 0311 0606 1922 0627 1322 0404 0000 0000 0000 0000 0111 0307 0614 0505
MAL, 0606 0404 0213 0101 1919 0411 0606 1416 0934 2431 0307 0811 0204 1414 2137 1111 0307 0710 0505
MAL, 0405 0405 0413 0103 1717 0304 1616 1016 0606 2139 0505 1010 0404 1422 1619 1114 0307 0709 0509
MAL, 0404 0404 0507 0101 1242 0911 0606 1415 0715 2136 0512 0000 0000 0000 0000 0000 0000 0000 0000
MAL, 0404 0405 0213 0103 1719 0303 0616 1018 1722 2125 0307 0814 0405 2225 1730 1111 0707 0809 0000

MAL, 0506 0506 0713 0303 1719 0404 0417 1819 0735 1125 0712 0812 0404 0220 0423 1113 0308 1013 0505
MAL, 0406 0506 0303 0101 1719 0404 0606 1616 0707 1113 0409 0813 0406 1414 1656 1112 0303 0709 0505
MAL, 0405 0404 0307 0101 1942 0404 0506 1418 1535 2442 0812 0816 0202 1428 4856 1011 0303 0707 0505
MAL, 0408 0405 0707 0303 1717 0409 0616 1418 0934 1121 0712 0813 0206 1414 1730 1111 0303 0909 0514
MAL, 0405 0406 0707 0101 1818 0416 0617 1822 0207 2134 0712 0813 0202 1214 1919 1113 0303 0709 0505
MAL, 0406 0404 0707 0101 1919 0411 0616 1919 1525 1321 0707 0813 0406 1414 1616 1111 0308 0709 0505
MAL, 0404 0404 0208 0101 1923 0404 0616 0815 2235 1125 0507 0813 0202 1417 1737 1111 0307 0707 0505
MAL, 0206 0404 0307 0101 1721 0922 0616 0818 0634 2136 0509 0000 0000 0000 0000 1111 0307 0607 0505
MAL, 0404 0406 0511 0101 1836 0409 0606 0000 2134 3741 0405 0813 0204 1224 1719 1111 0707 0707 0505
MAL, 0404 0405 0305 0103 1717 0422 0616 1018 1734 1143 0409 0816 0204 1414 1626 0112 0707 0709 0505
MAL, 0405 0404 0307 0101 1717 0404 0606 1419 3434 1321 0709 1111 0202 1622 1717 1113 0707 0713 0000
MAL, 0506 0405 1112 0101 1718 1115 0606 1010 0634 3538 0204 0813 0202 1717 1621 0511 0307 0913 0505
MAL, 0406 0405 0313 0103 0619 0409 0614 0815 0606 2132 0405 0813 0404 1417 1719 1111 0707 0910 0000
MAL, 0404 0405 0707 0101 1717 0404 0616 1627 0606 2534 0512 0813 0204 1214 1635 0111 0307 0910 0000
MAL, 0406 0305 0202 0101 1719 0404 0406 0826 2334 4244 0512 0812 0204 1515 1728 0111 0303 0910 0505
MAL, 0404 0406 0305 0103 1737 0416 0406 1218 0623 1121 0410 0816 0405 1414 1717 1111 0303 0713 0000
MAL, 0505 0306 0712 0103 1821 0312 0616 0823 3535 3339 0304 1011 0406 1214 2238 1111 0303 0707 0505
MAL, 0406 0406 0413 0101 1919 0411 0606 1322 0715 2535 0404 1010 0406 1422 1515 1111 0307 0708 0000
MAL, 0406 0404 0712 0103 1717 0916 0616 1019 0717 1121 0712 0811 0205 1414 1737 1111 0303 0707 0513
MAL, 0406 0405 0712 0303 1217 0303 0416 0514 0715 2125 0505 0000 0000 0000 0000 0512 0307 0910 0000
MAL, 0506 0404 0707 0103 1717 0304 0616 1010 0735 2142 0507 0000 0000 0000 0000 1111 0707 0710 0205
MAL, 0506 0404 0710 0103 0817 0404 0606 1522 0717 2125 0712 0000 0000 0000 0000 1111 0305 0610 0000
MAL, 0406 0405 0308 0101 1717 0404 0606 0527 1722 0913 0508 0813 0404 1414 1621 0111 0307 0712 0000
MAL, 0506 0305 0311 0101 1717 0404 0616 1217 0735 1125 0505 0813 0206 0214 1726 1213 0307 0709 0000
MAL, 0304 0404 0211 0103 1717 1111 0606 1819 0615 2125 0307 0813 0204 1214 1619 1112 0708 0607 0505
MAL, 0406 0404 0307 0101 1921 0909 0406 0000 0616 2121 0307 0813 0204 1426 1820 0513 0708 0710 0202
MAL, 0506 0506 0305 0103 1717 0911 0616 1016 0634 1539 0712 0808 0406 1414 1719 1111 0707 0709 0505
MAL, 0506 0404 0707 0103 1736 0416 0406 1014 0634 2133 0307 1313 0306 1422 1922 0511 0708 0713 0505
MAL, 0304 0405 0313 0303 1717 0309 0000 0000 0916 2124 0207 0813 0406 1425 1420 0511 0707 0707 0205
MAL, 0404 0304 0204 0103 1717 0411 0000 0000 1536 2125 0507 0000 0000 0000 0000 1113 0307 0909 0505
MAL, 0406 0404 0207 0103 1717 0409 0000 0000 0634 1121 0707 1218 0406 1214 1619 1111 0307 0710 0000
MAL, 0405 0405 0000 0101 1721 0409 0616 0811 1717 1121 0304 1113 0204 1414 1737 0505 0707 0710 0505
POP
Mosquito, 0406 0405 0513 0303 1821 0311 0606 1425 1317 0505 0404 1324 0405 1422 0216 0000 0000 0000 0505
Mosquito, 0406 0505 0307 0303 1719 0311 0606 1420 1313 0505 0204 1250 0404 2225 1720 1111 0709 0808 0505
Mosquito, 0406 0204 1313 0303 0818 0311 0606 2222 1317 0507 0204 1319 0205 2022 1733 1113 0307 0412 0505
Mosquito, 0407 0404 0713 0303 1718 1111 0606 0922 1317 0507 0405 2122 0505 2222 2020 1111 0707 0809 0505
Mosquito, 0406 0405 1313 0303 1018 0311 0606 1530 1517 0505 0405 2251 0202 2222 1621 1115 0307 0808 0508
Mosquito, 0406 0505 0707 0303 1717 0311 0606 1414 1315 0505 0407 1125 0405 1422 1721 1111 0307 0408 0508
Mosquito, 0404 0506 0313 0303 1130 1111 0506 2122 1015 0505 0404 2646 0404 1922 1753 1111 0307 0808 0000
Mosquito, 0406 0505 0707 0303 1819 0311 0606 1421 0713 0505 0407 1655 0204 2022 1742 1113 0307 0808 0505
Mosquito, 0404 0506 0713 0303 1818 0411 0606 2225 1315 0505 0407 1126 0505 2022 1617 1111 0303 0808 0505
Mosquito, 0408 0507 0307 0103 1718 0304 0606 1322 1313 0405 0404 0000 0000 0000 0000 1111 0307 0809 0505
Mosquito, 0406 0505 1314 0103 1718 1111 0606 1421 1317 0505 0207 0913 0204 2222 0917 1111 0307 0808 0505
Mosquito, 0404 0405 1313 0303 1719 0304 0506 1416 1313 0505 0207 0509 0204 2022 1719 1111 0708 0412 0505
Mosquito, 0406 0405 0713 0303 1719 0311 0606 1426 1313 0505 0304 0946 0202 2222 1230 1113 0307 0408 0505
Mosquito, 0607 0405 0713 0303 1018 0311 0606 2026 1315 0505 0405 1119 0404 2223 0812 1113 0303 0808 0505
Mosquito, 0404 0405 0713 0103 1119 0311 0606 1420 0413 0505 0407 2022 0404 1722 0917 1113 0307 0808 0000
Mosquito, 0607 0505 0713 0103 1919 0311 0606 1019 1313 0505 0404 0913 0405 2222 1719 1111 0307 0808 0505
Mosquito, 0607 0405 0713 0103 1819 1111 0606 1422 1315 0509 0405 1643 0404 1422 1919 1111 0707 0809 0505
Mosquito, 0406 0506 0707 0303 1721 0311 0606 2022 0000 0000 0000 1334 0205 2225 1717 1111 0303 0808 0508
Mosquito, 0406 0506 0707 0303 1018 1111 0606 2222 0717 0507 0404 1113 0204 1422 0921 1113 0707 0408 0505
Mosquito, 0407 0506 0713 0303 1719 0311 0606 1420 1515 0505 0407 1932 0205 2222 1717 1113 0307 0408 0505
Mosquito, 0404 0404 0713 0303 1718 0311 0606 1422 0711 0507 0507 1313 0202 1417 2040 1113 0708 0808 0508
Mosquito, 0406 0506 1313 0303 1921 0311 0607 0921 1313 0505 0408 0913 0204 2325 2024 1111 0303 0404 0505
Mosquito, 0606 0404 0707 0303 1517 0303 0606 1414 0717 0505 0404 1322 0404 1422 0730 1111 0307 0408 0505
Mosquito, 0406 0505 1113 0303 0817 0411 0606 1621 1517 0507 0407 2548 0405 2222 1919 1111 0307 0808 0505
Mosquito, 0406 0505 1313 0303 1719 0411 0606 1014 0713 0507 0204 1322 0205 1222 2626 1111 0303 0808 0505
Mosquito, 0407 0505 1313 0303 1015 0303 0606 1430 1113 0505 0404 1122 0404 2225 2020 1113 0303 0808 0000
Mosquito, 0404 0405 1314 0303 1719 0411 0606 1213 0415 0505 0407 0000 0000 0000 0000 1111 0309 0808 0505
Mosquito, 0406 0405 0413 0303 1717 1111 0606 1419 0713 0505 0404 0000 0000 0000 0000 1111 0707 0408 0505
Mosquito, 0407 0404 0303 0303 1517 1111 0606 1421 1313 0505 0405 0922 0205 2222 1717 1111 0307 0808 0000
Mosquito, 0404 0405 0713 0103 1930 1111 0606 2022 0713 0505 0204 0000 0000 0000 0000 1111 0303 0408 0505
POP
Nimpo, 0606 0404 0707 0303 0618 1211 0000 0000 1517 0505 0303 1414 0202 1414 1423 1414 0707 0808 0808
Nimpo, 0606 0404 0707 0303 1818 1111 0606 1616 1515 0505 0303 0000 0000 0000 0000 0000 0000 0000 0808
Nimpo, 0606 0404 0707 0303 1818 1111 0606 1620 1516 0505 0303 1414 0202 1414 1919 0411 0000 0000 0808
Nimpo, 0606 0404 0707 0303 0621 1111 0606 1616 1515 0505 0303 1414 0202 1214 1919 0000 0000 0000 0808
Nimpo, 0606 0404 0707 0303 1818 1111 0000 0000 1517 0505 0303 0000 0000 0000 0000 1414 0707 0808 0808
Nimpo, 0606 0404 0707 0303 1818 1111 0000 1616 1517 0505 0303 1419 0202 1414 1919 1414 0707 0808 0808

Nimpo, 0606 0404 0707 0303 1821 1109 0606 1616 1517 0505 0303 1414 0202 1414 0919 1414 0707 0808 0808
Nimpo, 0606 0404 0707 0303 1818 1111 0606 1920 1517 0505 0303 1414 0202 1414 1919 1414 0707 0808 0000
Nimpo, 0606 0404 0707 0303 0618 1111 0000 0000 1517 0505 0303 0000 0000 0000 0000 1414 0707 0808 0000
Nimpo, 0606 0404 0707 0303 1818 1111 0606 1819 1515 0505 0303 0000 0000 0000 0000 1414 0707 0808 0000
Nimpo, 0000 0000 0000 0000 0000 0000 0606 1819 1515 0505 0303 1415 0202 1414 1919 1414 0707 0808 0808
Nimpo, 0000 0000 0000 0000 0000 0000 0606 1619 1515 0505 0303 0000 0000 0000 0000 0000 0000 0000 0808
POP
Tlell, 0406 0606 0207 0303 1017 0312 0606 1022 0331 1323 0707 0812 0206 1422 1216 1515 0707 0808 0505
Tlell, 0407 0506 0307 0303 1119 0404 0606 0000 0912 0505 0507 1722 0202 1416 1639 1115 0307 0808 0505
Tlell, 0406 0505 0613 0303 1731 0304 0000 0000 1629 0515 0507 0837 0202 1417 0816 1515 0307 1010 0000
Tlell, 0406 0506 0202 0303 1218 0304 0000 0000 1620 1125 0707 1112 0205 1922 1214 1515 0303 0408 0505
Tlell, 0606 0406 0707 0303 0817 0403 0000 0000 0316 0525 0305 0812 0202 1422 1617 1115 0303 1010 0505
Tlell, 0406 0505 0207 0303 1718 0303 0606 1921 0930 0505 0510 1618 0506 1622 2139 1315 0307 0810 0505
Tlell, 0506 0405 0212 0303 0817 0303 0606 2327 0303 0521 0405 1117 0202 1417 1751 1111 0307 0810 0000
Tlell, 0406 0405 0202 0303 1823 0305 0606 1418 1629 1921 0507 0817 0202 1719 1628 1517 0711 0608 0510
Tlell, 0507 0404 0202 0303 1717 0911 0606 1819 1212 1313 0707 0000 0000 0000 0000 1515 0707 0808 0000
Tlell, 0506 0404 0207 0103 0633 0311 0606 1619 1337 0525 0507 1116 0406 1422 1237 1113 0303 0808 0505
Tlell, 0406 0404 1214 0303 0612 0303 0616 1422 0317 2123 0505 1135 0204 1216 1947 1215 0303 0708 0505
Tlell, 0505 0405 0207 0101 1218 0303 0606 0922 0317 0505 0507 1212 0206 1616 1316 1111 0307 0408 0208
Tlell, 0606 0606 0707 0303 1719 0303 0506 0922 0337 0505 0505 1213 0406 1422 1214 1515 0305 0810 0508
Tlell, 0606 0405 0713 0303 0814 0304 0606 0909 0322 0505 0407 0819 0406 1214 1316 1115 0708 0808 0505
Tlell, 0606 0406 0713 0303 0617 0303 0606 2223 0303 2525 0707 1123 0205 1419 1316 1515 0303 0808 0510
Tlell, 0506 0406 0713 0303 0000 0311 0000 0000 0332 0525 0707 1123 0205 1419 1316 1515 0307 0808 0510
Tlell, 0606 0404 0212 0303 1018 0303 0506 0914 1332 1325 0405 1215 0405 1419 1313 1517 0307 0608 0508
Tlell, 0505 0405 0214 0103 0606 0412 0506 1419 1731 0813 0507 0817 0204 1417 1234 1515 0307 0408 0505
Tlell, 0000 0000 0000 0000 0000 0000 0606 1420 3131 1325 0405 1113 0205 1414 1321 1515 0305 0808 0505
Tlell, 0406 0104 0207 0103 1923 0303 0506 1819 0322 1325 0303 0815 0204 1622 1728 1115 0707 0408 0505

Appendix 7 Genotypes of 14 microsatellite loci genotyped for *Cottus asper* in southwestern British Columbia, in GENEPOP format.

MSA-Output from file: all_07MSA.txt 2001,2002,2003 written by Daniel Dieringer
Cott170
Cott207
Cott54
CottE30
LCE54
Cott153
Cott213
CottE13
LCE81
Cott348
Cott50
Cott686
CottE11
CottE23
POP
AlouetteLake, 0203 0404 0105 0000 0406 4551 0505 1319 1733 2020 1414 0203 0708 0811
AlouetteLake, 0305 0304 0505 1361 0404 1212 0507 1313 1726 1625 1416 0202 1843 0913
AlouetteLake, 0305 0203 0105 7373 0606 1342 0507 0407 1630 2020 1819 0202 1618 0809
AlouetteLake, 0505 0404 0505 1515 0404 1212 0105 1113 1617 1720 1419 0202 1618 0809
AlouetteLake, 0305 0303 0505 1423 0608 1212 0507 1313 0515 2020 1414 0202 0718 0509
AlouetteLake, 0505 0306 0505 0000 0606 1242 0505 0919 1515 2022 0714 0202 1616 0209
AlouetteLake, 0305 0306 0105 0000 0606 1925 0505 1014 1535 2020 1619 0202 0816 0911
AlouetteLake, 0205 0307 0505 1855 0406 1230 0101 1313 1819 2020 1416 0202 0717 0909
AlouetteLake, 0305 0304 0105 6574 0406 1258 0507 1313 1718 2020 1314 0202 0819 0505
AlouetteLake, 0505 0303 0105 6767 0406 1213 0505 1319 1024 2021 1415 0202 1316 0909
AlouetteLake, 0505 0303 0105 4143 0404 0912 0101 1318 2635 2020 1414 0202 0817 0909
AlouetteLake, 0505 0304 0505 2675 0406 1212 0107 1313 1416 2020 1414 0202 0708 0809
AlouetteLake, 0305 0306 0505 4078 0606 1212 0105 1011 1619 0820 1416 0202 0818 0509
AlouetteLake, 0305 0303 0101 7070 0404 1213 0506 0713 1515 2727 1414 0202 0815 0809
AlouetteLake, 0505 0304 0101 0000 0406 1253 0707 1215 1517 2020 1416 0202 1319 0809
AlouetteLake, 0307 0303 0105 1414 0406 1324 0105 1313 1520 2020 1415 0202 0819 0509
AlouetteLake, 0505 0306 0105 2368 0404 1426 0507 1319 1315 2020 1421 0202 1822 0209
AlouetteLake, 0305 0406 0101 0000 0404 1222 0105 1313 1717 2029 1414 0202 1922 0909
AlouetteLake, 0305 0303 0105 6064 0404 1235 0101 1313 1931 1220 0715 0202 1619 0911
AlouetteLake, 0305 0303 0505 6666 0406 1215 0101 1313 1313 2020 1419 0202 0808 0911
AlouetteLake, 0204 0303 0101 2626 0404 1325 0505 1313 1618 2020 1414 0202 0716 0909
AlouetteLake, 0505 0304 0105 3939 0606 1324 0108 1113 1629 2020 1419 0202 1616 0209
AlouetteLake, 0305 0303 0505 2340 0406 1212 0105 1313 1729 2028 1415 0202 0816 0711
AlouetteLake, 0505 0407 0105 1841 0406 2525 0105 1313 1717 1420 1414 0202 1622 0505
AlouetteLake, 0303 0303 0105 0000 0606 1224 0505 1313 1829 2028 0718 0202 1721 0909
POP
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StaveLake, 0305 0303 0105 2626 0404 1254 0707 1313 0820 2020 0514 0202 0816 0511
StaveLake, 0505 0305 0105 4074 0606 0909 0101 1313 0816 1720 1414 0206 0718 0509
StaveLake, 0505 0303 0105 4075 0606 0912 0507 1317 0808 2020 1214 0202 0722 0511
StaveLake, 0305 0406 0105 2373 0406 1243 0107 1319 0828 2020 1314 0202 2223 0612
StaveLake, 0205 0303 0105 4062 0406 0959 0107 1313 1515 2020 0715 0202 0742 0211
StaveLake, 0505 0303 0505 3030 0406 1328 0107 1313 1818 2020 1419 0202 0817 0811
StaveLake, 0505 0305 0505 6265 0406 1226 0105 1213 1517 0820 1414 0202 1617 0910
StaveLake, 0305 0303 0105 1426 0606 0915 0707 0713 1523 2020 1419 0202 0716 0209
StaveLake, 0505 0304 0105 1570 0406 1130 0505 1414 1840 2020 1418 0202 1722 0511
StaveLake, 0000 0304 0105 0000 0000 0000 0105 0000 0000 1720 1320 0206 0216 0109
StaveLake, 0305 0304 0101 2323 0606 0909 0101 1317 1621 2020 1414 0202 0708 0913
StaveLake, 0305 0304 0101 2365 0406 1047 0507 1313 1021 1320 0516 0202 0716 0909
StaveLake, 0406 0303 0101 0000 0606 1212 0000 1819 0829 2020 1414 0202 4142 1111
StaveLake, 0505 0304 0105 3030 0406 1237 0507 1313 0817 0817 1414 0202 1628 1113
StaveLake, 0707 0303 0505 2369 0404 1230 0105 0713 1523 2020 1419 0202 1622 0203
StaveLake, 0505 0303 0505 1414 0606 1244 0505 1313 1833 2020 1416 0202 1717 0809
StaveLake, 0303 0304 0105 2328 0406 1014 0505 1317 0815 2020 0513 0202 1919 0508

Appendix 8 Morphometric landmark data from 733 sculpins (20 landmarks).

	0.43079 0.19305	1.65779 -0.06109	1.55123 0.18912	-0.71298 -0.00195	SCALE=0.031240	1.14015 -0.23846
	1.16246 0.17949	1.29049 -0.28165	1.62481 0.01428	-0.58167 0.09401	LM=20	1.29679 -0.09047
	1.05694 -0.08736	1.04247 -0.34010	1.54981 -0.05205	-0.34989 0.09104	1.76749 0.07240	1.38422 0.13244
	1.10252 -0.18826	0.56218 -0.32030	1.46100 -0.05351	0.40290 0.16075	1.65955 -0.06771	1.55058 0.18607
LM=20	1.26251 -0.07357	-0.42907 -0.09581	IMAGE=CL23_l.JPG	1.19042 0.19893	1.30601 -0.26601	1.65966 0.01912
1.78061 0.06322	1.38203 0.13993	-0.57995 -0.08231	ID=12	1.08164 -0.06557	1.05206 -0.34685	1.59182 -0.06949
1.66345 -0.05976	1.54990 0.18205	-0.71385 0.00124	SCALE=0.037287	1.12330 -0.18429	0.56520 -0.34895	1.50578 -0.08769
1.27604 -0.27059	1.62882 0.01385	-0.58947 0.08949	LM=20	1.27493 -0.03678	-0.44865 -0.10139	IMAGE=CL8_l.JPG
1.06712 -0.32156	1.55438 -0.07294	-0.30046 0.10801	1.77252 0.06077	1.40643 0.17849	-0.59010 -0.08828	ID=24
0.47291 -0.30965	1.46957 -0.07577	0.50474 0.15621	1.65310 -0.04425	1.57381 0.19195	-0.70780 0.00150	SCALE=0.031211
-0.42228 -0.09822	IMAGE=CL13_l.JPG	1.17836 0.13649	1.26967 -0.22044	1.64888 0.02304	-0.57951 0.09853	LM=20
-0.59614 -0.07833	ID=3	1.10196 -0.09435	1.04838 -0.28701	1.59275 -0.04977	-0.30672 0.11533	1.76555 0.04734
-0.73213 0.00182	SCALE=0.031041	1.14281 -0.20978	0.53150 -0.28655	1.49729 -0.06451	0.48743 0.15817	1.66765 -0.04382
-0.58104 0.09555	LM=20	1.26508 -0.07329	-0.41890 -0.09684	IMAGE=CL28_l.JPG	1.19035 0.14548	1.29449 -0.25188
-0.33014 0.11055	1.77107 0.06132	1.37982 0.13291	-0.59089 -0.08274	ID=16	1.11479 -0.11036	1.05152 -0.31889
0.39324 0.17634	1.66319 -0.06096	1.55105 0.17455	-0.72502 -0.00049	SCALE=0.037297	1.14979 -0.21375	0.56320 -0.29364
1.14213 0.14324	1.29293 -0.26603	1.63592 0.00769	-0.57119 0.08613	LM=20	1.27138 -0.09818	-0.43947 -0.08697
1.04372 -0.10252	1.06373 -0.33303	1.56557 -0.07624	-0.36176 0.10007	1.76107 0.07868	1.38990 0.11457	-0.57304 -0.08112
1.09109 -0.23041	0.51571 -0.31243	1.47203 -0.08525	0.43836 0.18278	1.65653 -0.05887	1.56305 0.16395	-0.71107 -0.00004
1.22804 -0.10787	-0.44655 -0.08866	IMAGE=CL18_l.JPG	1.16222 0.18467	1.28236 -0.26547	1.65024 0.01120	-0.57492 0.08707
1.36298 0.13090	-0.58378 -0.07886	ID=7	1.06494 -0.03102	1.04442 -0.32236	1.57717 -0.07302	-0.33975 0.10515
1.56848 0.19047	-0.72098 -0.00328	SCALE=0.031046	1.10156 -0.14961	0.53056 -0.32170	1.48337 -0.09018	0.46220 0.17991
1.63856 -0.01445	-0.57898 0.08797	LM=20	1.20687 -0.01237	-0.42591 -0.08964	IMAGE=CL5_l.JPG	1.17783 0.17807
1.57389 -0.08589	-0.34409 0.10098	1.76183 0.06658	1.36962 0.18963	-0.58516 -0.07364	ID=21	1.09962 -0.05637
1.47699 -0.09498	0.46395 0.16312	1.65314 -0.05312	1.56736 0.20202	-0.72241 0.00128	SCALE=0.031249	1.14389 -0.17608
IMAGE=CL10_l.JPG	1.20127 0.17222	1.26751 -0.26347	1.63640 0.03143	-0.58668 0.09148	LM=20	1.27493 -0.03973
ID=0	1.12075 -0.08823	1.03047 -0.33524	1.55252 -0.03204	-0.32780 0.10766	1.76341 0.06242	1.38644 0.17561
SCALE=0.030325	1.16243 -0.20160	0.53395 -0.32256	1.45611 -0.01969	0.46435 0.15730	1.65636 -0.06404	1.56654 0.20073
LM=20	1.30534 -0.07675	-0.44108 -0.09014	IMAGE=CL24_l.JPG	1.15444 0.14509	1.26786 -0.29040	1.65102 0.02932
1.78221 0.07128	1.40311 0.15628	-0.56877 -0.07481	ID=13	1.06505 -0.08728	1.01881 -0.34562	1.57280 -0.05289
1.68230 -0.05267	1.56621 0.17870	-0.72853 0.00141	SCALE=0.037276	1.11297 -0.21400	0.52890 -0.33164	1.47364 -0.05703
1.33027 -0.29973	1.65908 0.01173	-0.57512 0.09439	LM=20	1.25605 -0.10013	-0.44349 -0.09249	IMAGE=CL9_l.JPG
1.03102 -0.38028	1.59783 -0.07033	-0.32924 0.10795	1.77756 0.06941	1.35297 0.12307	-0.58149 -0.08459	ID=25
0.55019 -0.34149	1.50538 -0.08704	0.46724 0.17851	1.64731 -0.05683	1.53656 0.18121	-0.71516 -0.00150	SCALE=0.031189
-0.44117 -0.10166	IMAGE=CL14_l.JPG	1.15619 0.16954	1.28734 -0.26352	1.63245 0.02375	-0.57152 0.09203	LM=20
-0.59062 -0.08658	ID=4	1.08337 -0.08167	1.04232 -0.33807	1.54977 -0.07286	-0.35691 0.10234	1.78461 0.07635
-0.73173 0.00660	SCALE=0.031248	1.11784 -0.19083	0.53705 -0.33608	1.45601 -0.08056	0.47132 0.15650	1.66279 -0.06826
-0.59649 0.09400	LM=20	1.25422 -0.07173	-0.43150 -0.08900	IMAGE=CL2_l.JPG	1.13332 0.14299	1.22804 -0.28103
-0.24327 0.10955	1.77605 0.06987	1.37825 0.14682	-0.59536 -0.07976	ID=17	1.05744 -0.10844	1.02504 -0.32981
0.50142 0.11944	1.66820 -0.06735	1.55218 0.16821	-0.71127 -0.00066	SCALE=0.031046	1.11436 -0.20690	0.52342 -0.29970
1.14394 0.17223	1.25629 -0.30301	1.62985 0.02039	-0.58295 0.08963	LM=20	1.26877 -0.06991	-0.43832 -0.07265
1.02589 -0.11685	1.04886 -0.34726	1.56065 -0.06851	-0.29874 0.10943	1.77083 0.06624	1.37832 0.12852	-0.59008 -0.07180
1.11671 -0.22577	0.48460 -0.30094	1.46033 -0.06806	0.42190 0.15416	1.64592 -0.05427	1.54896 0.17361	-0.73209 0.00390
1.22372 -0.06892	-0.44745 -0.09693	IMAGE=CL19_l.JPG	1.15069 0.19728	1.25007 -0.24144	1.64149 0.00772	-0.57123 0.08783
1.36323 0.18476	-0.58048 -0.08751	ID=8	1.07306 -0.06384	1.01029 -0.29440	1.56674 -0.07385	-0.32016 0.09344
1.50905 0.18696	-0.71836 0.00282	SCALE=0.031018	1.11183 -0.18060	0.53187 -0.27600	1.47532 -0.07664	0.46467 0.15022
1.64154 0.02656	-0.58315 0.09944	LM=20	1.21350 -0.04335	-0.43736 -0.08558	IMAGE=CL6_l.JPG	1.17876 0.12696
1.58339 -0.06424	-0.38025 0.11012	1.76642 0.06112	1.34468 0.17770	-0.57234 -0.07658	ID=22	1.09450 -0.12374
1.49020 -0.09052	0.48151 0.11993	1.65474 -0.05762	1.55526 0.22292	-0.72605 0.00044	SCALE=0.031228	1.12388 -0.24965
IMAGE=Cl11_l.JPG	1.12417 0.14926	1.28818 -0.24703	1.63093 0.03908	-0.56637 0.09107	LM=20	1.24137 -0.12188
ID=1	1.02351 -0.14109	1.03836 -0.32188	1.56791 -0.04614	-0.35212 0.11000	1.77127 0.06147	1.34431 0.10827
SCALE=0.031395	1.09700 -0.25330	0.55477 -0.29699	1.47160 -0.05347	0.47673 0.19048	1.64906 -0.06269	1.53505 0.16753
LM=20	1.22168 -0.08354	-0.42187 -0.08916	IMAGE=CL25_l.JPG	1.16955 0.18356	1.25964 -0.27522	1.64144 -0.01002
1.75516 0.07046	1.32377 0.15957	-0.59456 -0.07537	ID=14	1.05468 -0.06474	1.04910 -0.32139	1.55422 -0.08946
1.66179 -0.06534	1.50074 0.17341	-0.72660 0.00145	SCALE=0.037287	1.09046 -0.18231	0.54547 -0.31354	1.45963 -0.09660
1.32282 -0.28600	1.64355 0.01545	-0.57511 0.08728	LM=20	1.23432 -0.05476	-0.43113 -0.10054	IMAGE=HL10_l.JPG
1.09735 -0.36044	1.57424 -0.07467	-0.31098 0.10665	1.77510 0.06251	1.36173 0.16509	-0.59347 -0.08916	ID=26
0.54012 -0.35118	1.48084 -0.07228	0.49136 0.16822	1.63555 -0.05567	1.55453 0.19061	-0.71765 -0.00099	SCALE=0.035605
-0.42268 -0.09882	IMAGE=CL15_l.JPG	1.17563 0.14254	1.28924 -0.22167	1.63149 0.01967	-0.57193 0.09585	LM=20
-0.58810 -0.08335	ID=5	1.08750 -0.08811	1.06220 -0.28102	1.54790 -0.06365	-0.37240 0.10873	1.79549 0.07121
-0.71942 -0.00128	SCALE=0.031342	1.12069 -0.20245	0.54237 -0.27875	1.45660 -0.04523	0.43699 0.16487	1.68338 -0.06450
-0.59582 0.09645	LM=20	1.26464 -0.09678	-0.38944 -0.09138	IMAGE=CL30_l.JPG	1.16359 0.15175	1.27004 -0.28853
-0.30087 0.11323	1.75457 0.06302	1.38698 0.12626	-0.57884 -0.08102	ID=18	1.06863 -0.10995	1.02111 -0.36713
0.47940 0.13768	1.65003 -0.06159	1.55426 0.16968	-0.73801 0.00034	SCALE=0.037012	1.12891 -0.21269	0.49868 -0.31929
1.16671 0.11849	1.28937 -0.26571	1.64376 0.00896	-0.60339 0.09078	LM=20	1.24859 -0.10457	-0.47224 -0.08266
1.09200 -0.13455	1.03139 -0.32603	1.56604 -0.06204	-0.35632 0.11013	1.76265 0.06927	1.37725 0.15269	-0.57559 -0.07547
1.14154 -0.24250	0.55040 -0.30602	1.47266 -0.07195	0.46549 0.19603	1.64890 -0.04888	1.53751 0.18333	-0.73343 0.00754
1.27761 -0.11922	-0.37582 -0.10426	IMAGE=CL1_l.JPG	1.16483 0.21635	1.26787 -0.25923	1.64124 0.00529	-0.57115 0.08572
1.37144 0.09658	-0.59260 -0.08585	ID=9	1.06024 -0.01186	1.02860 -0.31796	1.56747 -0.08073	-0.33329 0.08544
1.54080 0.14885	-0.73808 -0.00090	SCALE=0.031131	1.09788 -0.13988	0.54547 -0.30235	1.46538 -0.09390	0.40837 0.10516
1.62935 -0.01482	-0.61159 0.09587	LM=20	1.22124 0.00455	-0.41720 -0.08996	IMAGE=CL7_l.JPG	1.16881 0.15239
1.57390 -0.08559	-0.31435 0.11845	1.76891 0.06911	1.34197 0.21718	-0.58882 -0.07954	ID=23	1.05901 -0.08956
1.49335 -0.10500	0.43428 0.18627	1.64155 -0.05658	1.54491 0.22351	-0.72481 -0.00102	SCALE=0.031211	1.09303 -0.22603
IMAGE=CL12_l.JPG	1.17628 0.17066	1.27549 -0.23926	1.62977 0.04861	-0.57635 0.08959	LM=20	1.20359 -0.05541
ID=2	1.07181 -0.07458	1.05153 -0.29408	1.55003 -0.02460	-0.33869 0.10578	1.77735 0.06147	1.27411 0.12613
SCALE=0.031446	1.13289 -0.18947	0.55231 -0.30051	1.46161 -0.01084	0.49422 0.17639	1.66939 -0.05948	1.51654 0.19112
LM=20	1.27687 -0.06195	-0.41342 -0.08751	IMAGE=CL27_l.JPG	1.13182 0.12130	1.26663 -0.30167	1.65374 0.02212
1.75545 0.06918	1.38457 0.16190	-0.58561 -0.07541	ID=15	1.04929 -0.12722	1.02926 -0.34561	1.57541 -0.06611
1.64471 -0.05863	1.53851 0.18663	-0.71998 -0.00047	SCALE=0.037309	1.10483 -0.20499	0.53131 -0.33410	1.45728 -0.06582
1.27436 -0.25573	1.63255 0.01513	-0.59399 0.08926	LM=20	1.24149 -0.07863	-0.44031 -0.09325	IMAGE=HL11_l.JPG
1.01372 -0.31981	1.57605 -0.05946	-0.29072 0.10940	1.77914 0.06535	1.35906 0.12269	-0.59032 -0.08215	ID=27
0.52015 -0.30871	1.48664 -0.06654	0.48704 0.17331	1.65640 -0.06170	1.53644 0.16769	-0.72528 -0.00340	SCALE=0.035682
-0.42067 -0.10356	IMAGE=CL16_l.JPG	1.17720 0.16777	1.25585 -0.24663	1.63159 0.01933	-0.58113 0.09615	LM=20
-0.58317 -0.08779	ID=6	1.07443 -0.05913	1.05177 -0.28992	1.54974 -0.06705	-0.31569 0.11130	1.79030 0.06649
-0.71968 -0.00009	SCALE=0.031054	1.11570 -0.18043	0.50400 -0.30047	1.45737 -0.07925	0.48408 0.15557	1.67484 -0.05660
-0.58460 0.09543	LM=20	1.23742 -0.04910	-0.44289 -0.10135	IMAGE=CL3_l.JPG	1.16851 0.14382	1.26256 -0.27566
-0.29371 0.12078	1.76158 0.05487	1.36272 0.15216	-0.58719 -0.08325	ID=19	1.08744 -0.13981	1.04060 -0.32886

0.47789 -0.28919 1.66089 -0.06145 SCALE=0.035711 1.47011 -0.03646 1.52098 0.18023 1.13354 -0.24963 0.40661 0.13584
-0.45524 -0.07903 1.26752 -0.29011 LM=20 IMAGE=HL26_l.JPG 1.63487 0.01524 1.26101 -0.09787 1.18853 0.12070
-0.58119 -0.07163 1.02536 -0.33951 1.77913 0.06915 ID=39 1.56007 -0.06923 1.33905 0.13636 1.07307 -0.13521
-0.72601 0.00437 0.52753 -0.30851 1.65579 -0.06056 SCALE=0.038731 1.45638 -0.06786 1.53299 0.17561 1.09118 -0.25023
-0.56763 0.08805 -0.44405 -0.08172 1.26271 -0.27250 LM=20 IMAGE=HL32_l.JPG 1.65911 0.01853 1.23865 -0.11741
-0.34322 0.09794 -0.57959 -0.07246 1.03768 -0.33364 1.79327 0.07141 ID=45 1.59131 -0.07876 1.34655 0.13138
0.47060 0.12406 -0.72639 -0.00200 0.53435 -0.30520 1.69232 -0.06078 SCALE=0.039369 1.49967 -0.08681 1.52766 0.18419
1.16708 0.12820 -0.56130 0.08532 -0.41123 -0.08465 1.31144 -0.29893 LM=20 IMAGE=HL43_l.JPG 1.64161 0.00941
1.05321 -0.13347 -0.31334 0.09401 -0.57392 -0.07203 1.06770 -0.38943 1.79870 0.06797 ID=52 1.56637 -0.08703
1.10852 -0.23844 0.42987 0.13129 -0.74563 0.00073 0.46725 -0.36945 1.69718 -0.06658 SCALE=0.037584 1.46925 -0.09216
1.20448 -0.11905 1.17864 0.12980 -0.55595 0.08512 -0.43993 -0.08814 1.25917 -0.32918 LM=20 IMAGE=HL47_l.JPG
1.34816 0.13323 1.08959 -0.12000 -0.30230 0.09617 -0.58001 -0.06826 1.00346 -0.38282 1.78137 0.06423 ID=56
1.52977 0.16390 1.12908 -0.21875 0.44471 0.14851 -0.74760 0.00734 0.45580 -0.32873 1.68243 -0.05639 SCALE=0.037556
1.64913 0.01005 1.25842 -0.08365 1.17491 0.13680 -0.58205 0.08837 -0.44896 -0.08779 1.28659 -0.30673 LM=20
1.58443 -0.06782 1.37073 0.13900 1.07069 -0.11192 -0.31323 0.08966 -0.58698 -0.07802 1.05099 -0.39281 1.77889 0.07140
1.47349 -0.07578 1.54836 0.16942 1.11205 -0.22487 0.43407 0.08777 -0.74036 0.00820 0.50684 -0.37777 1.66097 -0.05146
IMAGE=HL12_l.JPG 1.64198 0.02079 1.22402 -0.09157 1.22310 0.10450 -0.59049 0.08427 -0.44588 -0.08004 1.24673 -0.26425
ID=28 1.57975 -0.07196 1.33454 0.13154 1.10780 -0.13813 -0.32429 0.09321 -0.56809 -0.06995 1.03820 -0.31603
SCALE=0.035670 1.48077 -0.07471 1.53050 0.17567 1.16402 -0.24877 0.40141 0.10520 -0.73818 -0.00534 0.55272 -0.30045
LM=20 IMAGE=HL18_l.JPG 1.63242 0.01626 1.27025 -0.10804 1.18276 0.13713 -0.57852 0.08829 -0.42346 -0.07728
1.76604 0.06662 ID=32 1.55049 -0.07532 1.36645 0.12525 1.07178 -0.12224 -0.29795 0.09750 -0.58053 -0.06563
1.65095 -0.06296 SCALE=0.035711 1.45512 -0.08797 1.54700 0.15999 1.11894 -0.25531 0.43738 0.08558 -0.72200 -0.00102
1.27033 -0.27906 LM=20 IMAGE=HL23_l.JPG 1.65901 0.01649 1.23552 -0.08312 1.19310 0.13041 -0.58277 0.08664
1.02892 -0.34066 1.77822 0.06968 ID=36 1.59267 -0.08917 1.32183 0.14634 1.08349 -0.10093 -0.28663 0.09641
0.54578 -0.31405 1.66868 -0.07509 SCALE=0.035700 1.48951 -0.09449 1.52476 0.19484 1.12073 -0.22994 0.45984 0.14157
-0.43501 -0.07651 1.24703 -0.31532 LM=20 IMAGE=HL28_l.JPG 1.66536 0.02068 1.23817 -0.09945 1.19391 0.14281
-0.57237 -0.06685 1.01485 -0.37299 1.78171 0.09266 ID=41 1.59836 -0.08477 1.33528 0.13822 1.09508 -0.09365
-0.72914 0.00084 0.49151 -0.31464 1.66215 -0.05476 SCALE=0.039292 1.50913 -0.09005 1.52155 0.18193 1.11422 -0.20781
-0.56321 0.08866 -0.42886 -0.07401 1.25631 -0.25326 LM=20 IMAGE=HL34_l.JPG 1.64765 0.01178 1.25117 -0.08322
-0.29682 0.09658 -0.58145 -0.06998 1.03562 -0.30447 1.79226 0.07753 ID=46 1.57466 -0.06938 1.36858 0.14447
0.47657 0.12451 -0.72512 0.00304 0.54572 -0.28120 1.66955 -0.05946 SCALE=0.031461 1.48196 -0.07390 1.55286 0.16411
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ID=118 1.58435 -0.06495 1.11729 -0.23873 -0.43073 -0.08544 LM=20 1.63515 0.01867 1.20409 0.24517
SCALE=0.034470 1.48388 -0.06830 1.25410 -0.09537 -0.58276 -0.07945 1.77739 0.07837 1.56821 -0.06531 1.08837 -0.00156
LM=20 IMAGE=mol25_l.JPG 1.36263 0.12348 -0.71458 -0.00101 1.66828 -0.06563 1.46687 -0.05284 1.11441 -0.14359
1.78176 0.07702 ID=122 1.56407 0.16306 -0.57583 0.09824 1.22967 -0.30459 IMAGE=MOL45_l.JP 1.25054 0.00658
1.65755 -0.06315 SCALE=0.034227 1.64991 -0.00530 -0.29742 0.10361 0.99684 -0.37193 G 1.35951 0.22845
1.27421 -0.27571 LM=20 1.58798 -0.08199 0.39682 0.14835 0.51458 -0.34978 ID=140 1.55346 0.21883
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1.47528 -0.02003
IMAGE=mol5_l.JPG
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-0.58099 -0.07323
-0.71355 -0.00261
-0.57538 0.08870
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0.36831 0.15868
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1.10929 -0.07142
1.14222 -0.19851
1.26970 -0.04008
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1.56415 0.19064
1.64298 0.02949
1.58258 -0.05904
1.48425 -0.06518
IMAGE=mol6_l.JPG
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LM=20
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1.25651 -0.24519
0.98912 -0.29364
0.51488 -0.27021
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1.07881 -0.00233
1.10686 -0.14948
1.25723 0.00207
1.35551 0.20916
1.54900 0.21464
1.65092 0.03725
1.57517 -0.04173
1.46526 -0.03357
IMAGE=mol8_l.JPG
ID=146
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LM=20
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1.24490 -0.27103
1.00510 -0.32231
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1.17979 0.18664
1.09528 -0.06782
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1.23864 -0.05654
1.36236 0.16108
1.54528 0.19106
1.63836 0.02109
1.56398 -0.07211
1.45597 -0.07026
IMAGE=mol9_l.JPG
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LM=20
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1.67720 -0.06778
1.31375 -0.30936
1.02993 -0.38994
0.49062 -0.34896
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-0.58578 -0.08368
-0.72080 0.00531
-0.59187 0.09034
-0.31821 0.09647
0.41927 0.09932
1.15703 0.12297
1.07896 -0.14371
1.11128 -0.24753
1.23643 -0.08032
1.33608 0.12762
1.51729 0.16516
1.65618 0.01823
1.57900 -0.07797
1.47110 -0.07168
IMAGE=OL10_l.JPG
ID=148
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LM=20
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1.20480 -0.28440
0.95463 -0.35160
0.47514 -0.31928
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-0.57187 -0.07676
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1.10733 0.17763
1.00166 -0.09160
1.03790 -0.21016
1.16201 -0.05805
1.29553 0.18456
1.49404 0.21082
1.61320 0.02461
1.53590 -0.06393
1.40748 -0.08334
IMAGE=OL11_l.JPG
ID=149
SCALE=0.032049
LM=20
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1.25842 -0.28172
1.03843 -0.34214
0.54778 -0.31716
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1.13756 0.11472
1.07211 -0.16531
1.10682 -0.24458
1.25014 -0.09278
1.35531 0.13550
1.53092 0.16292
1.64015 0.02725
1.56837 -0.05957
1.46234 -0.07361
IMAGE=OL12_l.JPG
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LM=20
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1.26348 -0.26962
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1.09100 -0.19817
1.22348 -0.07010
1.34378 0.15119
1.53987 0.17929
1.64303 0.03089
1.56250 -0.06350
1.45252 -0.06788
IMAGE=OL14_l.JPG
ID=151
SCALE=0.032009
LM=20
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1.65315 -0.05547
1.23305 -0.25674
1.00069 -0.31460
0.48589 -0.26450
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-0.55471 -0.07627
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0.40773 0.15927
1.15257 0.18163
1.04072 -0.07875
1.05843 -0.18818
1.20052 -0.05171
1.31281 0.19244
1.48886 0.21346
1.63176 0.03968
1.55377 -0.05167
1.44309 -0.04973
IMAGE=OL1_l.JPG
ID=152
SCALE=0.037874
LM=20
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1.66173 -0.05935
1.28282 -0.27677
1.06554 -0.33179
0.50690 -0.30321
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-0.57289 -0.06916
-0.70885 -0.00183
-0.55135 0.07863
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1.11284 -0.21462
1.19576 -0.04146
1.31972 0.16472
1.50031 0.18417
1.61536 0.04920
1.55215 -0.04269
1.44051 -0.04074
IMAGE=OL2_l.JPG
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LM=20
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1.24962 -0.31978
1.02313 -0.39070
0.49262 -0.34218
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-0.57455 -0.07958
-0.68478 0.00064
-0.58342 0.09094
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1.10314 0.11634
1.02090 -0.14164
1.04892 -0.26409
1.15153 -0.08641
1.25548 0.13241
1.46252 0.16551
1.61599 0.01340
1.52379 -0.08179
1.40526 -0.06861
IMAGE=OL3_l.JPG
ID=154
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LM=20
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1.72722 -0.07552
1.35088 -0.36734
1.05794 -0.45469
0.46997 -0.38698
-0.47546 -0.08596
-0.59187 -0.08347
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-0.59985 0.09239
-0.31147 0.09666
0.43359 0.07585
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1.18354 -0.33072
1.31873 -0.15829
1.39563 0.08679
1.56207 0.14337
1.68560 0.03442
1.63888 -0.09262
1.55072 -0.10317
IMAGE=OL4_l.JPG
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LM=20
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1.21384 -0.27165
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-0.58483 -0.08330
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1.02174 -0.11872
1.05405 -0.22247
1.17869 -0.06156
1.27428 0.17272
1.49532 0.20223
1.60957 0.01588
1.53245 -0.06565
1.42732 -0.06694
IMAGE=OL5_l.JPG
ID=156
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LM=20
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1.24004 -0.30752
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0.50413 -0.35482
-0.43875 -0.07636
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1.11519 0.13649
1.06270 -0.15589
1.08988 -0.25714
1.20945 -0.08828
1.28753 0.15413
1.48732 0.19549
1.63927 0.02312
1.56274 -0.07440
1.43920 -0.07840
IMAGE=OL6_l.JPG
ID=157
SCALE=0.032051
LM=20
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1.30545 -0.32424
1.03303 -0.40209
0.51546 -0.38448
-0.48052 -0.09070
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-0.71981 0.00386
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1.30545 -0.32424
1.68091 -0.06938
1.77216 0.07511
IMAGE=streamBCRD
11_l.JPG
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LM=20
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1.66658 -0.05408
1.23530 -0.24787
IMAGE=streamBCRD
16_l.JPG
ID=165
SCALE=0.035169
LM=20
1.77513 0.05587
1.28461 -0.20026
IMAGE=streamBCRD
20_l.JPG
ID=170
SCALE=0.034401
LM=20
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1.65460 -0.05866
1.26997 -0.24705
1.03274 -0.32287
0.57185 -0.32839
-0.45390 -0.09222
-0.57807 -0.08782
-0.71173 0.00159
-0.56064 0.10147
-0.35870 0.11607
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1.20919 0.19104
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1.15212 -0.18474
1.29360 -0.02977
1.40065 0.17775
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1.58055 -0.05073
1.50167 -0.05265
IMAGE=streamBCRD
21_l.JPG
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1.06336 -0.27835
0.55168 -0.30250
-0.45702 -0.09111
-0.56285 -0.08528
-0.56797 0.09574
-0.30862 0.11734
0.49827 0.18233
1.77590 0.07592
1.65758 -0.05755
1.29377 -0.24893
1.04357 -0.32424
0.59238 -0.32343
-0.45518 -0.09596
-0.56994 -0.09478
-0.70686 0.00136
-0.57344 0.10643
-0.33783 0.13165
0.46711 0.18676
1.21071 0.16302
1.10030 -0.12209
1.14146 -0.20627
1.30354 -0.07779
1.40604 0.15096
1.56438 0.18394
1.64044 0.01068
1.58262 -0.06090
1.49923 -0.07231
IMAGE=streamBCRD
15_l.JPG
ID=164
SCALE=0.034799
LM=20
1.77086 0.05581
1.66535 -0.05303
1.26422 -0.22234
IMAGE=streamBCRD
1_l.JPG
ID=169
SCALE=0.035682
LM=20
1.76672 0.07271
1.66003 -0.04917
1.25655 -0.26797
1.03039 -0.33423
0.52510 -0.32873
-0.47130 -0.09234
-0.57124 -0.08341
-0.71275 0.00134
-0.55258 0.08418
-0.33867 0.11041
0.46765 0.15840
1.24766 0.16476
1.14518 -0.08903
1.15377 -0.19652
1.29515 -0.05020
1.39946 0.15317
1.56668 0.17300
1.63861 0.02172
1.58373 -0.04761
1.49506 -0.05781
IMAGE=streamBCRD
20_l.JPG
ID=170
SCALE=0.034401
LM=20
1.76632 0.06377
1.65460 -0.05866
1.26997 -0.24705
1.03274 -0.32287
0.57185 -0.32839
-0.45390 -0.09222
-0.57807 -0.08782
-0.71173 0.00159
-0.56064 0.10147
-0.35870 0.11607
0.45591 0.16819
1.20919 0.19104
1.12573 -0.07860
1.15212 -0.18474
1.29360 -0.02977
1.40065 0.17775
1.55625 0.19160
1.63589 0.02069
1.58055 -0.05073
1.50167 -0.05265
IMAGE=streamBCRD
21_l.JPG
ID=171
SCALE=0.034234
LM=20
1.77202 0.06026
1.65787 -0.04302
1.28947 -0.21920
1.06336 -0.27835
0.55168 -0.30250
-0.45702 -0.09111
-0.56285 -0.08528
-0.56797 0.09574
-0.30862 0.11734
0.49827 0.18233
1.58675 -0.05933
1.48666 -0.07312
IMAGE=streamBCRD
19_l.JPG
ID=168
SCALE=0.034880
LM=20
1.77533 0.06249
1.66408 -0.04855
1.32610 -0.25532
1.07814 -0.32964
0.51067 -0.33348
-0.45773 -0.09135
-0.57404 -0.08403
-0.70244 0.00120
-0.56847 0.08866
-0.33625 0.10779
0.45826 0.14916
1.23584 0.15141
1.12795 -0.07952
1.15013 -0.18727
1.28103 -0.04017
1.39881 0.16884
1.55245 0.18610
1.64051 0.02478
1.58653 -0.04252
1.50168 -0.04314
IMAGE=streamBCRD
1_l.JPG
ID=169
SCALE=0.035682
LM=20
1.76672 0.07271
1.66003 -0.04917
1.25655 -0.26797
1.03039 -0.33423
0.52510 -0.32873
-0.47130 -0.09234
-0.57124 -0.08341
-0.71275 0.00134
-0.55258 0.08418
-0.33867 0.11041
0.46765 0.15840
1.24766 0.16476
1.14518 -0.08903
1.15377 -0.19652
1.29515 -0.05020
1.39946 0.15317
1.56668 0.17300
1.63861 0.02172
1.58373 -0.04761
1.49506 -0.05781
IMAGE=streamBCRD
20_l.JPG
ID=170
SCALE=0.034401
LM=20
1.76632 0.06377
1.65460 -0.05866
1.26997 -0.24705
1.03274 -0.32287
0.57185 -0.32839
-0.45390 -0.09222
-0.57807 -0.08782
-0.71173 0.00159
-0.56064 0.10147
-0.35870 0.11607
0.45591 0.16819
1.20919 0.19104
1.12573 -0.07860
1.15212 -0.18474
1.29360 -0.02977
1.40065 0.17775
1.55625 0.19160
1.63589 0.02069
1.58055 -0.05073
1.50167 -0.05265
IMAGE=streamBCRD
21_l.JPG
ID=171
SCALE=0.034234
LM=20
1.77202 0.06026
1.65787 -0.04302
1.28947 -0.21920
1.06336 -0.27835
0.55168 -0.30250
-0.45702 -0.09111
-0.56285 -0.08528
-0.56797 0.09574
-0.30862 0.11734
0.49827 0.18233

1.21330 0.18267 0.51285 -0.31690 ID=179 1.41274 0.14713 -0.55426 0.09501 1.67906 -0.05656 1.48111 -0.06720
1.13875 -0.07753 -0.45106 -0.08974 SCALE=0.034639 1.56438 0.17295 -0.33938 0.10725 1.31643 -0.26170 IMAGE=streamFC16_
1.15474 -0.16974 -0.57279 -0.08252 LM=20 1.64691 0.01337 0.44338 0.19049 1.07101 -0.33790 l.JPG
1.29298 -0.03672 -0.71842 0.00175 1.80179 0.07559 1.58086 -0.04589 1.26213 0.19317 0.56379 -0.34970 ID=194
1.39376 0.16794 -0.57130 0.08982 1.69221 -0.06592 1.49675 -0.06010 1.15865 -0.04253 -0.45269 -0.10607 SCALE=0.034715
1.56393 0.18049 -0.33124 0.11365 1.32368 -0.25950 IMAGE=streamBCRD 1.17088 -0.14740 -0.58044 -0.08927 LM=20
1.64138 0.02628 0.43357 0.15882 1.06579 -0.34507 38_l.JPG 1.31788 -0.00849 -0.71607 0.00360 1.77440 0.06616
1.58040 -0.03605 1.25046 0.14669 0.56204 -0.34909 ID=183 1.43899 0.18287 -0.58539 0.10011 1.66182 -0.05800
1.49586 -0.03556 1.17529 -0.10267 -0.45945 -0.09635 SCALE=0.034721 1.59802 0.18142 -0.34706 0.11299 1.28245 -0.25439
IMAGE=streamBCRD 1.20883 -0.21113 -0.57410 -0.08904 LM=20 1.65081 0.02497 0.43052 0.16162 1.03117 -0.31591
24_l.JPG 1.35621 -0.06900 -0.74409 0.00896 1.76828 0.06117 1.58463 -0.03525 1.21016 0.14987 0.59429 -0.30599
ID=172 1.42587 0.13727 -0.58816 0.09529 1.66581 -0.05199 1.51345 -0.03969 1.11194 -0.08844 -0.42387 -0.09775
SCALE=0.034669 1.57763 0.16034 -0.38209 0.11746 1.30686 -0.24893 IMAGE=streamBCRD 1.14628 -0.19725 -0.59790 -0.08516
LM=20 1.65988 -0.00500 0.45887 0.16648 1.05253 -0.31909 7_l.JPG 1.25794 -0.07292 -0.72434 0.00100
1.78210 0.06691 1.60874 -0.06544 1.22120 0.16779 0.57209 -0.31784 ID=187 1.38017 0.14164 -0.57510 0.09799
1.68579 -0.06851 1.53285 -0.08517 1.12773 -0.10180 -0.46302 -0.09341 SCALE=0.035404 1.56375 0.17158 -0.33604 0.11753
1.30932 -0.26985 IMAGE=streamBCRD 1.14908 -0.19906 -0.57825 -0.08854 LM=20 1.66189 0.02717 0.47632 0.19098
1.06401 -0.32820 29_l.JPG 1.28496 -0.07041 -0.70602 0.00201 1.78021 0.06331 1.59373 -0.05773 1.18905 0.15774
0.53295 -0.32702 ID=176 1.36266 0.12990 -0.56827 0.09009 1.67703 -0.05280 1.48993 -0.06621 1.11007 -0.08662
-0.45934 -0.08205 SCALE=0.034944 1.56741 0.18840 -0.33805 0.10799 1.33323 -0.26525 IMAGE=streamFC13_ 1.14246 -0.19117
-0.59870 -0.07029 LM=20 1.68008 -0.02268 0.46261 0.16423 1.05540 -0.34468 l.JPG 1.29834 -0.09764
-0.72490 0.00691 1.80512 0.08475 1.62046 -0.06442 1.25267 0.14862 0.55636 -0.33645 ID=191 1.41845 0.14524
-0.58934 0.09708 1.71063 -0.06964 1.53138 -0.06769 1.14359 -0.10415 -0.45035 -0.09472 SCALE=0.034001 1.56548 0.16890
-0.33297 0.10578 1.33164 -0.29183 IMAGE=streamBCRD 1.17584 -0.19314 -0.57232 -0.08311 LM=20 1.65508 0.00906
0.45053 0.13063 1.08134 -0.35578 34_l.JPG 1.28777 -0.04013 -0.72546 0.00415 1.77551 0.07349 1.59090 -0.06475
1.22095 0.13526 0.52512 -0.34742 ID=180 1.39649 0.15705 -0.57590 0.09260 1.66064 -0.05711 1.50027 -0.07072
1.14848 -0.14193 -0.47248 -0.09037 SCALE=0.034923 1.54583 0.17398 -0.33759 0.11002 1.28231 -0.25171 IMAGE=streamFC17_
1.18294 -0.24151 -0.59329 -0.08106 LM=20 1.64893 0.00602 0.46825 0.15040 1.04348 -0.32469 l.JPG
1.30991 -0.08648 -0.74647 0.01262 1.77442 0.07638 1.59007 -0.05020 1.25299 0.15541 0.58880 -0.33953 ID=195
1.39495 0.13322 -0.60115 0.09925 1.65921 -0.05170 1.50216 -0.05773 1.17610 -0.09900 -0.43203 -0.09939 SCALE=0.034007
1.56554 0.16527 -0.35646 0.10680 1.30138 -0.22621 IMAGE=streamBCRD 1.19244 -0.20146 -0.57494 -0.08451 LM=20
1.67883 0.01653 0.44934 0.12475 1.08735 -0.28911 4_l.JPG 1.31457 -0.06163 -0.72906 -0.00190 1.77174 0.07050
1.62442 -0.06589 1.27969 0.13122 0.57127 -0.30735 ID=184 1.41699 0.15157 -0.58652 0.10268 1.65850 -0.05755
1.54571 -0.08888 1.16267 -0.14997 -0.45524 -0.08530 SCALE=0.035197 1.58013 0.16442 -0.31236 0.12625 1.25881 -0.24870
IMAGE=streamBCRD 1.20866 -0.24778 -0.56962 -0.07775 LM=20 1.66904 0.01540 0.45016 0.17604 1.01120 -0.30152
25_l.JPG 1.33316 -0.09600 -0.70763 0.00152 1.78822 0.06264 1.60831 -0.06109 1.20287 0.15255 0.51493 -0.28870
ID=173 1.42766 0.12495 -0.57473 0.09870 1.65060 -0.04342 1.51602 -0.06798 1.11817 -0.09980 -0.45838 -0.09420
SCALE=0.035180 1.58609 0.16153 -0.35534 0.10971 1.23969 -0.17464 IMAGE=streamBCRD 1.14459 -0.19532 -0.57906 -0.09059
LM=20 1.68998 0.02944 0.49660 0.18644 1.04108 -0.20697 8_l.JPG 1.29364 -0.07458 -0.70625 -0.00212
1.78312 0.08624 1.63500 -0.07261 1.22792 0.14884 0.55766 -0.22736 ID=188 1.38194 0.11585 -0.57148 0.09906
1.66105 -0.05992 1.55949 -0.08830 1.13268 -0.07904 -0.44846 -0.08955 SCALE=0.035180 1.55101 0.16754 -0.35998 0.11282
1.28280 -0.24486 IMAGE=streamBCRD 1.16178 -0.17823 -0.56757 -0.08243 LM=20 1.64642 0.01751 0.39380 0.19565
1.04285 -0.31357 31_l.JPG 1.29429 -0.07201 -0.70826 0.00091 1.77517 0.07171 1.58302 -0.05766 1.18169 0.16668
0.53677 -0.31227 ID=177 1.40926 0.13438 -0.56936 0.09045 1.65327 -0.05102 1.48974 -0.07145 1.07811 -0.08650
-0.45018 -0.08182 SCALE=0.034535 1.57460 0.16327 -0.35940 0.11745 1.26438 -0.23085 IMAGE=streamFC14_ 1.11383 -0.18457
-0.56162 -0.08038 LM=20 1.64891 0.01471 0.39449 0.24343 1.05045 -0.29094 l.JPG 1.24600 -0.04754
-0.71653 0.00062 1.77396 0.06544 1.58405 -0.05234 1.26713 0.26177 0.60110 -0.30070 ID=192 1.35679 0.16169
-0.56616 0.09626 1.66052 -0.04301 1.49805 -0.05750 1.15991 -0.00215 -0.45782 -0.09165 SCALE=0.033919 1.52409 0.18949
-0.38226 0.11419 1.26061 -0.20616 IMAGE=streamBCRD 1.16228 -0.10889 -0.56493 -0.08859 LM=20 1.63435 0.01885
0.46091 0.17542 1.03707 -0.25273 35_l.JPG 1.31197 0.01978 -0.71365 0.00135 1.77686 0.07668 1.57006 -0.05128
1.23476 0.14640 0.57998 -0.26455 ID=181 1.44952 0.21508 -0.55796 0.08731 1.66198 -0.04832 1.47859 -0.06179
1.15790 -0.10155 -0.46647 -0.09145 SCALE=0.034475 1.60497 0.20815 -0.39679 0.10668 1.28843 -0.24461 IMAGE=streamFC18_
1.16950 -0.20805 -0.56561 -0.09032 LM=20 1.64921 0.02997 0.45028 0.20118 1.03371 -0.31551 l.JPG
1.31221 -0.11570 -0.71181 0.00157 1.77433 0.06322 1.58029 -0.03412 1.20673 0.20070 0.52153 -0.33901 ID=196
1.43262 0.12448 -0.54712 0.09950 1.66346 -0.04862 1.50252 -0.02732 1.11682 -0.05244 -0.43407 -0.10806 SCALE=0.034013
1.58609 0.15928 -0.30891 0.12822 1.31491 -0.23529 IMAGE=streamBCRD 1.14390 -0.15421 -0.57553 -0.08514 LM=20
1.66511 0.00831 0.46794 0.22744 1.07338 -0.31548 5_l.JPG 1.28186 -0.01908 -0.72491 0.00219 1.76488 0.07438
1.58904 -0.07308 1.23468 0.19234 0.53566 -0.31872 ID=185 1.38368 0.18926 -0.58405 0.10254 1.67049 -0.05981
1.50678 -0.08821 1.14878 -0.06574 -0.46266 -0.08657 SCALE=0.035605 1.55326 0.20371 -0.37477 0.11031 1.32229 -0.26075
IMAGE=streamBCRD 1.17605 -0.16074 -0.56799 -0.08432 LM=20 1.63956 0.02507 0.43813 0.16622 1.04707 -0.34778
26_l.JPG 1.31794 -0.05333 -0.71235 0.00127 1.77331 0.07299 1.57757 -0.03507 1.19509 0.18931 0.53688 -0.33348
ID=174 1.43989 0.15346 -0.55716 0.09563 1.66300 -0.05495 1.49338 -0.04337 1.09312 -0.03772 -0.42830 -0.10296
SCALE=0.034430 1.59267 0.17888 -0.32824 0.10478 1.30452 -0.24380 IMAGE=streamBCRD 1.12584 -0.16639 -0.57942 -0.08956
LM=20 1.66143 0.01444 0.47337 0.15641 1.05329 -0.31597 9_l.JPG 1.26201 -0.02495 -0.72720 0.00264
1.80223 0.06308 1.60003 -0.04883 1.23002 0.17258 0.55915 -0.32604 ID=189 1.37436 0.18927 -0.58004 0.09915
1.70223 -0.07417 1.51412 -0.05533 1.14505 -0.09074 -0.46025 -0.08841 SCALE=0.035203 1.54993 0.20797 -0.32121 0.12115
1.34993 -0.27697 IMAGE=streamBCRD 1.17721 -0.19418 -0.57329 -0.08302 LM=20 1.63944 0.01562 0.47498 0.17091
1.07738 -0.36293 32_l.JPG 1.30650 -0.02137 -0.71124 0.00171 1.78190 0.07494 1.58064 -0.04190 1.19110 0.14106
0.54539 -0.37531 ID=178 1.40018 0.17212 -0.56279 0.09952 1.66381 -0.05227 1.48956 -0.05139 1.11729 -0.11540
-0.46586 -0.09674 SCALE=0.034709 1.56374 0.18153 -0.36595 0.11337 1.28557 -0.24859 IMAGE=streamFC15_ 1.13065 -0.21258
-0.61347 -0.08868 LM=20 1.65466 0.02230 0.48776 0.16439 1.04125 -0.32766 l.JPG 1.25922 -0.09384
-0.73961 0.01084 1.78133 0.05856 1.59313 -0.04308 1.19833 0.15400 0.56305 -0.32315 ID=193 1.36437 0.13387
-0.60015 0.10340 1.67043 -0.04756 1.50233 -0.04461 1.12171 -0.10691 -0.44382 -0.10300 SCALE=0.034475 1.53014 0.16210
-0.35960 0.11656 1.28117 -0.24029 IMAGE=streamBCRD 1.16121 -0.18745 -0.56782 -0.09466 LM=20 1.64691 0.01048
0.43081 0.13734 1.04410 -0.29849 37_l.JPG 1.28112 -0.03080 -0.72157 0.00267 1.77757 0.07742 1.58720 -0.07005
1.24318 0.13815 0.55406 -0.27365 ID=182 1.39201 0.17128 -0.57734 0.09654 1.66353 -0.05526 1.47684 -0.09098
1.16355 -0.14155 -0.45797 -0.08548 SCALE=0.034701 1.55975 0.18515 -0.35975 0.11714 1.29115 -0.24639 IMAGE=streamFC1_l.
1.18855 -0.24052 -0.57883 -0.08914 LM=20 1.64379 0.02177 0.41762 0.19055 1.04389 -0.32036 JPG
1.32437 -0.07851 -0.70655 0.00226 1.77373 0.06127 1.58333 -0.04938 1.18078 0.18006 0.54053 -0.32129 ID=197
1.41667 0.13451 -0.57586 0.09456 1.65811 -0.04548 1.49803 -0.06122 1.08377 -0.06580 -0.45122 -0.09441 SCALE=0.033975
1.58119 0.16207 -0.36362 0.11498 1.28015 -0.22262 IMAGE=streamBCRD 1.10301 -0.18007 -0.59409 -0.09112 LM=20
1.68352 -0.02700 0.43268 0.21909 1.01717 -0.29332 6_l.JPG 1.25759 -0.03937 -0.70780 0.00185 1.77484 0.05938
1.63108 -0.07837 1.22417 0.20204 0.54167 -0.29234 ID=186 1.39234 0.19080 -0.57535 0.09843 1.65469 -0.05437
1.53080 -0.09429 1.12252 -0.07864 -0.43239 -0.09149 SCALE=0.035404 1.53653 0.19816 -0.35098 0.10836 1.29897 -0.23042
IMAGE=streamBCRD 1.14610 -0.17963 -0.58405 -0.08326 LM=20 1.64304 0.03008 0.45758 0.16484 1.01949 -0.31251
28_l.JPG 1.28952 -0.03624 -0.72318 0.00053 1.77176 0.05855 1.57562 -0.05031 1.19000 0.15729 0.51889 -0.29634
ID=175 1.40682 0.17379 -0.57536 0.09977 1.65896 -0.04584 1.48360 -0.05339 1.08323 -0.08356 -0.46182 -0.10020
SCALE=0.034681 1.56676 0.19120 -0.32017 0.12922 1.27382 -0.20677 IMAGE=streamFC10_ 1.12250 -0.18995 -0.56943 -0.09719
LM=20 1.64477 0.01770 0.41418 0.20206 1.04967 -0.25311 l.JPG 1.27140 -0.06480 -0.71363 0.00224
1.75928 0.06897 1.58039 -0.05529 1.25078 0.18949 0.51615 -0.25912 ID=190 1.39316 0.14970 -0.56553 0.10499
1.66850 -0.05656 1.49677 -0.06171 1.13532 -0.06226 -0.45578 -0.08284 SCALE=0.034239 1.55265 0.17677 -0.31852 0.12750
1.32770 -0.25998 IMAGE=streamBCRD 1.15683 -0.18741 -0.56904 -0.07923 LM=20 1.64630 0.02452 0.39506 0.20690
1.00815 -0.35773 33_l.JPG 1.30400 -0.06252 -0.70528 0.00014 1.78900 0.06327 1.57762 -0.05555 1.17882 0.18786

1.09763 -0.07765 -0.44848 -0.10731 SCALE=0.034243 1.52225 0.22170 -0.34489 0.09812 1.32713 -0.21824 IMAGE=streamLCRD
1.13673 -0.17594 -0.58368 -0.09924 LM=20 1.65889 0.03096 0.44905 0.10640 1.10023 -0.29128 22_l.JPG
1.27900 -0.03458 -0.72583 0.00506 1.78077 0.07323 1.59290 -0.04437 1.21452 0.15138 0.60627 -0.31257 ID=222
1.40524 0.17812 -0.58899 0.09676 1.65947 -0.06635 1.50648 -0.05625 1.14843 -0.12003 -0.42630 -0.08802 SCALE=0.034655
1.56978 0.19668 -0.34099 0.11343 1.28122 -0.26819 IMAGE=streamFC6_l. 1.19073 -0.22097 -0.58142 -0.07189 LM=20
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1.58737 -0.05602 1.16815 0.17235 0.52257 -0.31186 ID=209 1.46280 0.15608 -0.58472 0.09409 1.68174 -0.05670
1.49986 -0.05793 1.11305 -0.09998 -0.45490 -0.10338 SCALE=0.034475 1.60685 0.18505 -0.29262 0.09385 1.28785 -0.23256
IMAGE=streamFC20_ 1.13904 -0.19866 -0.55937 -0.09136 LM=20 1.68435 0.01355 0.49499 0.14911 1.06644 -0.29739
l.JPG 1.25424 -0.08456 -0.72330 0.00233 1.78712 0.07447 1.63461 -0.06107 1.21146 0.19621 0.55161 -0.29849
ID=198 1.38336 0.13722 -0.57753 0.09575 1.67694 -0.05347 1.54985 -0.07925 1.13479 -0.04047 -0.43869 -0.09671
SCALE=0.034701 1.54592 0.16968 -0.35985 0.10929 1.25205 -0.26224 IMAGE=streamLCRD 1.18901 -0.14384 -0.57328 -0.07474
LM=20 1.64894 0.01215 0.44625 0.16502 1.01216 -0.30716 12_l.JPG 1.33670 -0.00119 -0.73247 0.00426
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IMAGE=streamLCRD 1.15783 -0.14744 -0.57572 -0.08436 LM=20 1.64496 0.00318 0.46415 0.16529 1.30271 -0.25430
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ID=230 1.41994 0.20778 -0.58847 0.10747 1.66547 -0.06124 1.47559 -0.07631 1.12085 -0.08133 0.53438 -0.30679
SCALE=0.034655 1.58805 0.21842 -0.31466 0.11612 1.28437 -0.26164 IMAGE=streamPER24 1.15835 -0.19468 -0.47598 -0.09250
LM=20 1.64782 0.03295 0.39810 0.19278 1.05486 -0.31814 _1.JPG 1.30048 -0.04391 -0.57508 -0.08137
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-0.45415 -0.09699 SCALE=0.035453 1.58153 0.20748 -0.33759 0.11000 1.28268 -0.24718 IMAGE=streamPER30 1.11412 -0.07641
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IMAGE=streamLCRD 1.15052 -0.14577 -0.57095 -0.07817 LM=20 1.66460 -0.00528 0.39802 0.13237 1.26732 -0.24746
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ID=232 1.43206 0.20062 -0.56008 0.09443 1.66271 -0.05769 1.50521 -0.08885 1.08502 -0.13579 0.51806 -0.27525
SCALE=0.035197 1.59677 0.20553 -0.37284 0.10901 1.25290 -0.26412 IMAGE=streamPER26 1.12426 -0.24011 -0.42570 -0.09254
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IMAGE=streamPER38 1.12850 -0.12834 -0.55890 -0.08482 LM=20 1.64926 0.02270 0.46942 0.14999 1.27651 -0.26946
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ID=259 1.41659 0.23053 -0.56776 0.09568 1.66692 -0.06271 1.49699 -0.05534 1.12363 -0.08715 0.53868 -0.34440
SCALE=0.039351 1.57371 0.22945 -0.35860 0.09670 1.26716 -0.25242 IMAGE=streamPER5_ 1.16644 -0.20914 -0.45216 -0.09490
LM=20 1.65770 0.03543 0.49111 0.11078 1.03995 -0.29832 1.JPG 1.27921 -0.06144 -0.54991 -0.08437
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1.27429 -0.24283 IMAGE=streamPER41 1.11747 -0.20384 -0.57208 -0.08305 LM=20 1.64454 0.02091 -0.32407 0.11131
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SCALE=0.039995 1.57464 0.16132 -0.35724 0.11140 1.29752 -0.27067 IMAGE=streamPER7_ 1.17329 -0.26599 -0.45556 -0.08035
LM=20 1.67360 0.01534 0.40464 0.14446 1.04606 -0.35502 1.JPG 1.26884 -0.10645 -0.57549 -0.07707
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IMAGE=streamPER3_ 1.17527 -0.19933 -0.58311 -0.08835 LM=20 1.64143 -0.01297 0.46747 0.17628 1.26277 -0.24813
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1.16836 -0.28058 0.51114 0.17596 -0.71289 -0.00118 0.55062 -0.27289 1.66675 -0.04774 1.48505 -0.03499 1.07464 -0.08768
1.28054 -0.12278 1.16299 0.15576 -0.56232 0.09188 -0.44063 -0.09068 1.27491 -0.23886 IMAGE=POIL10_l.JP 1.12558 -0.18973
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1.61220 -0.09256 1.36770 0.15730 1.07437 -0.10322 -0.32428 0.11884 -0.59642 -0.08646 LM=20 1.64788 0.00836
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IMAGE=PL25_l.JPG 1.63547 0.02088 1.20861 -0.05352 1.17671 0.21884 -0.57463 0.09516 1.66725 -0.04367 1.47167 -0.07477
ID=321 1.54295 -0.05671 1.32019 0.15321 1.06744 0.00060 -0.34060 0.12422 1.27568 -0.23199 IMAGE=POIL14_l.JP
SCALE=0.033509 1.43637 -0.04824 1.53414 0.18734 1.10317 -0.12887 0.41746 0.19064 0.98441 -0.29899 G
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1.66386 -0.04852 SCALE=0.033520 1.45511 -0.05299 1.56988 0.20522 1.14507 -0.16384 -0.58400 -0.07926 LM=20
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1.48413 -0.06756 1.55243 0.17765 1.11311 -0.17458 0.47150 0.13148 -0.58550 -0.07750 LM=20 1.64358 0.03280
IMAGE=PL26_l.JPG 1.65337 0.02612 1.20989 -0.01341 1.16093 0.14104 -0.70851 0.00202 1.77435 0.05754 1.58248 -0.05441
ID=322 1.58896 -0.07112 1.34700 0.17737 1.07146 -0.10335 -0.56261 0.09893 1.65838 -0.05094 1.48282 -0.05411
SCALE=0.033333 1.49131 -0.07545 1.55126 0.19729 1.12187 -0.21420 -0.26704 0.12347 1.25083 -0.25533 IMAGE=POIL15_l.JP
LM=20 IMAGE=PL30_l.JPG 1.64759 0.03029 1.22747 -0.05186 0.43426 0.15337 0.99020 -0.30301 G
1.77580 0.07423 ID=327 1.56077 -0.04731 1.34852 0.15480 1.23423 0.16919 0.52225 -0.29015 ID=346
1.66141 -0.05306 SCALE=0.033556 1.45264 -0.03019 1.54165 0.17867 1.14091 -0.09512 -0.43804 -0.09395 SCALE=0.034470
1.26743 -0.27825 LM=20 IMAGE=PL3_l.JPG 1.63743 0.02161 1.18516 -0.21130 -0.58131 -0.08330 LM=20

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1.66640 -0.05167
1.24110 -0.25134
0.98570 -0.32203
0.51662 -0.32064
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-0.57066 -0.08688
-0.72190 0.00188
-0.56731 0.09530
-0.37219 0.11586
0.42388 0.18746
1.16198 0.18226
1.07714 -0.08341
1.11491 -0.18806
1.24774 -0.06433
1.37761 0.14490
1.56441 0.16381
1.65788 0.01827
1.58001 -0.07098
1.46410 -0.07285
IMAGE=POIL16_1.JP
G
ID=347
SCALE=0.034234
LM=20
1.77116 0.06089
1.65995 -0.05339
1.22427 -0.25841
0.99089 -0.29758
0.53721 -0.29462
-0.45586 -0.08478
-0.57586 -0.08086
-0.70449 0.00162
-0.55254 0.09467
-0.34012 0.11714
0.42397 0.19693
1.19619 0.17925
1.07508 -0.10520
1.12308 -0.18942
1.28396 -0.09000
1.40417 0.12728
1.57085 0.15392
1.65387 0.01306
1.57929 -0.07315
1.47251 -0.07779
IMAGE=POIL17_1.JP
G
ID=348
SCALE=0.034365
LM=20
1.77162 0.06631
1.65391 -0.05384
1.26168 -0.25639
0.99959 -0.32213
0.50958 -0.32407
-0.42755 -0.09122
-0.57132 -0.08018
-0.72123 0.00058
-0.57881 0.09728
-0.36805 0.11600
0.42031 0.16146
1.13778 0.16556
1.05157 -0.09377
1.10299 -0.19606
1.26825 -0.06825
1.38361 0.14360
1.54956 0.16237
1.64799 0.01091
1.56782 -0.07131
1.47096 -0.07629
IMAGE=POIL19_1.JP
G
ID=349
SCALE=0.034182
LM=20
1.78691 0.06372
1.69630 -0.07129
1.29446 -0.30556
1.03639 -0.37189
0.51684 -0.36848
-0.46122 -0.08994
-0.60268 -0.08612
-0.72393 0.00845
-0.58125 0.10010
-0.35394 0.10914
0.41158 0.12639
1.17814 0.12404
1.07378 -0.15762
1.14168 -0.25161
1.29858 -0.12558
1.41745 0.13748
1.56599 0.15608
1.68618 0.02018
1.61951 -0.08903
1.52015 -0.09752
IMAGE=POIL20_1.JP
G
ID=350
SCALE=0.034470
LM=20
1.78562 0.06862
1.67531 -0.05819
1.28363 -0.25976
1.00816 -0.33014
0.54056 -0.32301
-0.43304 -0.09617
-0.58163 -0.08230
-0.72813 0.00346
-0.57863 0.09975
-0.33573 0.11657
0.41965 0.16046
1.19716 0.16846
1.10836 -0.09473
1.14426 -0.20559
1.29993 -0.06883
1.40810 0.14485
1.58198 0.17058
1.66826 0.00816
1.59832 -0.07499
1.51443 -0.06337
IMAGE=POIL21_1.JP
G
ID=351
SCALE=0.033985
LM=20
1.77004 0.06413
1.66103 -0.04813
1.25250 -0.23972
0.99134 -0.31168
0.53618 -0.30537
-0.42951 -0.08833
-0.58241 -0.07990
-0.71897 -0.00083
-0.57711 0.09860
-0.33557 0.12175
0.41484 0.18590
1.18370 0.18313
1.10683 -0.07671
1.13370 -0.17972
1.29025 -0.07091
1.41520 0.14468
1.57558 0.16667
1.65518 0.01609
1.57771 -0.06234
1.47896 -0.06956
IMAGE=POIL22_1.JP
G
ID=352
SCALE=0.034475
LM=20
1.77784 0.07866
1.67048 -0.05706
1.28098 -0.28078
1.04776 -0.36063
0.52075 -0.38495
-0.45969 -0.08999
-0.57628 -0.07783
-0.70841 -0.00169
-0.55619 0.09694
-0.34320 0.11096
0.45168 0.13206
1.14867 0.15385
1.07675 -0.12580
1.12563 -0.22253
1.25265 -0.07596
1.36745 0.13403
1.54143 0.17280
1.66058 0.02725
1.58299 -0.06914
1.46094 -0.07793
IMAGE=POIL23_1.JP
G
ID=353
SCALE=0.034207
LM=20
1.78654 0.07070
1.66598 -0.05829
1.20383 -0.27803
1.00203 -0.32854
0.52662 -0.32552
-0.46032 -0.08634
-0.58325 -0.08472
-0.70572 0.00131
-0.56756 0.10417
-0.36024 0.12309
0.42556 0.16520
1.14013 0.13720
1.05193 -0.13431
1.09818 -0.23805
1.23064 -0.09068
1.35083 0.12144
1.53633 0.14366
1.63481 -0.00310
1.56679 -0.08385
1.46503 -0.07876
IMAGE=POIL24_1.JP
G
ID=354
SCALE=0.034167
LM=20
1.78075 0.06910
1.68517 -0.05732
1.29938 -0.27538
1.03866 -0.34636
0.48167 -0.34499
-0.45468 -0.09076
-0.59254 -0.08649
-0.72100 0.00546
-0.58842 0.09631
-0.39144 0.10819
0.44383 0.14713
1.17830 0.15724
1.10217 -0.11460
1.15348 -0.20505
1.29146 -0.06781
1.39407 0.13357
1.57024 0.15850
1.67808 0.02224
1.60578 -0.06696
1.48606 -0.09025
IMAGE=POIL25_1.JP
G
ID=355
SCALE=0.034417
LM=20
1.77644 0.06492
1.66244 -0.05725
1.22634 -0.25377
0.96234 -0.31666
0.47297 -0.30006
-0.41632 -0.09392
-0.57709 -0.08749
-0.73095 0.00236
-0.59346 0.10177
-0.38704 0.13010
0.37205 0.21959
1.13383 0.21174
1.03445 -0.08929
1.09034 -0.18627
1.22988 -0.03996
1.35173 0.17951
1.54059 0.18564
1.66003 0.02416
1.57041 -0.06115
1.44630 -0.06053
IMAGE=POIL26_1.JP
G
ID=356
SCALE=0.033101
LM=20
1.77506 0.07017
1.66490 -0.05500
1.27230 -0.25574
1.01054 -0.33204
0.52904 -0.31576
-0.43347 -0.10466
-0.58288 -0.08688
-0.71351 0.00337
-0.58963 0.09959
-0.36879 0.11589
0.39641 0.17350
1.14543 0.18416
1.05447 -0.07794
1.10815 -0.19475
1.27858 -0.05760
1.39166 0.15383
1.55840 0.18106
1.65451 0.01551
1.58753 -0.06212
1.48961 -0.07155
IMAGE=POIL27_1.JP
G
ID=357
SCALE=0.033321
LM=20
1.79588 0.06827
1.66587 -0.05475
1.22764 -0.26202
0.98394 -0.31225
0.55743 -0.31756
-0.45913 -0.08786
-0.59581 -0.08565
-0.71135 0.00210
-0.56442 0.09912
-0.36687 0.11003
0.41032 0.18377
1.15388 0.18045
1.07436 -0.09741
1.09789 -0.19991
1.23853 -0.07655
1.36419 0.14633
1.55847 0.16430
1.64610 0.00773
1.57034 -0.07212
1.46379 -0.06936
IMAGE=POIL28_1.JP
G
ID=358
SCALE=0.033315
LM=20
1.80948 0.07283
1.70532 -0.06749
1.32157 -0.30675
1.02065 -0.38684
0.52556 -0.37515
-0.46578 -0.08463
-0.60174 -0.08132
-0.73531 0.00980
-0.59624 0.09867
-0.37056 0.11241
0.39473 0.13216
1.16828 0.13482
1.10097 -0.14301
1.16550 -0.23438
1.32571 -0.08869
1.42457 0.12148
1.58618 0.15888
1.68517 0.02439
1.62739 -0.07929
1.52130 -0.09838
IMAGE=POIL29_1.JP
G
ID=359
SCALE=0.032662
LM=20
1.78241 0.07057
1.68073 -0.05879
1.26382 -0.26193
0.97378 -0.34905
0.48007 -0.34618
-0.46923 -0.09840
-0.59154 -0.08732
-0.71908 0.00450
-0.57324 0.10104
-0.36303 0.11092
0.37792 0.16397
1.18018 0.15136
1.10774 -0.11789
1.13851 -0.20552
1.31003 -0.08129
1.41673 0.14033
1.57791 0.17242
1.66970 0.01366
1.59371 -0.06375
1.50081 -0.07412
IMAGE=POIL30_1.JP
G
ID=360
SCALE=0.033333
LM=20
1.77683 0.06335
1.65710 -0.05284
1.23317 -0.24201
0.98958 -0.29609
0.50446 -0.28397
-0.44166 -0.10029
-0.59634 -0.09269
-0.71301 0.00183
-0.57460 0.09690
-0.32930 0.12731
0.38629 0.22212
1.16741 0.22302
1.08643 -0.03302
1.09953 -0.14901
1.26382 -0.01483
1.39444 0.18042
1.56946 0.18027
1.64536 0.02075
1.57093 -0.04527
1.45811 -0.04586
IMAGE=POIL31_1.JP
G
ID=361
SCALE=0.033746
LM=20
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1.66008 -0.05421
1.30706 -0.21770
1.00711 -0.29912
0.51425 -0.29574
-0.44093 -0.09008
-0.58412 -0.08713
-0.71037 0.00075
-0.56475 0.09499
-0.34359 0.11825
0.40894 0.18040
1.18200 0.18218
1.07556 -0.08760
1.13244 -0.18665
1.28053 -0.05067
1.39628 0.15303
1.55845 0.17330
1.66020 0.02106
1.57483 -0.06100
1.47067 -0.06108
IMAGE=POIL32_1.JP
G
ID=362
SCALE=0.033315
LM=20
1.77397 0.05935
1.66397 -0.05447
1.24315 -0.25832
1.01874 -0.31858
0.53401 -0.31572
-0.45341 -0.09584
-0.58103 -0.08326
-0.70946 -0.00295
-0.57663 0.09534
-0.34939 0.11591
0.41833 0.17421
1.16112 0.20295
1.07766 -0.04299
1.10925 -0.16458
1.27119 -0.01127
1.38369 0.18517
1.54810 0.19798
1.66676 0.03687
1.58309 -0.05076
1.48149 -0.04579
IMAGE=POIL33_1.JP
G
ID=363
SCALE=0.033330
LM=20
1.76750 0.06290
1.65965 -0.05785
1.24664 -0.25303
1.01405 -0.30878
0.56260 -0.31959
-0.42910 -0.09407
-0.59860 -0.08772
-0.71635 0.00129
-0.58154 0.09601
-0.35831 0.12078
0.42714 0.19115
1.20730 0.17510
1.07759 -0.08301
1.12741 -0.19565
1.29535 -0.08290
1.40897 0.13607
1.57424 0.16801
1.65146 0.00416
1.58279 -0.08095
1.48684 -0.08845
IMAGE=POIL34_1.JP
G
ID=364
SCALE=0.033286
LM=20
1.78620 0.07557
1.69192 -0.06264
1.28774 -0.29232
1.03582 -0.37209
0.50605 -0.37184
-0.45770 -0.09907
-0.59223 -0.08645
-0.72255 0.00689
-0.59019 0.09803
-0.33173 0.11436
0.43952 0.13954
1.14717 0.11440
1.07586 -0.15076
1.13329 -0.25178
1.26234 -0.11331
1.37076 0.12822
1.53540 0.15308
1.65931 0.01535
1.59472 -0.07533
1.49409 -0.09073
IMAGE=POIL35_1.JP
G
ID=365
SCALE=0.033106
LM=20
1.77166 0.07346
1.66292 -0.05194
1.27325 -0.25895
1.04795 -0.32626
0.52850 -0.34040
-0.44695 -0.09026
-0.59180 -0.07645
-0.71655 -0.00208
-0.57756 0.09903
-0.35636 0.11416
0.43983 0.15728
1.16673 0.17266
1.09929 -0.10393
1.12874 -0.19365
1.28398 -0.07574
1.38856 0.15029
1.55361 0.17190
1.66195 0.01890
1.58018 -0.06054
1.47218 -0.07683
IMAGE=POIL36_1.JP
G
ID=366
SCALE=0.033094
LM=20
1.78100 0.06502
1.67419 -0.05865
1.26818 -0.26191
1.01732 -0.32983
0.50305 -0.32992
-0.43841 -0.09650
-0.59224 -0.08680
-0.71740 0.00391
-0.59043 0.09825
-0.36256 0.11692
0.39702 0.17983
1.15540 0.17562
1.06729 -0.09569
1.11433 -0.20557
1.26061 -0.02743
1.37240 0.16705
1.55080 0.18933
1.66914 0.02365
1.58707 -0.05928
1.48516 -0.08235
IMAGE=POIL37_1.JP
G
ID=367
SCALE=0.033094
LM=20
1.77719 0.07149
1.66712 -0.05880
1.23993 -0.28100
0.98496 -0.34555
0.55125 -0.33186
-0.45029 -0.10318
-0.58839 -0.08617
-0.71533 -0.00168
-0.56631 0.09833
-0.38971 0.11250
0.42851 0.16724
1.13650 0.14013
1.06073 -0.13058
1.07764 -0.23634
1.25662 -0.11403
1.37296 0.10537
1.54664 0.15093
1.64871 -0.00547
1.56978 -0.08601
1.47191 -0.09662
IMAGE=POIL40_1.JP
G
ID=369
SCALE=0.033327
LM=20
1.76855 0.07491
1.66345 -0.04871
1.27822 -0.25601
1.02161 -0.33123
0.49619 -0.32910
-0.47319 -0.09738
-0.57461 -0.08332
-0.70852 0.00070
-0.58360 0.09034
-0.34881 0.12305
0.40391 0.18098
1.15038 0.17862
1.06607 -0.09580
1.11534 -0.18834
1.24892 -0.05250
1.38395 0.15530
1.54716 0.17124
1.64948 0.01233
1.56748 -0.06026
1.45904 -0.06613
IMAGE=POIL41_1.JP
G
ID=370
SCALE=0.033260
LM=20
1.77587 0.06402
1.66855 -0.06669
1.26520 -0.26738
0.98078 -0.33069
0.52169 -0.32039
-0.45983 -0.10037
-0.59368 -0.09316
-0.70273 0.00302
-0.56815 0.09584
-0.35592 0.11508
0.41056 0.18035
1.18916 0.17100
1.09778 -0.11549
1.13891 -0.22098
1.29267 -0.07973
1.40315 0.14714
1.55754 0.16539
1.66052 0.00876
1.59052 -0.06894
1.49392 -0.08864
IMAGE=POIL42_1.JP
G
ID=371
SCALE=0.032883
LM=20
1.77217 0.05196
1.66930 -0.05549
1.27111 -0.24949
1.00252 -0.31364
0.52679 -0.31085
-0.41845 -0.09805
-0.61166 -0.08582
-0.72561 0.00389
-0.59680 0.09964
-0.35046 0.12457
0.38959 0.18975
1.18895 0.19037
1.10013 -0.05067
1.13775 -0.16214
1.30039 -0.01468
1.40372 0.17484
1.57273 0.17676
1.65983 0.02563
1.58186 -0.04713
1.50886 -0.03245
IMAGE=POIL43_1.JP
G
ID=372
SCALE=0.032849
LM=20
1.77374 0.06410
1.65369 -0.05228
1.24547 -0.24711
0.98994 -0.30436
0.52555 -0.31322
-0.45870 -0.09296
-0.57807 -0.08516
-0.71128 0.00055
-0.57291 0.09667
-0.37778 0.11917
0.42256 0.20285
1.15921 0.19700
1.06054 -0.05384
1.10304 -0.16674
1.26119 -0.03747
1.38482 0.16938
1.56207 0.18286
1.65183 0.02063
1.57033 -0.05528
1.45835 -0.07185
IMAGE=POIL44_1.JP
G
ID=373
SCALE=0.032883
LM=20
1.79096 0.07185

1.67894 -0.05501 1.49439 -0.08101 1.35068 0.15604 1.18036 0.20159 0.48938 -0.38932 ID=394 1.39164 0.14263
1.23986 -0.25862 IMAGE=POIL49_1.JP 1.51604 0.17426 1.09847 -0.06977 -0.46487 -0.09211 SCALE=0.032662 1.57912 0.19913
0.99114 -0.31348 G 1.62728 0.01774 1.13399 -0.19174 -0.58656 -0.09139 LM=20 1.65896 0.00645
0.54497 -0.31011 ID=377 1.56174 -0.06558 1.24984 -0.04035 -0.70837 0.00505 1.78299 0.06429 1.57546 -0.06688
-0.43161 -0.08925 SCALE=0.032892 1.46312 -0.07139 1.37880 0.17928 -0.58290 0.09334 1.65517 -0.07386 1.48733 -0.08244
-0.60645 -0.08957 LM=20 IMAGE=POIL7_1.JPG 1.55912 0.20139 -0.30051 0.10941 1.23834 -0.27560 IMAGE=PROL26_1.JP
-0.72011 0.00253 1.79335 0.06734 ID=381 1.65710 0.02580 0.45280 0.12131 0.98933 -0.34310 G
-0.56879 0.10242 1.67505 -0.05970 SCALE=0.034681 1.58850 -0.04156 1.15975 0.14637 0.50942 -0.32273 ID=398
-0.33813 0.12673 1.21405 -0.27215 LM=20 1.49715 -0.05524 1.07107 -0.13387 -0.43921 -0.09082 SCALE=0.035641
0.43607 0.20119 1.00947 -0.30677 1.77073 0.06072 IMAGE=PROL11_1.JP 1.12564 -0.25286 -0.58428 -0.09135 LM=20
1.18073 0.18395 0.50734 -0.29055 1.66054 -0.05949 G 1.22978 -0.09419 -0.70819 -0.00114 1.78950 0.06945
1.07274 -0.10366 -0.44457 -0.08944 1.24816 -0.27679 ID=385 1.33851 0.14608 -0.57129 0.10601 1.66997 -0.04925
1.11618 -0.20443 -0.58838 -0.08712 0.99934 -0.33602 SCALE=0.032869 1.52159 0.18715 -0.35959 0.12331 1.28858 -0.25380
1.27683 -0.07537 -0.71427 0.00310 0.51604 -0.33882 LM=20 1.65398 0.01404 0.43893 0.21827 1.04061 -0.31424
1.38909 0.14789 -0.57120 0.09818 -0.47087 -0.08595 1.79467 0.08016 1.58924 -0.07795 1.18619 0.17514 0.55052 -0.31700
1.57801 0.18098 -0.35873 0.10527 -0.57612 -0.08128 1.68502 -0.06979 1.50106 -0.08139 1.08191 -0.09641 -0.44538 -0.09416
1.65810 -0.00760 0.40839 0.17771 -0.69183 0.00206 1.30360 -0.31092 IMAGE=PROL16_1.JP 1.11999 -0.21840 -0.57901 -0.08683
1.58574 -0.06987 1.17924 0.16668 -0.57711 0.09901 1.05743 -0.38250 G 1.27725 -0.10710 -0.71351 -0.00166
1.48375 -0.07301 1.06510 -0.12980 -0.31633 0.12567 0.51924 -0.39158 ID=390 1.39939 0.12817 -0.57005 0.09746
IMAGE=POIL45_1.JP 1.10826 -0.22661 0.42822 0.15136 -0.44722 -0.09576 SCALE=0.032793 1.57401 0.17474 -0.34740 0.10650
G 1.28355 -0.10099 1.13619 0.18168 -0.58003 -0.08160 LM=20 1.65408 -0.00775 0.46220 0.19242
ID=374 1.38169 0.13733 1.05573 -0.06898 -0.73166 0.00620 1.78429 0.07016 1.57210 -0.09257 1.21995 0.16709
SCALE=0.032877 1.55514 0.15802 1.09720 -0.18515 -0.59115 0.10621 1.66463 -0.05215 1.48650 -0.10190 1.12622 -0.09217
LM=20 1.66772 -0.01213 1.22839 -0.02250 -0.36190 0.11526 1.26610 -0.27901 IMAGE=PROL23_1.JP 1.15731 -0.20024
1.77202 0.06023 1.58305 -0.07727 1.35637 0.16110 0.47730 0.15175 1.02002 -0.37431 G 1.30522 -0.07917
1.65481 -0.05317 1.48784 -0.08562 1.52767 0.16973 1.16218 0.13636 0.48233 -0.36313 ID=395 1.41542 0.13778
1.24009 -0.23884 IMAGE=POIL4_1.JPG 1.65231 0.01902 1.08236 -0.13604 -0.45276 -0.09982 SCALE=0.032655 1.58587 0.17155
0.99592 -0.31582 ID=378 1.56190 -0.05854 1.12851 -0.26379 -0.58004 -0.08766 LM=20 1.67247 0.01431
0.52595 -0.31944 SCALE=0.033746 1.43944 -0.06247 1.25031 -0.09897 -0.69991 0.00062 1.78414 0.05824 1.60037 -0.06134
-0.43846 -0.09246 LM=20 IMAGE=POIL8_1.JPG 1.35391 0.13022 -0.55877 0.10386 1.65324 -0.05564 1.51312 -0.07268
-0.57413 -0.08598 1.80588 0.06973 ID=382 1.54632 0.17653 -0.34180 0.11415 1.25471 -0.24773 IMAGE=PROL27_1.JP
-0.72807 -0.00084 1.71467 -0.08402 SCALE=0.033707 1.67986 0.01430 0.43899 0.17635 1.01734 -0.32064 G
-0.56712 0.09937 1.31375 -0.33459 LM=20 1.60509 -0.07837 1.16608 0.20469 0.49917 -0.31293 ID=399
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0.43332 0.19769 0.50275 -0.43643 1.66022 -0.05454 IMAGE=PROL12_1.JP 1.13234 -0.22024 -0.58408 -0.08147 LM=20
1.16947 0.20101 -0.48204 -0.09319 1.26705 -0.25508 G 1.26612 -0.07401 -0.71253 -0.00201 1.78306 0.07748
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1.09855 -0.15573 -0.73763 0.01359 0.55061 -0.33874 SCALE=0.032673 1.55108 0.20563 -0.36194 0.11614 1.27210 -0.28533
1.25264 -0.03932 -0.59055 0.09236 -0.44559 -0.09466 LM=20 1.66181 0.01137 0.45878 0.18775 1.04345 -0.35489
1.38119 0.16734 -0.34960 0.10535 -0.57534 -0.08774 1.78878 0.06291 1.58727 -0.07147 1.17033 0.18303 0.51824 -0.36545
1.55915 0.17311 0.43819 0.08301 -0.69687 0.00067 1.66021 -0.05102 1.49811 -0.07772 1.08851 -0.09084 -0.43560 -0.09836
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1.45879 -0.04690 1.15004 -0.28001 0.43215 0.17464 0.53077 -0.30018 ID=391 1.37178 0.16419 -0.56937 0.10607
IMAGE=POIL47_1.JP 1.25897 -0.10133 1.17787 0.17740 -0.45209 -0.08794 SCALE=0.032461 1.56485 0.20169 -0.32440 0.11221
G 1.35476 0.12576 1.08667 -0.07977 -0.57261 -0.08578 LM=20 1.65767 0.00912 0.46155 0.19206
ID=375 1.53635 0.14097 1.12280 -0.19219 -0.70918 0.00128 1.78274 0.06287 1.58113 -0.05997 1.20041 0.16999
SCALE=0.032860 1.68113 -0.03537 1.26513 -0.05940 -0.57348 0.09913 1.68089 -0.06982 1.49243 -0.05147 1.11220 -0.11278
LM=20 1.61462 -0.09809 1.38189 0.14602 -0.35508 0.12332 1.33636 -0.28182 IMAGE=PROL24_1.JP 1.15982 -0.22763
1.77275 0.06888 1.50678 -0.10858 1.55803 0.16806 0.45591 0.20096 1.03625 -0.37170 G 1.28054 -0.08303
1.67387 -0.06267 IMAGE=POIL5_1.JPG 1.65041 0.01306 1.18481 0.20690 0.53386 -0.37589 ID=396 1.38510 0.13776
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1.01441 -0.40206 SCALE=0.034709 1.47626 -0.05833 1.10690 -0.17268 -0.59628 -0.09000 LM=20 1.65669 0.00403
0.48857 -0.39767 LM=20 IMAGE=POIL9_1.JPG 1.24764 -0.04329 -0.72303 0.00670 1.79143 0.06692 1.58748 -0.08378
-0.43492 -0.09583 1.81473 0.07603 ID=383 1.39017 0.17994 -0.59572 0.10224 1.66341 -0.06233 1.49815 -0.09359
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-0.59566 0.09942 1.02982 -0.40559 1.77841 0.05372 1.57511 -0.06049 1.18424 0.18478 0.49501 -0.33450 ID=400
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1.12278 -0.27002 -0.59581 0.09826 -0.43830 -0.09038 SCALE=0.033040 1.52734 0.19299 -0.36450 0.12713 1.23584 -0.25316
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1.65962 0.01330 1.07944 -0.14338 -0.33243 0.11383 1.28133 -0.23978 IMAGE=PROL1_1.JP 1.09250 -0.22456 -0.59049 -0.09142
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IMAGE=POIL48_1.JP 1.35857 0.13311 1.10820 -0.08438 -0.44122 -0.09411 SCALE=0.032756 1.53351 0.20060 -0.33749 0.10850
G 1.54421 0.16549 1.13956 -0.20106 -0.57243 -0.09298 LM=20 1.65240 0.01262 0.47467 0.20135
ID=376 1.68991 0.03315 1.27328 -0.05856 -0.71613 0.00073 1.77466 0.05785 1.57913 -0.07126 1.18004 0.17741
SCALE=0.032888 1.62082 -0.07936 1.39212 0.17096 -0.57470 0.09894 1.65083 -0.04751 1.46517 -0.07382 1.07696 -0.09631
LM=20 1.53195 -0.10991 1.56242 0.18848 -0.31123 0.11815 1.26682 -0.25046 IMAGE=PROL25_1.JP 1.12707 -0.21053
1.79283 0.07290 IMAGE=POIL6_1.JPG 1.65543 0.00461 0.46788 0.19764 1.01575 -0.31817 G 1.25665 -0.08036
1.68403 -0.06558 ID=380 1.57592 -0.06601 1.16782 0.20874 0.53025 -0.31242 ID=397 1.38080 0.14330
1.30217 -0.30176 SCALE=0.034443 1.48597 -0.07007 1.07547 -0.06472 -0.44071 -0.09147 SCALE=0.035641 1.55945 0.17989
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1.22939 -0.07071 0.42251 0.16403 -0.57250 -0.08644 LM=20 1.65218 0.03171 0.45825 0.22177 1.04903 -0.34003
1.34437 0.14240 1.13597 0.18180 -0.71643 0.00056 1.78828 0.06580 1.57723 -0.03815 1.18661 0.17677 0.51711 -0.32355
1.53296 0.17174 1.03482 -0.08261 -0.56681 0.09837 1.67494 -0.06362 1.49190 -0.02783 1.08380 -0.08812 -0.43520 -0.09883
1.66686 0.02375 1.08442 -0.20223 -0.32325 0.11760 1.30665 -0.31485 IMAGE=PROL22_1.JP 1.11599 -0.20843 -0.57517 -0.09465
1.59280 -0.07547 1.23181 -0.05107 0.47108 0.19307 1.07596 -0.40018 G 1.27319 -0.08630 -0.72096 0.00317

-0.60159 0.09492
-0.34806 0.11546
0.44927 0.17629
1.16246 0.18920
1.06603 -0.09949
1.11769 -0.20813
1.22374 -0.05535
1.33804 0.16996
1.52456 0.20032
1.65317 0.01246
1.58607 -0.07483
1.48851 -0.07466
IMAGE=PROL2_1.JP
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ID=402
SCALE=0.033327
LM=20
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1.65196 -0.06373
1.24379 -0.26509
0.99470 -0.32606
0.50793 -0.30504
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-0.57676 0.09299
-0.33094 0.10800
0.43108 0.18058
1.15699 0.19616
1.08091 -0.07118
1.10902 -0.19197
1.25656 -0.05191
1.34895 0.16985
1.53871 0.19071
1.65194 0.00931
1.57067 -0.05948
1.47301 -0.06570
IMAGE=PROL30_1.JP
G
ID=403
SCALE=0.035439
LM=20
1.77358 0.06209
1.64551 -0.05097
1.25837 -0.24065
1.02251 -0.31575
0.54209 -0.30974
-0.42526 -0.09203
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-0.72613 -0.00091
-0.56851 0.10177
-0.30298 0.11185
0.47388 0.18332
1.16330 0.20351
1.08020 -0.04830
1.11216 -0.17552
1.24405 -0.04868
1.36982 0.18026
1.55331 0.19095
1.64691 0.02233
1.56305 -0.05213
1.47092 -0.06072
IMAGE=PROL31_1.JP
G
ID=404
SCALE=0.035711
LM=20
1.77645 0.06351
1.64670 -0.06083
1.21322 -0.25268
1.02179 -0.29868
0.48351 -0.30206
-0.42070 -0.08888
-0.58936 -0.08724
-0.73121 -0.00116
-0.57209 0.09684
-0.32930 0.11712
0.42778 0.20266
1.16376 0.20456
1.04112 -0.07168
1.08485 -0.18849
1.24166 -0.07000
1.36977 0.16451
1.53560 0.19602
1.64371 0.00703
1.56507 -0.06777
1.47024 -0.07518
IMAGE=PROL33_1.JP
G
ID=406
SCALE=0.035711
LM=20
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1.68372 -0.05520
1.28937 -0.29377
1.04854 -0.35218
0.55414 -0.32350
-0.41991 -0.08642
-0.59345 -0.08222
-0.74369 0.00509
-0.59557 0.10364
-0.30984 0.11190
0.47625 0.14674
1.18876 0.11831
1.10328 -0.16348
1.14941 -0.26128
1.25533 -0.12408
1.35791 0.11336
1.54295 0.15883
1.66123 0.01449
1.59721 -0.07667
1.50888 -0.09549
IMAGE=PROL34_1.JP
G
ID=407
SCALE=0.034962
LM=20
1.78272 0.06517
1.65195 -0.06650
1.23326 -0.26015
0.96776 -0.30734
0.48418 -0.28628
-0.45135 -0.09711
-0.57847 -0.09172
-0.71310 0.00165
-0.57024 0.10010
-0.36811 0.12526
0.43025 0.22967
1.15521 0.19346
1.04791 -0.07424
1.09480 -0.21380
1.24877 -0.08715
1.39014 0.15490
1.57219 0.18575
1.65422 -0.00322
1.57851 -0.07444
1.48905 -0.09052
IMAGE=PROL35_1.JP
G
ID=408
SCALE=0.035706
LM=20
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1.66887 -0.05799
1.29542 -0.26064
0.99609 -0.33603
0.51531 -0.31450
-0.43080 -0.08908
-0.57882 -0.08458
-0.72892 0.00147
-0.58158 0.09661
-0.33269 0.10986
0.42089 0.19592
1.19068 0.17223
1.08488 -0.10755
1.14150 -0.21594
1.29525 -0.06172
1.40105 0.16694
1.56665 0.19322
1.66634 0.00638
1.59200 -0.06586
1.50175 -0.07769
IMAGE=PROL36_1.JP
G
ID=409
SCALE=0.035457
LM=20
1.77383 0.06029
1.66003 -0.05213
1.25675 -0.25161
1.05020 -0.31220
0.48126 -0.31645
-0.43614 -0.09710
-0.59151 -0.09406
-0.71776 0.00101
-0.57784 0.11011
-0.33373 0.12135
0.46232 0.21382
1.19741 0.21441
1.08793 -0.08001
1.14218 -0.18823
1.27638 -0.05226
1.40586 0.18203
1.57335 0.18588
1.66114 0.01270
1.59246 -0.05875
1.50437 -0.06200
IMAGE=PROL37_1.JP
G
ID=410
SCALE=0.035457
LM=20
1.78386 0.06996
1.67556 -0.06451
1.27461 -0.28776
1.02197 -0.36461
0.50728 -0.35567
-0.44808 -0.09468
-0.59794 -0.08743
-0.73347 0.00480
-0.57351 0.09945
-0.33681 0.10826
0.46051 0.15208
1.19910 0.12574
1.09267 -0.15081
1.14388 -0.25923
1.27533 -0.11095
1.38728 0.12094
1.55041 0.16397
1.67183 0.01108
1.59813 -0.07803
1.52226 -0.09049
IMAGE=PROL38_1.JP
G
ID=411
SCALE=0.035197
LM=20
1.78695 0.07068
1.66399 -0.05536
1.26520 -0.25746
1.01756 -0.31746
0.54568 -0.31830
-0.42519 -0.09342
-0.60431 -0.08748
-0.73535 0.00393
-0.59239 0.10438
-0.35047 0.11988
0.45042 0.20786
1.18305 0.20275
1.08427 -0.07400
1.12358 -0.18869
1.25304 -0.04625
1.36784 0.17621
1.54930 0.18888
1.65349 0.01624
1.58092 -0.05352
1.49142 -0.05788
IMAGE=PROL39_1.JP
G
ID=412
SCALE=0.035203
LM=20
1.79358 0.06075
1.69097 -0.08277
1.36092 -0.31323
1.03237 -0.42847
0.50651 -0.42089
-0.45353 -0.10153
-0.59095 -0.09153
-0.72808 0.00732
-0.58345 0.09673
-0.32086 0.08702
0.44869 0.08236
1.17374 0.11940
1.10098 -0.16411
1.16317 -0.27329
1.27135 -0.10639
1.35754 0.13322
1.53802 0.17112
1.67806 -0.01916
1.61788 -0.08949
1.52119 -0.10822
IMAGE=PROL3_1.JP
G
ID=413
SCALE=0.033106
LM=20
1.78017 0.06845
1.65083 -0.04999
1.26458 -0.24785
1.02184 -0.32211
0.52715 -0.31904
-0.42071 -0.09003
-0.58035 -0.08513
-0.72410 -0.00082
-0.57739 0.10185
-0.34115 0.11991
0.44694 0.19398
1.18680 0.18756
1.08423 -0.06927
1.11461 -0.18211
1.24015 -0.04476
1.36573 0.18739
1.55434 0.19770
1.64703 0.02364
1.57486 -0.04941
1.46944 -0.06255
IMAGE=PROL40_1.JP
G
ID=414
SCALE=0.035197
LM=20
1.77769 0.06034
1.66767 -0.06474
1.26254 -0.30334
1.00285 -0.38248
0.51950 -0.36683
-0.43973 -0.09390
-0.58139 -0.08248
-0.72456 -0.00314
-0.57786 0.09449
-0.30834 0.10926
0.47611 0.14319
1.20435 0.16442
1.08727 -0.13550
1.14671 -0.25083
1.27727 -0.08588
1.36332 0.13506
1.52915 0.17998
1.65600 0.00734
1.59340 -0.07343
1.50046 -0.09111
IMAGE=PROL41_1.JP
G
ID=415
SCALE=0.034944
LM=20
1.77763 0.06129
1.65962 -0.04783
1.23478 -0.24456
0.99657 -0.30731
0.53202 -0.29792
-0.43356 -0.09367
-0.57021 -0.08097
-0.72124 -0.00187
-0.57948 0.09729
-0.33210 0.10892
0.45723 0.18782
1.17366 0.20125
1.06228 -0.06156
1.11705 -0.17433
1.25318 -0.04174
1.36818 0.18370
1.54156 0.19554
1.64897 0.02260
1.57682 -0.04819
1.47779 -0.05210
IMAGE=PROL42_1.JP
G
ID=416
SCALE=0.036839
LM=20
1.78202 0.07696
1.64786 -0.04960
1.24191 -0.21883
1.02026 -0.26955
0.56415 -0.26362
-0.43812 -0.09006
-0.57708 -0.08730
-0.71827 0.00157
-0.56849 0.09587
-0.34382 0.12509
0.45278 0.22078
1.15124 0.24535
1.05467 -0.03520
1.11111 -0.13264
1.26603 -0.00152
1.42524 0.22896
1.59238 0.22478
1.65548 0.02206
1.57127 -0.04549
1.47292 -0.04139
IMAGE=PROL43_1.JP
G
ID=417
SCALE=0.035189
LM=20
1.79221 0.07331
1.65625 -0.04802
1.23540 -0.24818
1.01364 -0.30326
0.52882 -0.29779
-0.44150 -0.08825
-0.58680 -0.08499
-0.71004 0.00167
-0.57104 0.09364
-0.32607 0.11116
0.46139 0.19792
1.16229 0.19795
1.06527 -0.06535
1.10296 -0.18263
1.22249 -0.05852
1.39568 0.20532
1.54942 0.21673
1.65397 0.02288
1.57563 -0.04591
1.48585 -0.04320
IMAGE=PROL45_1.JP
G
ID=418
SCALE=0.034709
LM=20
1.78274 0.07032
1.67273 -0.06935
1.28814 -0.28312
1.05245 -0.35501
0.49488 -0.35435
-0.44375 -0.09205
-0.58889 -0.08824
-0.72330 0.00348
-0.57767 0.09871
-0.32756 0.11603
0.44774 0.16330
1.18631 0.15506
1.07529 -0.12430
1.11869 -0.23469
1.26802 -0.09588
1.37230 0.14816
1.53851 0.17691
1.66958 0.01051
1.59933 -0.06800
1.51395 -0.06611
IMAGE=PROL46_1.JP
G
ID=419
SCALE=0.035197
LM=20
1.77922 0.06482
1.66219 -0.05909
1.27035 -0.26128
1.01235 -0.33123
0.53217 -0.32788
-0.42220 -0.09164
-0.58363 -0.08532
-0.72878 0.00208
-0.57524 0.09617
-0.30978 0.12154
0.46838 0.19601
1.16229 0.19795
1.06527 -0.06535
1.10296 -0.18263
1.22249 -0.05852
1.39568 0.20532
1.54942 0.21673
1.65397 0.02288
1.57563 -0.04591
1.48585 -0.04320
IMAGE=PROL47_1.JP
G
ID=420
SCALE=0.034701
LM=20
1.78274 0.07032
1.67273 -0.06935
1.28814 -0.28312
1.05245 -0.35501
0.49488 -0.35435
-0.44375 -0.09205
-0.58889 -0.08824
-0.72330 0.00348
-0.57767 0.09871
-0.32756 0.11603
0.44774 0.16330
1.18631 0.15506
1.07529 -0.12430
1.11869 -0.23469
1.26802 -0.09588
1.37230 0.14816
1.53851 0.17691
1.66958 0.01051
1.59933 -0.06800
1.51395 -0.06611
IMAGE=PROL48_1.JP
G
ID=421
SCALE=0.034944
LM=20
1.78078 0.07261
1.64970 -0.05292
1.27448 -0.23757
1.00153 -0.30970
0.50418 -0.30604
-0.42186 -0.09499
-0.58054 -0.09118
-0.73172 0.00066
-0.57396 0.09461
-0.35043 0.11772
0.42885 0.22867
1.15234 0.20820
1.06325 -0.06695
1.10532 -0.19026
1.23660 -0.05738
1.36378 0.17729
1.55276 0.19770
1.64815 0.02774
1.56571 -0.05666
1.46640 -0.05655
IMAGE=PROL49_1.JP
G
ID=422
SCALE=0.035457
LM=20
1.80076 0.07144
1.69557 -0.07062
1.32759 -0.32914
1.01854 -0.42227
0.43774 -0.39257
-0.42384 -0.10315
-0.60026 -0.07635
1.78397 0.06295
-0.62411 0.09849
-0.37885 0.11285
0.38675 0.11654
1.15132 0.13652
1.09538 -0.16254
1.16109 -0.26492
1.26313 -0.08683
1.35924 0.14161
1.53937 0.17812
1.68258 0.02335
1.62567 -0.07837
1.53155 -0.09700
IMAGE=PROL5_1.JP
G
ID=424
SCALE=0.032877
LM=20
1.78802 0.06027
1.64955 -0.05473
1.22779 -0.24264
1.00146 -0.30244
0.52264 -0.29144
-0.43805 -0.09223
-0.58412 -0.08703
1.76642 0.06539
-0.56506 0.10187
-0.34242 0.12219
0.43561 0.22045
1.16788 0.20655
1.05687 -0.06814
1.10254 -0.19088
1.24268 -0.05684
1.37930 0.18843
1.56282 0.20775
1.64991 0.01632
1.56979 -0.05069
1.47307 -0.05155
IMAGE=PROL6_1.JP
G
ID=425
SCALE=0.032860
LM=20
1.78078 0.05737
1.64802 -0.04708
1.25438 -0.20477
IMAGE=RL10_1.JPG
ID=429
SCALE=0.033919
LM=20
1.79156 0.08006
1.66790 -0.05477
1.22316 -0.27767
1.02690 -0.32916
0.53080 -0.31363
-0.42172 -0.08689
-0.57870 -0.08043
-0.72039 -0.00188
-0.59441 0.09044
-0.30568 0.09592
1.52735 0.23374
1.64186 0.03892
1.55972 -0.02347
1.47729 -0.00592
IMAGE=PROL7_1.JP
G
ID=426
SCALE=0.032673
LM=20
1.78840 0.07131
1.67759 -0.05908
1.27103 -0.28855
1.01371 -0.36761
0.50947 -0.36366
-0.44536 -0.10079
-0.58514 -0.08505
-0.72477 -0.00399
-0.58986 0.09567
-0.32148 0.10875
0.39956 0.14660
1.19152 0.16732
1.09170 -0.12600
1.14522 -0.24325
1.25286 -0.08412
1.37737 0.16711
1.55159 0.19104
1.65923 -0.01018
1.59348 -0.07031
1.50310 -0.07628
IMAGE=PROL8_1.JP
G
ID=427
SCALE=0.032677
LM=20
1.78397 0.06295
1.66256 -0.05368
1.26457 -0.24430
1.01283 -0.32000
0.51404 -0.31453
-0.45482 -0.09526
-0.56028 -0.08854
-0.71083 0.00050
-0.56808 0.10106
-0.35243 0.12135
0.43518 0.21371
1.16884 0.20519
1.05082 -0.07368
1.11017 -0.17436
1.22588 -0.02248
1.36156 0.19835
1.55066 0.20655
1.64927 0.02297
1.57419 -0.05421
1.47625 -0.05496
IMAGE=PROL9_1.JP
G
ID=428
SCALE=0.032892
LM=20
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1.64957 -0.06537
1.25332 -0.28606
1.00935 -0.34560
0.51261 -0.31172
-0.41564 -0.09419
-0.58983 -0.07757
-0.71995 -0.00210
-0.57899 0.08290
-0.31690 0.09594
0.47200 0.14700
1.15971 0.14359
1.05990 -0.10068
1.10510 -0.20984
1.22933 -0.10083
1.35267 0.11067
1.53926 0.14757
1.64258 0.00854
1.55116 -0.07215
1.45137 -0.07514

0.46834 0.13892 -0.73030 -0.00105 0.54815 -0.28940 1.66229 -0.04923 SCALE=0.034007 1.41855 -0.02673 1.52786 0.15702
1.21163 0.14008 -0.58560 0.08217 -0.44285 -0.08952 1.24943 -0.23787 LM=20 IMAGE=RL38_l.JPG 1.64156 0.00750
1.10829 -0.13336 -0.31220 0.11005 -0.57873 -0.07685 1.01325 -0.29368 1.78052 0.05988 ID=451 1.55882 -0.07965
1.12920 -0.23608 0.42901 0.20801 -0.71881 -0.00029 0.48403 -0.28450 1.66262 -0.05400 SCALE=0.034150 1.45858 -0.06859
1.24230 -0.12142 1.16272 0.20704 -0.56436 0.08955 -0.44764 -0.08378 1.24207 -0.28401 LM=20 IMAGE=RL41_l.JPG
1.37092 0.13011 1.05003 -0.01630 -0.33655 0.09713 -0.58496 -0.08032 1.02310 -0.35367 1.78088 0.06000 ID=455
1.55661 0.16590 1.09432 -0.13372 0.42689 0.18339 -0.71669 -0.00111 0.54751 -0.34525 1.65911 -0.05368 SCALE=0.033783
1.65608 0.00773 1.21231 -0.01314 1.15378 0.17490 -0.55861 0.08766 -0.47531 -0.09629 1.22300 -0.25348 LM=20
1.57780 -0.07577 1.35943 0.20947 1.05521 -0.10005 -0.32112 0.11020 -0.60815 -0.07844 1.02630 -0.29863 1.77602 0.08611
1.48726 -0.08128 1.56222 0.20739 1.08826 -0.19015 0.46803 0.18983 -0.70839 -0.00143 0.54786 -0.26933 1.65397 -0.05891
IMAGE=RL11_l.JPG 1.64830 0.03510 1.21116 -0.05787 1.17647 0.16817 -0.55839 0.09339 -0.45057 -0.07858 1.23898 -0.32087
ID=430 1.56059 -0.03426 1.36052 0.15545 1.06724 -0.10197 -0.30939 0.10907 -0.58542 -0.08027 0.98724 -0.37799
SCALE=0.034243 1.45814 -0.02090 1.54870 0.17231 1.11638 -0.19288 0.48652 0.13783 -0.71605 -0.00160 0.48447 -0.34519
LM=20 IMAGE=RL18_l.JPG 1.65209 0.01628 1.21850 -0.05936 1.11599 0.13784 -0.53902 0.08748 -0.43013 -0.09000
1.78506 0.06120 ID=435 1.56874 -0.06286 1.36397 0.15719 1.02915 -0.09886 -0.32708 0.09696 -0.58038 -0.07604
1.65992 -0.05489 SCALE=0.033496 1.45572 -0.05547 1.55831 0.18119 1.06789 -0.21265 0.45166 0.17860 -0.73789 -0.00253
1.21960 -0.26016 LM=20 IMAGE=RL24_l.JPG 1.65501 0.02709 1.19377 -0.05561 1.18450 0.16980 -0.56364 0.09855
0.95971 -0.31766 1.78253 0.05693 ID=440 1.56493 -0.05374 1.34032 0.14118 1.08531 -0.08797 -0.31034 0.09981
0.53946 -0.30090 1.65641 -0.05349 SCALE=0.033781 1.47062 -0.04830 1.53631 0.15429 1.10897 -0.18263 0.43917 0.12281
-0.44845 -0.08582 1.26500 -0.26710 LM=20 IMAGE=RL2_l.JPG 1.64826 0.00781 1.23015 -0.02574 1.11564 0.12550
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-0.56504 0.09068 -0.45643 -0.08814 1.28373 -0.25249 LM=20 IMAGE=RL34_l.JPG 1.64759 0.03441 1.14368 -0.06351
-0.34609 0.10475 -0.59524 -0.07692 1.04108 -0.32702 1.84414 0.08606 ID=448 1.56097 -0.05355 1.27276 0.16565
0.46089 0.18672 -0.70870 -0.00083 0.57513 -0.32788 1.75484 -0.08863 SCALE=0.033994 1.46138 -0.02755 1.48516 0.18520
1.20172 0.17052 -0.54612 0.08689 -0.45961 -0.08374 1.40294 -0.37210 LM=20 IMAGE=RL39_l.JPG 1.61996 0.03515
1.09449 -0.08381 -0.30628 0.10906 -0.56959 -0.06895 1.08963 -0.46570 1.77659 0.05971 ID=452 1.53047 -0.05637
1.12556 -0.20673 0.47483 0.15783 -0.72050 0.00109 0.49857 -0.40868 1.66528 -0.04538 SCALE=0.033950 1.42143 -0.04576
1.24253 -0.05408 1.14982 0.13571 -0.56367 0.08525 -0.47397 -0.08623 1.27891 -0.24251 LM=20 IMAGE=RL42_l.JPG
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1.56874 0.17384 1.07334 -0.18625 0.50378 0.13855 -0.75756 0.02129 0.54454 -0.28954 1.66727 -0.05561 SCALE=0.033756
1.64958 0.01145 1.19593 -0.04725 1.18025 0.16978 -0.62405 0.09983 -0.42823 -0.09133 1.22925 -0.28277 LM=20
1.55321 -0.06498 1.33712 0.15594 1.09385 -0.07477 -0.33323 0.09430 -0.58875 -0.08677 1.01123 -0.32485 1.78240 0.06193
1.47636 -0.04753 1.54262 0.17347 1.12044 -0.17390 0.45127 0.08883 -0.70797 0.00064 0.51050 -0.30195 1.65480 -0.04740
IMAGE=RL13_l.JPG 1.65037 0.00847 1.22456 -0.02365 1.20971 0.07437 -0.59649 0.09527 -0.44755 -0.08600 1.22070 -0.23904
ID=431 1.55491 -0.06503 1.35476 0.17713 1.15212 -0.24218 -0.28320 0.13060 -0.57262 -0.07735 0.98084 -0.27953
SCALE=0.034182 1.44247 -0.06263 1.55914 0.18448 1.20939 -0.33785 0.42073 0.20432 -0.70550 0.00126 0.50032 -0.27809
LM=20 IMAGE=RL19_l.JPG 1.65357 0.02877 1.30933 -0.16585 1.22961 0.20544 -0.58351 0.09272 -0.45823 -0.08342
1.77519 0.06145 ID=436 1.57149 -0.04300 1.38119 0.09281 1.12165 -0.04661 -0.34772 0.09647 -0.59213 -0.07973
1.65887 -0.05666 SCALE=0.033330 1.46207 -0.02994 1.58447 0.14424 1.17470 -0.16119 0.44134 0.15716 -0.71890 0.00074
1.27615 -0.24873 LM=20 IMAGE=RL25_l.JPG 1.72898 0.05044 1.25847 -0.03289 1.18848 0.14163 -0.56712 0.08928
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-0.42848 -0.08321 1.23686 -0.25122 LM=20 IMAGE=RL31_l.JPG 1.66020 0.02660 1.26084 -0.11347 1.18155 0.21422
-0.59086 -0.07675 1.01100 -0.30510 1.77402 0.07168 ID=445 1.58139 -0.04345 1.36917 0.13013 1.07754 -0.03813
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1.11427 -0.18675 0.42461 0.18888 -0.72789 -0.00256 0.50280 -0.32369 1.63321 -0.07078 SCALE=0.034043 1.44845 -0.02701
1.25477 -0.03248 1.18390 0.20386 -0.57481 0.08312 -0.44654 -0.08667 1.23101 -0.25883 LM=20 IMAGE=RL43_l.JPG
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1.55812 -0.05978 1.33912 0.18717 1.07739 -0.13525 -0.29876 0.11138 -0.58801 -0.08302 1.01197 -0.35250 1.79308 0.07063
1.46112 -0.04817 1.54452 0.19675 1.10197 -0.23692 0.43677 0.17441 -0.73161 -0.00091 0.50118 -0.33687 1.68218 -0.06020
IMAGE=RL14_l.JPG 1.64332 0.02524 1.17807 -0.12924 1.18482 0.17607 -0.55385 0.08773 -0.45248 -0.08053 1.27511 -0.26762
ID=432 1.56078 -0.05120 1.33483 0.10980 1.08995 -0.07196 -0.33973 0.09281 -0.57452 -0.07729 0.99647 -0.32894
SCALE=0.034007 1.45136 -0.03544 1.52930 0.14771 1.10655 -0.20889 0.45654 0.17770 -0.72416 -0.00137 0.50503 -0.30464
LM=20 IMAGE=RL1_l.JPG 1.64336 0.01026 1.21031 -0.06332 1.16337 0.20788 -0.56761 0.09615 -0.47140 -0.08293
1.77417 0.06608 ID=437 1.56136 -0.08429 1.33572 0.16610 1.06048 -0.00685 -0.32507 0.10031 -0.58278 -0.08348
1.65545 -0.05289 SCALE=0.033530 1.44467 -0.09820 1.54249 0.19123 1.10139 -0.13772 0.44384 0.14641 -0.69888 -0.00317
1.25597 -0.25169 LM=20 IMAGE=RL26_l.JPG 1.64892 0.01039 1.21009 0.00143 1.17023 0.14925 -0.58496 0.08676
1.00835 -0.31696 1.78451 0.06769 ID=442 1.56233 -0.07321 1.34973 0.21601 1.06219 -0.10831 -0.32544 0.10531
0.52093 -0.30968 1.65193 -0.05720 SCALE=0.033297 1.46664 -0.04410 1.52512 0.22024 1.10133 -0.23965 0.42875 0.15946
-0.43649 -0.08471 1.24555 -0.24034 LM=20 IMAGE=RL32_l.JPG 1.63680 0.05319 1.21222 -0.09081 1.14943 0.15104
-0.57586 -0.07283 1.00494 -0.29973 1.77156 0.06347 ID=446 1.54962 -0.03576 1.32347 0.15893 1.04907 -0.11131
-0.72353 -0.00249 0.52214 -0.28375 1.65888 -0.05922 SCALE=0.033975 1.44343 -0.01662 1.52437 0.16705 1.10191 -0.23568
-0.56418 0.09371 -0.45999 -0.09087 1.28104 -0.24091 LM=20 IMAGE=RL37_l.JPG 1.64813 0.01245 1.21672 -0.09747
-0.31579 0.10335 -0.57830 -0.08291 1.05057 -0.31703 1.77613 0.07106 ID=450 1.56683 -0.06685 1.33076 0.15726
0.44675 0.16274 -0.70160 -0.00043 0.58161 -0.32534 1.65764 -0.05421 SCALE=0.034715 1.46043 -0.05615 1.52746 0.19021
1.17678 0.17237 -0.56640 0.08868 -0.44461 -0.07974 1.26813 -0.27088 LM=20 IMAGE=RL40_l.JPG 1.67036 0.01187
1.06110 -0.05703 -0.34261 0.10037 -0.58791 -0.07031 1.00647 -0.34070 1.79156 0.08269 ID=454 1.58404 -0.06627
1.09473 -0.17916 0.43340 0.18550 -0.70095 0.00052 0.53056 -0.31215 1.65111 -0.05515 SCALE=0.033756 1.48065 -0.05961
1.20761 -0.04460 1.15560 0.22776 -0.58673 0.09380 -0.42631 -0.08769 1.24268 -0.27614 LM=20 IMAGE=RL45_l.JPG
1.35335 0.17667 1.06282 -0.01421 -0.34758 0.10009 -0.59412 -0.07805 1.00489 -0.34049 1.76906 0.07243 ID=458
1.53532 0.18254 1.10507 -0.12105 0.50212 0.14780 -0.73541 0.00095 0.54680 -0.34109 1.66366 -0.06427 SCALE=0.034010
1.65008 0.02555 1.21694 0.04083 1.15069 0.15488 -0.58641 0.09706 -0.40835 -0.08959 1.27646 -0.30552 LM=20
1.55680 -0.05606 1.33671 0.21755 1.06525 -0.07958 -0.32138 0.11080 -0.58610 -0.07504 1.01549 -0.36269 1.78236 0.06558
1.45254 -0.04882 1.55176 0.22442 1.11086 -0.17195 0.47363 0.17025 -0.73628 -0.00364 0.50648 -0.33539 1.65752 -0.05794
IMAGE=RL16_l.JPG 1.65886 0.05054 1.19783 -0.04804 1.17167 0.13757 -0.57879 0.09685 -0.45574 -0.09167 1.22306 -0.27896
ID=434 1.57018 -0.03153 1.33278 0.16270 1.07295 -0.10587 -0.30999 0.10070 -0.58716 -0.08785 0.99398 -0.33906
SCALE=0.033315 1.46100 -0.01095 1.54092 0.17652 1.11303 -0.22464 0.48505 0.14862 -0.71439 -0.00174 0.48363 -0.31071
LM=20 IMAGE=RL21_l.JPG 1.65011 0.03434 1.22269 -0.10102 1.15136 0.15293 -0.56170 0.09587 -0.46525 -0.08530
1.79054 0.07179 ID=438 1.56100 -0.04834 1.34916 0.12897 1.04740 -0.07597 -0.29574 0.10929 -0.56685 -0.08210
1.65508 -0.04586 SCALE=0.033746 1.44410 -0.04783 1.54928 0.16641 1.05015 -0.20264 0.51227 0.12870 -0.70230 -0.00096
1.22353 -0.20783 LM=20 IMAGE=RL27_l.JPG 1.64008 0.01506 1.15550 -0.04065 1.16080 0.12422 -0.58280 0.09691
1.02757 -0.24966 1.78239 0.06494 ID=443 1.55812 -0.06920 1.28202 0.18675 1.07339 -0.11676 -0.32856 0.10478
0.50792 -0.24517 1.66608 -0.04614 SCALE=0.033721 1.46128 -0.06180 1.51405 0.21676 1.11396 -0.21561 0.41517 0.17449
-0.41072 -0.08324 1.25189 -0.23443 LM=20 IMAGE=RL33_l.JPG 1.63811 0.04059 1.22911 -0.08887 1.16617 0.18244
-0.57432 -0.07397 1.01884 -0.28885 1.78088 0.06020 ID=447 1.53162 -0.05585 1.33347 0.13771 1.05119 -0.09507

1.08760 -0.22484
1.23102 -0.04557
1.33436 0.18328
1.53627 0.20469
1.64649 0.01512
1.55158 -0.06201
1.44744 -0.04148
IMAGE=RL46_l.JPG
ID=459
SCALE=0.034007
LM=20
1.77431 0.08446
1.66493 -0.04825
1.29148 -0.27422
1.03749 -0.35644
0.50389 -0.35203
-0.42971 -0.09836
-0.57784 -0.08916
-0.72357 -0.00246
-0.56638 0.09418
-0.30967 0.10703
0.48015 0.14633
1.18676 0.15395
1.10805 -0.11888
1.13347 -0.23133
1.23557 -0.10464
1.34860 0.12800
1.55186 0.17540
1.65411 0.01318
1.57697 -0.06798
1.47878 -0.06639
IMAGE=RL47_l.JPG
ID=460
SCALE=0.034001
LM=20
1.78938 0.08287
1.66348 -0.05500
1.23480 -0.27646
1.01300 -0.33858
0.57935 -0.33942
-0.46463 -0.09842
-0.57077 -0.08474
-0.70855 -0.00272
-0.56297 0.08379
-0.31642 0.09328
0.47829 0.14502
1.18193 0.12865
1.06532 -0.14423
1.11623 -0.22480
1.21020 -0.08015
1.34008 0.12438
1.55703 0.15497
1.65145 0.00430
1.55838 -0.07445
1.44460 -0.07000
IMAGE=RL4_l.JPG
ID=461
SCALE=0.033950
LM=20
1.77123 0.05462
1.65784 -0.07537
1.27581 -0.30975
1.02412 -0.37792
0.48572 -0.34951
-0.43708 -0.08137
-0.58102 -0.07477
-0.71684 -0.00084
-0.58071 0.08625
-0.32000 0.09319
0.46086 0.11332
1.18800 0.11827
1.11184 -0.15025
1.13725 -0.24981
1.24812 -0.09027
1.35413 0.12661
1.54529 0.15211
1.64725 0.01087
1.57218 -0.07833
1.47765 -0.07766
IMAGE=RL5_l.JPG
ID=462
SCALE=0.033814
LM=20
1.79223 0.06037
1.66992 -0.05475
1.26799 -0.23140
1.02507 -0.29512
0.56219 -0.29552
-0.44629 -0.09008
-0.59486 -0.08166
-0.72033 -0.00454
-0.57480 0.08919
-0.33577 0.10759
0.44048 0.19004
1.19594 0.19819
1.08465 -0.03571
1.12329 -0.14241
1.25369 -0.02694
1.38247 0.17298
1.57671 0.17262
1.66641 0.02248
1.56480 -0.05643
1.47406 -0.04304
IMAGE=RL6_l.JPG
ID=463
SCALE=0.033781
LM=20
1.78278 0.05226
1.65213 -0.05523
1.25106 -0.22664
1.00378 -0.29969
0.52823 -0.28576
-0.45837 -0.08873
-0.58347 -0.08078
-0.70036 -0.00172
-0.56249 0.09308
-0.31753 0.11631
0.46758 0.20092
1.18138 0.19158
1.07892 -0.04615
1.10249 -0.16035
1.23091 -0.00798
1.36201 0.17791
1.56996 0.17092
1.64608 0.01649
1.56104 -0.05478
1.46298 -0.02707
IMAGE=RL7_l.JPG
ID=464
SCALE=0.033901
LM=20
1.77881 0.06717
1.65608 -0.05782
1.24477 -0.26703
1.00621 -0.32925
0.53669 -0.33063
-0.44432 -0.09574
-0.57731 -0.08159
-0.71461 -0.00148
-0.57487 0.09114
-0.32401 0.11205
0.49663 0.15885
1.11112 0.14313
1.03540 -0.09450
1.07032 -0.18673
1.16256 -0.04062
1.30658 0.15463
1.50923 0.17062
1.63483 0.02080
1.53555 -0.04844
1.42275 -0.03606
IMAGE=RL9_l.JPG
ID=465
SCALE=0.034207
LM=20
1.78438 0.06588
1.65910 -0.06493
1.19443 -0.28475
1.00771 -0.34359
0.48825 -0.32515
-0.44078 -0.08446
-0.59944 -0.08151
-0.72970 0.00341
-0.57437 0.09406
-0.30840 0.11581
0.43089 0.16011
1.16096 0.17424
1.04723 -0.08960
1.06846 -0.21717
1.19467 -0.05751
1.33411 0.15643
1.52884 0.16694
1.65454 0.01783
1.56182 -0.07996
1.45617 -0.08777
IMAGE=SL10_l.JPG
ID=466
SCALE=0.036307
LM=20
1.75777 0.07585
1.65525 -0.05928
1.22593 -0.32207
0.98557 -0.38106
0.49176 -0.35654
-0.43609 -0.09032
-0.55896 -0.06880
-0.72853 -0.00113
-0.57260 0.08245
-0.33659 0.09256
0.42720 0.12761
1.15108 0.12561
1.06540 -0.10895
1.08818 -0.22917
1.22819 -0.06325
1.34638 0.13168
1.53510 0.16112
1.65037 0.00831
1.56540 -0.07678
1.43990 -0.09671
IMAGE=SL11_l.JPG
ID=467
SCALE=0.036231
LM=20
1.76912 0.08185
1.66622 -0.05531
1.22932 -0.30064
0.99813 -0.35498
0.49917 -0.33855
-0.41849 -0.09008
-0.57316 -0.07359
-0.74291 -0.00229
-0.58065 0.08225
-0.30536 0.10492
0.44777 0.15357
1.14076 0.16252
1.07361 -0.08220
1.10354 -0.21230
1.23213 -0.05489
1.36289 0.15578
1.55262 0.18375
1.65888 0.02406
1.56898 -0.06707
1.45498 -0.06291
IMAGE=SL12_l.JPG
ID=468
SCALE=0.036488
LM=20
1.77297 0.06921
1.66433 -0.05903
1.24275 -0.26824
1.02648 -0.32247
0.52288 -0.30866
-0.44102 -0.08214
-0.56885 -0.07699
-0.72014 -0.00279
-0.57190 0.08634
-0.30497 0.09740
0.44262 0.16448
1.19048 0.15768
1.11577 -0.07951
1.15267 -0.20401
1.27194 -0.05842
1.38623 0.14504
1.56018 0.17061
1.65702 0.01709
1.58142 -0.06872
1.47393 -0.07023
IMAGE=SL13_l.JPG
ID=469
SCALE=0.036137
LM=20
1.75763 0.07572
1.63847 -0.05481
1.22714 -0.25355
0.98882 -0.32196
0.52072 -0.31146
-0.41466 -0.08285
-0.57734 -0.08020
-0.71435 -0.00112
-0.59728 0.08538
-0.31520 0.10645
0.44022 0.16697
1.12899 0.18641
1.04297 -0.05848
1.08247 -0.16306
1.18918 -0.00799
1.33544 0.17308
1.52289 0.18966
1.62535 0.02831
1.53355 -0.04933
1.42860 -0.03832
IMAGE=SL15_l.JPG
ID=470
SCALE=0.036449
LM=20
1.77548 0.08309
1.66381 -0.05346
1.25228 -0.28256
0.98635 -0.34886
0.47807 -0.30742
-0.44319 -0.08304
-0.58126 -0.07531
-0.71745 0.00099
-0.56449 0.08601
-0.29836 0.10371
0.41074 0.16225
1.16486 0.16114
1.08035 -0.07999
1.10679 -0.20636
1.23019 -0.03981
1.34503 0.15264
1.52140 0.18644
1.64106 0.01991
1.57032 -0.06631
1.46468 -0.06326
IMAGE=SL16_l.JPG
ID=471
SCALE=0.036250
LM=20
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1.65124 -0.06012
1.22500 -0.28087
0.99985 -0.33628
0.49519 -0.30693
-0.42474 -0.08258
-0.58137 -0.07888
-0.72570 0.00213
-0.57786 0.08420
-0.28230 0.10424
0.37884 0.14360
1.17135 0.15797
1.07608 -0.09045
1.09222 -0.20955
1.21253 -0.07713
1.33410 0.15334
1.52350 0.18895
1.64251 0.01881
1.56796 -0.07329
1.46827 -0.05636
IMAGE=SL17_l.JPG
ID=472
SCALE=0.036217
LM=20
1.77190 0.07822
1.65405 -0.06554
1.25479 -0.28674
1.00136 -0.35871
0.50500 -0.33982
-0.41505 -0.08326
-0.57198 -0.07470
-0.72154 -0.00059
-0.56496 0.08361
-0.31506 0.09949
0.39888 0.13378
1.16945 0.15554
1.07931 -0.08021
1.09694 -0.22575
1.23836 -0.06109
1.33681 0.15692
1.53873 0.18836
1.64416 0.02227
1.56253 -0.07450
1.46902 -0.05399
IMAGE=SI18_l.JPG
ID=473
SCALE=0.036756
LM=20
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1.66370 -0.05730
1.24731 -0.30515
0.96103 -0.37822
0.45650 -0.35493
-0.44207 -0.08148
-0.59263 -0.07228
-0.71261 -0.00184
-0.57358 0.09885
-0.31577 0.11165
0.43853 0.12998
1.14806 0.12663
1.05641 -0.13750
1.10287 -0.25396
1.21494 -0.08492
1.34144 0.14268
1.52237 0.17144
1.65071 0.01177
1.57420 -0.07183
1.47673 -0.07942
IMAGE=SL19_l.JPG
ID=474
SCALE=0.035938
LM=20
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1.66034 -0.07149
1.21090 -0.29452
1.00686 -0.34638
0.50010 -0.34137
-0.42761 -0.08972
-0.58314 -0.07901
-0.72535 -0.00185
-0.57614 0.09096
-0.32180 0.09983
0.39193 0.12892
1.16060 0.12796
1.06363 -0.13174
1.09894 -0.24097
1.24404 -0.08126
1.33974 0.14485
1.53885 0.17919
1.65445 0.01340
1.57346 -0.08050
1.46979 -0.06692
IMAGE=SL1_l.JPG
ID=475
SCALE=0.036481
LM=20
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1.65401 -0.06258
1.19965 -0.27052
1.00843 -0.31758
0.51703 -0.30777
-0.41560 -0.09047
-0.60288 -0.08363
-0.74433 0.00224
-0.56683 0.08989
-0.28274 0.10435
0.41857 0.16787
1.15887 0.15772
1.06536 -0.10690
1.09109 -0.21719
1.21467 -0.07692
1.34333 0.14309
1.54415 0.16921
1.63252 0.01758
1.55370 -0.07412
1.45035 -0.07354
IMAGE=Sl20_l.JPG
ID=476
SCALE=0.035968
LM=20
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1.64971 -0.05773
1.19160 -0.25647
1.77849 0.08023
1.65496 -0.05761
1.24989 -0.26756
1.03623 -0.31845
0.56622 -0.30152
-0.41577 -0.08689
-0.58955 -0.08350
-0.71817 0.00344
-0.58348 0.09156
-0.24234 0.11739
0.46820 0.16953
1.16742 0.15716
1.06611 -0.08132
1.09849 -0.18471
1.21311 -0.05487
1.34964 0.14337
1.54564 0.17150
1.63942 0.02344
1.55105 -0.06575
1.44572 -0.05561
IMAGE=SL29_l.JPG
ID=482
SCALE=0.036208
LM=20
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1.64520 -0.08018
1.21119 -0.28948
0.99316 -0.33955
0.51661 -0.33035
-0.42674 -0.09045
-0.58248 -0.08250
-0.72583 0.00056
-0.57348 0.09382
-0.41773 0.15367
1.16218 0.15145
1.04024 -0.08116
1.06875 -0.22087
1.20094 -0.09398
1.33725 0.13321
1.52981 0.16044
1.62943 0.00478
1.55299 -0.08393
1.43842 -0.09271
IMAGE=SL2_l.JPG
ID=483
SCALE=0.036434
LM=20
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1.66531 -0.05410
1.25883 -0.27529
1.05189 -0.35311
0.55199 -0.34421
-0.42254 -0.08197
-0.59499 -0.08154
-0.71423 -0.00065
-0.57044 0.08777
-0.26849 0.10764
0.48492 0.13701
1.16932 0.12329
1.07647 -0.12707
1.11120 -0.24540
1.23701 -0.09661
1.35113 0.12248
1.54682 0.15971
1.65635 0.01135
1.56448 -0.07658
1.45690 -0.08250
IMAGE=SL30_l.JPG
ID=484
SCALE=0.035878
LM=20
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1.65635 -0.05211
1.25704 -0.26076
1.01445 -0.33453
0.53005 -0.33160
-0.44302 -0.09317
-0.57739 -0.08025
-0.72448 -0.00288
-0.56083 0.08675
-0.29483 0.10797
0.45035 0.16439
1.18404 0.16641
1.09028 -0.07460
1.11314 -0.19810
1.22874 -0.05049
1.36988 0.15005
1.55333 0.17767
1.64904 0.01653
1.56267 -0.06042
1.46022 -0.05977
IMAGE=SL31_l.JPG
ID=485
SCALE=0.035948
LM=20
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1.65919 -0.05548
1.22563 -0.26603
0.99124 -0.32072
0.51055 -0.30802
-0.42883 -0.07884
-0.58410 -0.07409
-0.72427 0.00060
-0.57630 0.08350
-0.31537 0.09962
0.46339 0.16772
1.16870 0.14400
1.07334 -0.09620
1.10837 -0.19952
1.23839 -0.09131
1.37309 0.12832
1.56451 0.16350
1.66273 0.01114
1.56791 -0.07569
1.45251 -0.09169
IMAGE=SL33_l.JPG
ID=486
SCALE=0.036155
LM=20
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1.64842 -0.06718
1.15962 -0.28252
0.97922 -0.33117
0.44133 -0.28262
-0.40347 -0.09013
-0.60229 -0.06506
-0.73070 -0.00346
-0.57899 0.08405
-0.32287 0.08352
0.43000 0.13695
1.11935 0.13580
1.01587 -0.11110
1.05945 -0.22857
1.15927 -0.10228
1.30732 0.12237

1.53997 0.16077 1.08623 -0.23004 0.45041 0.11847 -0.72385 0.00316 0.56056 -0.33311 1.67315 -0.06183 SCALE=0.032860
1.62282 0.02007 1.19598 -0.08497 1.16635 0.13267 -0.57701 0.07612 -0.45309 -0.08871 1.14657 -0.29236 LM=20
1.53491 -0.08228 1.34024 0.13170 1.09693 -0.13457 -0.29155 0.09396 -0.57662 -0.08251 0.99917 -0.34917 1.77032 0.06921
1.42470 -0.06353 1.53197 0.16894 1.13039 -0.24644 0.44999 0.15815 -0.70397 0.00195 0.47252 -0.34745 1.66459 -0.05847
IMAGE=SL35_l.JPG 1.63746 0.00689 1.21750 -0.07864 1.17341 0.14577 -0.56785 0.08894 -0.45345 -0.08013 1.30090 -0.24473
ID=488 1.54430 -0.09049 1.35665 0.13176 1.06607 -0.09755 -0.31915 0.09501 -0.58421 -0.07935 1.02550 -0.33741
SCALE=0.035970 1.43518 -0.08402 1.55799 0.17439 1.09510 -0.19990 0.47750 0.13515 -0.70679 -0.00110 0.55647 -0.33126
LM=20 IMAGE=SL39_l.JPG 1.67388 0.02024 1.22082 -0.05284 1.19890 0.16661 -0.56918 0.09179 -0.42835 -0.09641
1.77417 0.07501 ID=492 1.58723 -0.07913 1.35884 0.13762 1.11064 -0.06713 -0.32557 0.10727 -0.57561 -0.07957
1.65026 -0.05959 SCALE=0.035938 1.46893 -0.08341 1.54996 0.17315 1.14022 -0.17790 0.39412 0.13008 -0.71706 -0.00198
1.27013 -0.27235 LM=20 IMAGE=SL43_l.JPG 1.65126 0.01457 1.25796 -0.02916 1.20281 0.14066 -0.57002 0.09355
1.02202 -0.33634 1.77413 0.07285 ID=496 1.56926 -0.07338 1.34745 0.15248 1.10035 -0.12232 -0.32129 0.10743
0.52197 -0.33216 1.65540 -0.06896 SCALE=0.035956 1.45863 -0.07391 1.54131 0.18026 1.15432 -0.21141 0.44338 0.15682
-0.43246 -0.08365 1.16496 -0.27935 LM=20 IMAGE=SL6_l.JPG 1.65411 0.02909 1.30244 -0.09074 1.20031 0.14593
-0.57686 -0.08132 0.98724 -0.33354 1.77675 0.06734 ID=501 1.58098 -0.04561 1.39952 0.14302 1.10039 -0.09103
-0.73165 0.00027 0.49758 -0.30529 1.66420 -0.05117 SCALE=0.035948 1.47292 -0.03696 1.55542 0.16074 1.15307 -0.20882
-0.55196 0.08966 -0.42491 -0.08889 1.21104 -0.27040 LM=20 IMAGE=TR12_l.JPG 1.66246 -0.00314 1.27660 -0.07603
-0.26107 0.10495 -0.59039 -0.07894 1.01719 -0.33290 1.76072 0.04912 ID=507 1.60151 -0.07139 1.40592 0.12744
0.42240 0.13962 -0.73259 -0.00122 0.49548 -0.31658 1.65069 -0.07367 SCALE=0.032352 1.50850 -0.09222 1.57249 0.17026
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1.05685 -0.08560 -0.30883 0.10472 -0.57398 -0.07712 1.03341 -0.34243 1.77364 0.06603 ID=512 1.58075 -0.07093
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1.55537 -0.06108 1.31786 0.13941 1.06754 -0.10481 -0.26954 0.08957 -0.56074 -0.08126 1.03881 -0.34285 1.78466 0.07768
1.44523 -0.05331 1.52937 0.15475 1.11362 -0.20904 0.48853 0.12510 -0.70117 -0.00178 0.50372 -0.35746 1.65074 -0.04642
IMAGE=SL36_l.JPG 1.62935 0.00231 1.23013 -0.03932 1.18021 0.15047 -0.56596 0.08794 -0.45816 -0.08898 1.27074 -0.23516
ID=489 1.54168 -0.08235 1.34756 0.16903 1.07169 -0.09357 -0.32368 0.09415 -0.58421 -0.08356 1.03928 -0.31031
SCALE=0.036228 1.43404 -0.07110 1.53721 0.19317 1.09533 -0.19936 0.43491 0.13014 -0.70553 -0.00156 0.50533 -0.30618
LM=20 IMAGE=SL3_l.JPG 1.64451 0.01993 1.19807 -0.03611 1.17441 0.13853 -0.57053 0.09570 -0.44767 -0.08369
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0.98908 -0.37530 1.78770 0.09407 ID=497 1.54098 -0.07094 1.35298 0.15295 1.11092 -0.09506 -0.28465 0.11812
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-0.27554 0.11507 -0.59454 -0.08357 0.98762 -0.33133 1.76955 0.07344 ID=509 1.59027 -0.05080 1.35184 0.17343
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1.05818 -0.24391 0.38977 0.15073 -0.71231 -0.00114 0.47505 -0.34848 1.66702 -0.04677 SCALE=0.032177 1.45388 -0.03558
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1.42718 -0.10436 1.55069 0.20157 1.12123 -0.20486 0.45050 0.10133 -0.72995 0.00079 0.54619 -0.32820 1.66147 -0.05471
IMAGE=SL37_l.JPG 1.66038 0.03395 1.23134 -0.07761 1.13475 0.12709 -0.58264 0.08176 -0.46595 -0.08930 1.28451 -0.23990
ID=490 1.56514 -0.04840 1.36891 0.14103 1.04733 -0.13883 -0.28593 0.08928 -0.57466 -0.08923 1.07696 -0.29494
SCALE=0.035938 1.44722 -0.04782 1.55747 0.17000 1.09554 -0.26125 0.43221 0.12852 -0.70163 -0.00045 0.50813 -0.29632
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SCALE=0.036198 1.43115 -0.07583 1.54943 0.16945 1.08679 -0.21612 0.49369 0.15153 -0.70933 0.00057 0.52083 -0.34107
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1.55710 -0.04629 1.30609 0.15016 1.08669 -0.04369 -0.35778 0.09862 -0.61875 -0.08054 1.06446 -0.33246 1.78008 0.05974
1.44141 -0.03434 1.50256 0.17825 1.11630 -0.16269 0.49216 0.15565 -0.74632 0.01747 0.55266 -0.32475 1.66734 -0.05384
IMAGE=TR3_l.JPG 1.62987 0.00113 1.24875 -0.01716 1.18701 0.16789 -0.62609 0.10407 -0.45344 -0.08900 1.28273 -0.24695
ID=521 1.55268 -0.07232 1.36312 0.20145 1.07966 -0.11297 -0.39613 0.09388 -0.57158 -0.07793 1.04595 -0.32231
SCALE=0.032271 1.45168 -0.07446 1.55522 0.21394 1.12640 -0.21173 0.45677 0.03792 -0.71948 -0.00215 0.53085 -0.30765
LM=20 IMAGE=TR7_l.JPG 1.65473 0.02975 1.23495 -0.08173 1.24093 0.08889 -0.55247 0.09189 -0.44401 -0.08427
1.76137 0.07352 ID=525 1.57568 -0.04895 1.38271 0.15436 1.17728 -0.19527 -0.36757 0.09384 -0.58528 -0.08234
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1.04911 -0.37628 1.76469 0.06612 ID=529 1.56834 -0.06103 1.42818 0.11604 1.13871 -0.09704 -0.34733 0.10208
0.52963 -0.37310 1.66938 -0.05798 SCALE=0.034001 1.47436 -0.07543 1.58945 0.16436 1.16445 -0.20755 0.51065 0.18136
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-0.57365 -0.08207 1.05650 -0.33627 1.77679 0.08177 ID=533 1.67322 -0.09314 1.39451 0.15677 1.11572 -0.08366
-0.71140 0.00127 0.50299 -0.31911 1.66345 -0.06444 SCALE=0.033273 1.57861 -0.10687 1.57407 0.19301 1.14491 -0.20383
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0.44196 0.13088 -0.70898 0.00135 0.52142 -0.33647 1.67853 -0.04573 SCALE=0.033482 1.49184 -0.06260 1.58542 0.18675
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1.48845 -0.06739 1.55057 0.18534 1.12233 -0.21714 0.49900 0.15421 -0.71966 0.00262 0.51761 -0.30405 1.66074 -0.06278
IMAGE=TR4_l.JPG 1.64888 0.00779 1.24991 -0.10066 1.16545 0.13707 -0.55772 0.09251 -0.45578 -0.08792 1.25924 -0.28120
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SCALE=0.032544 1.48933 -0.04706 1.56086 0.19047 1.11503 -0.19070 0.43447 0.15932 -0.71448 0.00087 0.50991 -0.32338
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0.53679 -0.28554 1.65620 -0.05867 SCALE=0.033321 1.44439 -0.06958 1.54853 0.21628 1.12457 -0.17368 0.48046 0.13142
-0.43948 -0.08195 1.28015 -0.25666 LM=20 IMAGE=GL16_l.JPG 1.65865 0.03379 1.24053 -0.01323 1.18566 0.10360
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1.23243 0.16409 -0.56930 0.10132 -0.45028 -0.08512 1.27921 -0.25258 LM=20 IMAGE=GL4_l.JPG 1.64750 0.00677
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1.15670 -0.17086 0.47570 0.17351 -0.70820 0.00190 0.51599 -0.31607 1.72809 -0.07264 SCALE=0.033330 1.46610 -0.09424
1.30566 -0.03443 1.18687 0.14815 -0.56597 0.09988 -0.43606 -0.09569 1.38829 -0.29829 LM=20 IMAGE=GL9_l.JPG
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1.65505 -0.04344
1.28659 -0.21628
1.06623 -0.28148
0.56249 -0.30868
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-0.57946 -0.08277
-0.71441 -0.00107
-0.57940 0.10115
-0.34514 0.11075
0.47609 0.19078
1.19315 0.18565
1.09088 -0.05592
1.12651 -0.16147
1.25977 -0.03546
1.37113 0.16612
1.55112 0.18624
1.64554 0.01952
1.56857 -0.04054
1.47795 -0.04461
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1.68292 -0.06133
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1.07963 -0.33707
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-0.58419 -0.09350
-0.71511 0.00676
-0.60093 0.10990
-0.37258 0.11969
0.41667 0.17493
1.18234 0.19194
1.10618 -0.09016
1.15200 -0.18354
1.26837 -0.01950
1.36591 0.19103
1.53725 0.20563
1.67864 0.02081
1.60460 -0.04488
1.51173 -0.04696
IMAGE=KR11_l.JPG
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1.28482 -0.28356
1.06972 -0.36425
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-0.58341 -0.09382
-0.70538 0.00222
-0.57638 0.09640
-0.27909 0.11257
0.47675 0.15154
1.20416 0.18241
1.12043 -0.04971
1.13475 -0.17648
1.26469 -0.03061
1.36239 0.17415
1.53242 0.20121
1.64384 0.02067
1.57558 -0.05503
1.48564 -0.04655
IMAGE=KR2_l.JPG
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1.04340 -0.31350
0.57747 -0.32034
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-0.57854 -0.09650
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1.09763 -0.07623
1.12694 -0.17053
1.24338 -0.02873
1.36003 0.17502
1.52850 0.18090
1.63918 0.02714
1.56645 -0.04407
1.46763 -0.03856
IMAGE=KR3_l.JPG
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1.65527 -0.05031
1.27745 -0.22613
1.02081 -0.30153
0.55369 -0.30027
-0.44666 -0.09876
-0.57167 -0.09117
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-0.56138 0.10084
-0.34128 0.11883
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1.13542 -0.07116
1.16541 -0.17001
1.31144 -0.05392
1.41776 0.16411
1.57068 0.17587
1.64931 0.01995
1.58771 -0.05105
1.50515 -0.05525
IMAGE=KR6_l.JPG
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LM=20
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1.31741 -0.26590
1.08243 -0.35384
0.51031 -0.37250
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-0.58549 -0.09266
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-0.34791 0.11490
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1.10489 -0.10017
1.14583 -0.22399
1.26208 -0.06810
1.35434 0.15835
1.52054 0.18344
1.64206 0.02671
1.58147 -0.05213
1.49794 -0.05923
IMAGE=KR7_l.JPG
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LM=20
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1.66146 -0.05207
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1.05807 -0.29766
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-0.56928 -0.09443
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-0.58205 0.10452
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0.43976 0.21131
1.21442 0.19425
1.12308 -0.04184
1.16976 -0.14469
1.29656 -0.01014
1.39867 0.19465
1.56348 0.20287
1.65685 0.02682
1.59324 -0.03653
1.50885 -0.02799
IMAGE=KR8_l.JPG
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0.49498 0.17235
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1.22719 -0.03912
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1.65118 0.02592
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1.46486 -0.04240
IMAGE=LAL11_l.JPG
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0.45614 0.17884
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1.21027 -0.05788
1.34792 0.15264
1.53127 0.19396
1.62479 0.02918
1.54866 -0.05612
1.44582 -0.06754
IMAGE=LAL12_l.JPG
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SCALE=0.032257
LM=20
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1.70400 -0.07109
1.30345 -0.31411
1.05601 -0.38930
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0.46460 0.10740
1.18350 0.11209
1.08135 -0.16619
1.11300 -0.26821
1.25758 -0.12626
1.38851 0.13272
1.55629 0.15495
1.67150 0.02605
1.61764 -0.09238
1.52040 -0.10521
IMAGE=LAL13_l.JPG
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LM=20
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-0.30656 0.10533
0.44561 0.15366
1.15632 0.13544
1.07197 -0.10131
1.10217 -0.21490
1.22729 -0.08249
1.36109 0.13565
1.55373 0.17418
1.64113 0.02566
1.56231 -0.05956
1.45371 -0.06876
IMAGE=LAL14_l.JPG
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1.27633 -0.26534
1.02332 -0.32667
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0.48140 0.14772
1.17955 0.17593
1.08312 -0.04878
1.10570 -0.16194
1.22208 0.00155
1.34240 0.18620
1.52997 0.19604
1.63715 0.04549
1.55510 -0.03599
1.45368 -0.02419
IMAGE=LAL15_l.JPG
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LM=20
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1.25288 -0.26681
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-0.31417 0.09615
0.47201 0.15311
1.15808 0.14763
1.06934 -0.07335
1.09446 -0.18912
1.23109 -0.05435
1.35993 0.14547
1.54327 0.17422
1.63781 0.01749
1.56583 -0.06023
1.44996 -0.06895
IMAGE=LAL16_l.JPG
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LM=20
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1.24979 -0.25227
0.98982 -0.32143
0.51430 -0.30174
-0.44037 -0.08586
-0.57503 -0.07988
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-0.56381 0.09493
-0.32464 0.11118
0.44828 0.17943
1.14013 0.16111
1.06475 -0.07417
1.09088 -0.18919
1.21518 -0.03099
1.35045 0.16477
1.54180 0.17974
1.63693 0.02277
1.55680 -0.05945
1.44903 -0.06128
IMAGE=LAL17_l.JPG
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1.04835 -0.17341
1.19100 -0.02659
1.33956 0.17827
1.53450 0.18855
1.64191 0.03284
1.55198 -0.06197
1.42123 -0.05817
IMAGE=LAL18_l.JPG
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1.56228 -0.06470
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IMAGE=LAL1_l.JPG
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1.33886 0.17806
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1.43902 -0.04468
IMAGE=LAL20_l.JP
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1.08777 -0.20585
1.21201 -0.04204
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1.52580 0.18423
1.62656 0.02151
1.55037 -0.05768
1.45230 -0.06513
IMAGE=LAL21_l.JP
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SCALE=0.032457
LM=20
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1.26878 -0.26416
1.01087 -0.32651
0.51713 -0.31406
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-0.57960 -0.08550
-0.69372 0.00120
-0.56006 0.09506
-0.33067 0.10416
0.47650 0.18209
1.16177 0.17649
1.06852 -0.07729
1.11079 -0.20045
1.24807 -0.05488
1.36158 0.17146
1.53117 0.19545
1.63878 0.02391
1.56699 -0.05768
1.47522 -0.06367
IMAGE=LAL22_l.JP
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1.26940 -0.26417
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-0.32682 0.10655
0.45258 0.16404
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1.10251 -0.19072
1.24863 -0.03243
1.37885 0.17752
1.56219 0.20188
1.65396 0.02041
1.56235 -0.06156
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IMAGE=LAL23_l.JP
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LM=20
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1.23274 -0.25380
0.97969 -0.32640
0.51266 -0.31365
-0.44866 -0.08652
-0.58166 -0.08104
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-0.56013 0.09030
-0.33229 0.10260
0.46753 0.17676
1.16002 0.16197
1.05598 -0.08160
1.09013 -0.20379
1.22468 -0.07306
1.35327 0.14544
1.53504 0.16946
1.62373 0.01104
1.55045 -0.07311
1.44540 -0.07208
IMAGE=LAL24_l.JP
G
ID=568
SCALE=0.032457
LM=20
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1.66680 -0.04939
1.27581 -0.25434
1.04434 -0.32261
0.54532 -0.30774
-0.44945 -0.09312
-0.59498 -0.08123
-0.70533 0.00160
-0.56378 0.08596
-0.31840 0.10192
0.46387 0.17165
1.09527 -0.05428
1.13961 -0.15503
1.26305 -0.00717
1.38661 0.17507
1.56520 0.19076
1.64720 0.02714
1.57484 -0.05012
1.47928 -0.03514
IMAGE=LAL25_l.JP
G
ID=569
SCALE=0.032434
LM=20
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1.64803 -0.05543
1.21457 -0.24188
0.98956 -0.28967
0.52657 -0.28197
SCALE=0.032461
LM=20
1.76640 0.05722
1.64660 -0.05402
1.26277 -0.24025
1.01061 -0.29542
0.53321 -0.28366
-0.41988 -0.09411
-0.59001 -0.08427
1.08738 -0.16546
1.22970 -0.02998
1.36572 0.17193
1.54125 0.18924
1.64418 0.02214
1.55994 -0.06261
1.43446 -0.06569
IMAGE=LAL27_l.JP
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ID=570
SCALE=0.032431
LM=20
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1.65759 -0.05790
1.29142 -0.26796
1.03966 -0.35798
0.53560 -0.34795
-0.44715 -0.09034
-0.57553 -0.08021
-0.71262 -0.00068
-0.57154 0.09167
-0.29351 0.09425
0.48093 0.11307
1.13151 0.14090
1.04702 -0.11252
1.10068 -0.22518
1.19636 -0.06429
1.32103 0.15775
1.51441 0.17970
1.63162 0.02807
1.55950 -0.06056
1.44715 -0.05400
IMAGE=LAL28_l.JP
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SCALE=0.032562
LM=20
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1.67268 -0.05685
1.28718 -0.28582
1.03681 -0.36021
0.52042 -0.36422
-0.45225 -0.08411
-0.57981 -0.08399
-0.70435 -0.00241
-0.56954 0.10034
-0.26788 0.10793
0.44749 0.13540
1.18170 0.14145
1.09392 -0.12032
1.13228 -0.24016
1.26605 -0.09552
1.36950 0.13988
1.55143 0.18256
1.65832 0.01317
1.58084 -0.07524
1.49156 -0.08259
IMAGE=LAL29_l.JP
G
ID=572
SCALE=0.032457
LM=20
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1.65608 -0.06364
1.27384 -0.27869
1.01485 -0.34801
0.50562 -0.31938
-0.43777 -0.08294
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-0.71571 0.00137
-0.57661 0.08429
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0.44534 0.13588
1.16069 0.15781
1.06550 -0.08866
1.08848 -0.22464
1.18498 -0.07739
1.32860 0.13646
1.53585 0.17613
1.63962 0.01430
1.55557 -0.07624
1.44362 -0.06554
IMAGE=LAL2_l.JPG
ID=573
SCALE=0.032461
LM=20
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1.26277 -0.24025
1.01061 -0.29542
0.53321 -0.28366
-0.41988 -0.09411
-0.59001 -0.08427

-0.72327 0.00042 0.47391 -0.31047 LM=20 1.65643 0.01649 0.37613 0.18196 1.08322 -0.41948 IMAGE=LCRU7_1.JP
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-0.30453 0.10412 -0.58034 -0.08471 1.66181 -0.04454 1.49373 -0.05125 1.12065 -0.00509 -0.48800 -0.08948 ID=598
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1.17801 0.19295 -0.57084 0.09154 1.05994 -0.21009 PG 1.29430 0.02266 -0.73125 0.01560 LM=20
1.08392 -0.02501 -0.35194 0.10609 0.50782 -0.21947 ID=585 1.42719 0.21264 -0.61639 0.10085 1.78469 0.06364
1.09972 -0.14926 0.44551 0.19211 -0.45992 -0.09173 SCALE=0.035705 1.59382 0.21131 -0.36660 0.09779 1.66251 -0.04697
1.22855 -0.00811 1.16677 0.17700 -0.59764 -0.08522 LM=20 1.65739 0.04046 0.41438 0.07975 1.24691 -0.21507
1.38613 0.17224 1.05234 -0.07933 -0.68356 0.00276 1.79231 0.04488 1.58370 -0.02806 1.21166 0.10457 1.02628 -0.26707
1.55223 0.17930 1.06148 -0.19774 -0.60274 0.08420 1.66691 -0.03489 1.49755 -0.02428 1.17866 -0.17946 0.54497 -0.26655
1.63913 0.03144 1.18889 -0.05339 -0.35176 0.10166 1.26421 -0.14008 IMAGE=LCRU21_1.J 1.21484 -0.26872 -0.43503 -0.08878
1.54877 -0.05188 1.33343 0.14261 0.38100 0.21256 1.03216 -0.17023 PG 1.36157 -0.10567 -0.58107 -0.08257
1.44024 -0.03864 1.53521 0.17503 1.20885 0.26088 0.64240 -0.17925 ID=590 1.43052 0.11771 -0.70964 0.00079
IMAGE=LAL3_1.JPG 1.62556 0.01405 1.11005 0.01958 -0.46612 -0.07912 SCALE=0.035439 1.59516 0.15920 -0.56434 0.09474
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SCALE=0.032623 1.43338 -0.06961 1.30439 0.02557 -0.70533 -0.00396 1.77436 0.04404 1.66927 -0.08563 0.42478 0.19757
LM=20 IMAGE=LAL8_1.JPG 1.43259 0.21729 -0.57370 0.08959 1.66060 -0.05186 1.58475 -0.11059 1.18127 0.21718
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1.65073 -0.04930 SCALE=0.032461 1.67197 0.04121 0.37457 0.24368 1.06020 -0.25170 G 1.10277 -0.13605
1.24319 -0.24496 LM=20 1.58960 -0.02478 1.23209 0.26146 0.58227 -0.24970 ID=595 1.25392 -0.00909
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0.50655 -0.30149 1.64854 -0.05017 IMAGE=LCRU13_1.J 1.14015 -0.07575 -0.56752 -0.08828 LM=20 1.56659 0.20264
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1.15333 0.20192 -0.56598 0.09348 1.29879 -0.25064 IMAGE=LCRU18_1.J 1.13528 -0.12688 -0.57683 -0.08277 SCALE=0.036666
1.03404 -0.03026 -0.31274 0.10555 1.03391 -0.31571 PG 1.28257 0.02805 -0.72813 0.00517 LM=20
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1.63677 0.04194 1.24026 -0.07647 -0.60625 0.09611 1.64746 -0.04753 1.49651 -0.02277 1.09641 -0.11672 0.59847 -0.24800
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IMAGE=LAL4_1.JPG 1.63634 0.01932 1.20867 0.17244 0.52142 -0.22667 ID=592 1.38715 0.15247 -0.70664 0.00107
ID=575 1.56122 -0.06505 1.09578 -0.10061 -0.43468 -0.09243 SCALE=0.035948 1.54517 0.16979 -0.58024 0.09549
SCALE=0.032443 1.45665 -0.06775 1.16724 -0.19368 -0.57968 -0.08131 LM=20 1.66601 0.01254 -0.37137 0.09954
LM=20 IMAGE=LAL9_1.JPG 1.32383 -0.06689 -0.70614 0.00228 1.80101 0.08123 1.61142 -0.06659 0.44852 0.18666
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1.02271 -0.33314 1.77028 0.06578 1.60874 -0.07052 1.16861 0.24704 0.49486 -0.40406 ID=596 1.26212 0.03331
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1.44447 -0.04713 1.56997 0.18515 -0.30596 0.09829 1.25729 -0.24544 IMAGE=LCRU25_1.J 1.12544 -0.19242 -0.46156 -0.07371
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ID=576 1.58021 -0.06674 1.19648 0.18290 0.48207 -0.28742 ID=593 1.40383 0.15531 -0.68578 -0.00061
SCALE=0.032399 1.49562 -0.06406 1.06388 -0.05082 -0.45015 -0.09074 SCALE=0.036223 1.57002 0.18134 -0.58203 0.10516
LM=20 IMAGE=LCRU10_1.J 1.12297 -0.14759 -0.58354 -0.08907 LM=20 1.64963 0.00842 -0.38512 0.10918
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1.44473 -0.06845 1.41576 0.11847 -0.57367 0.09233 1.66223 -0.04538 1.47895 -0.04614 1.09193 -0.02317 0.48761 -0.29310
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SCALE=0.032457 1.64740 -0.08948 1.22558 0.19264 0.52430 -0.26735 ID=594 1.40968 0.21995 -0.70091 0.00089
LM=20 1.55377 -0.10189 1.10732 -0.03617 -0.45544 -0.08863 SCALE=0.035878 1.58733 0.21266 -0.57838 0.09970
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1.24458 -0.25815 ID=581 1.43251 0.18656 -0.58470 0.09181 1.73220 -0.08150 1.49949 -0.02969 1.15658 0.18970
0.97830 -0.32372 SCALE=0.036400 1.59961 0.18967 -0.34366 0.11277 1.38548 -0.32006 1.06242 -0.08346

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1.57513 -0.06439 1.17060 0.14771 -0.45391 -0.09561 SCALE=0.033330 1.54576 0.21364 0.40887 0.12134 0.49108 -0.24103
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G 1.28728 -0.08133 -0.58282 0.09376 1.66379 -0.05213 1.48704 -0.05096 1.17611 -0.23164 -0.69514 0.00099
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SCALE=0.033550 1.56747 0.20885 0.43923 0.15039 0.99121 -0.28963 G 1.38285 0.13213 -0.37258 0.10686
LM=20 1.71868 0.04500 1.19738 0.16309 0.50683 -0.27549 ID=619 1.57366 0.18651 0.34621 0.20635
1.78064 0.06415 1.66083 -0.07607 1.11143 -0.07963 -0.46249 -0.09373 SCALE=0.033333 1.68606 0.02339 1.23010 0.21792
1.66465 -0.05028 1.56418 -0.09502 1.15530 -0.18659 -0.57353 -0.08540 LM=20 1.63303 -0.08088 1.08035 -0.04482
1.27053 -0.22912 IMAGE=MAL17_l.JP 1.29277 -0.05344 -0.69176 0.00050 1.78372 0.06608 1.53637 -0.09440 1.10749 -0.14493
0.99284 -0.29487 G 1.38310 0.16174 -0.58504 0.09934 1.66615 -0.06053 IMAGE=MAL36_l.JP 1.26705 -0.02079
0.50246 -0.27353 ID=607 1.56411 0.19317 -0.36838 0.11958 1.28484 -0.25375 G 1.42413 0.20800
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-0.56467 -0.08180 1.77467 0.07148 G 1.29837 -0.00411 -0.70158 0.00158 1.76689 0.05869 1.56697 -0.03288
-0.69418 0.00088 1.65602 -0.06050 ID=645 1.42843 0.20286 -0.58633 0.09227 1.64943 -0.04611 1.47458 -0.01528
-0.57243 0.09492 1.30046 -0.23745 SCALE=0.036740 1.57971 0.21327 -0.38814 0.11390 1.25613 -0.20467 IMAGE=MALI3_1.JP
-0.36200 0.10815 1.04274 -0.31222 LM=20 1.65806 0.01996 0.39678 0.18870 0.87676 -0.26989 G
0.45489 0.16802 0.50516 -0.29435 1.77408 0.06228 1.58246 -0.04547 1.18394 0.20071 0.51582 -0.27896 ID=663
1.18660 0.18508 -0.45134 -0.09173 1.66058 -0.05634 1.48969 -0.03966 1.12577 -0.02507 -0.44123 -0.10373 SCALE=0.038451
1.09599 -0.05364 -0.57342 -0.08324 1.29865 -0.20534 IMAGE=MALI15_1.JP 1.14863 -0.13140 -0.58671 -0.09292 LM=20
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1.26223 -0.02645 -0.56775 0.09625 0.51786 -0.29394 ID=649 1.41142 0.20085 -0.60738 0.10438 1.65684 -0.03780
1.38035 0.17722 -0.38034 0.11023 -0.43792 -0.10702 SCALE=0.036756 1.56587 0.20980 -0.35893 0.10921 1.29444 -0.18848
1.55629 0.20852 0.44694 0.17826 -0.57457 -0.09147 LM=20 1.66022 0.02930 0.32042 0.21038 1.06706 -0.25599
1.65330 0.02533 1.17059 0.19103 -0.71644 0.00244 1.79581 0.05210 1.58432 -0.04156 1.20028 0.23518 0.54861 -0.26675
1.58044 -0.03786 1.08845 -0.06498 -0.59354 0.10623 1.66145 -0.03673 1.49661 -0.03756 1.10111 -0.00702 -0.45577 -0.09497
1.48070 -0.04427 1.12873 -0.18636 -0.34617 0.12399 1.28517 -0.14844 IMAGE=MALI21_1.JP 1.15300 -0.12346 -0.56822 -0.08275
IMAGE=MAL48_1.JP 1.27133 -0.06720 0.38350 0.20884 1.01334 -0.19398 G 1.29105 0.02002 -0.70279 -0.00037
G 1.37993 0.15927 1.18424 0.21921 0.54165 -0.19137 ID=655 1.39247 0.22251 -0.57429 0.09452
ID=636 1.54116 0.20524 1.08368 -0.07721 -0.45111 -0.09240 SCALE=0.036481 1.58628 0.23511 -0.32661 0.11675
SCALE=0.033330 1.65143 0.01081 1.14708 -0.16913 -0.57875 -0.07783 LM=20 1.65517 0.01788 0.44933 0.20382
LM=20 1.57398 -0.05546 1.27760 -0.02364 -0.70898 0.00376 1.77878 0.05816 1.59074 -0.04005 1.19066 0.20355
1.78578 0.06328 1.49061 -0.06037 1.37991 0.19463 -0.58932 0.09975 1.66047 -0.05849 1.49270 -0.03664 1.13202 -0.02627
1.64686 -0.04977 IMAGE=MAL6_1.JPG 1.57217 0.21822 -0.40824 0.11976 1.29443 -0.22668 IMAGE=MALI26_1.JP 1.16351 -0.12484
1.25012 -0.20627 ID=642 1.65303 0.01184 0.36546 0.29416 0.98520 -0.29001 G 1.31826 -0.01565
0.98261 -0.27440 SCALE=0.033094 1.58810 -0.06045 1.25152 0.27051 0.54096 -0.27695 ID=659 1.43028 0.17773
0.50062 -0.27949 LM=20 1.49282 -0.05460 1.11739 0.04298 -0.45097 -0.11109 SCALE=0.035968 1.58449 0.19803
-0.46263 -0.09699 1.79239 0.07966 IMAGE=MALI11_1.JP 1.14510 -0.07326 -0.58829 -0.09627 LM=20 1.65511 0.02424
-0.57555 -0.09593 1.66384 -0.04185 G 1.30322 0.05735 -0.70114 0.00287 1.79011 0.06513 1.58633 -0.04177
-0.69375 0.00137 1.27426 -0.21772 ID=646 1.43647 0.23357 -0.58800 0.09850 1.65670 -0.04007 1.49682 -0.04493
-0.56131 0.10420 1.02684 -0.27295 SCALE=0.036472 1.62146 0.23230 -0.36545 0.10873 1.27937 -0.20801 IMAGE=MALI4_1.JP
-0.35899 0.12007 0.51878 -0.27455 LM=20 1.67632 0.02835 0.33187 0.16571 1.02138 -0.27672 G

ID=664
SCALE=0.038433
LM=20
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1.65060 -0.04859
1.19696 -0.19232
0.96891 -0.22913
0.53636 -0.22124
-0.44740 -0.08751
-0.56222 -0.08124
-0.71518 0.00058
-0.56612 0.10037
-0.36387 0.11246
0.39087 0.20806
1.15191 0.23260
1.01945 -0.03187
1.07123 -0.14152
1.20697 0.00101
1.36468 0.23226
1.54189 0.22379
1.64360 0.02914
1.56237 -0.03679
1.46038 -0.03147
IMAGE=MALI5_1.JPG
ID=665
SCALE=0.038389
LM=20
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1.68463 -0.06525
1.31667 -0.27475
1.03576 -0.34815
0.42948 -0.36384
-0.47705 -0.09243
-0.58960 -0.08538
-0.71284 0.00713
-0.58739 0.09333
-0.37134 0.10064
0.07483 0.11120
1.17314 0.15314
1.08172 -0.08463
1.12276 -0.20141
1.24668 -0.05911
1.35625 0.16391
1.52570 0.17430
1.66593 0.02440
1.59672 -0.06453
1.47871 -0.08281
IMAGE=MALI6_1.JPG
ID=666
SCALE=0.038421
LM=20
1.77476 0.05862
1.65427 -0.05335
1.27396 -0.22464
1.04540 -0.29796
0.56138 -0.30414
-0.46097 -0.08951
-0.56475 -0.07994
-0.70244 0.00109
-0.57398 0.10172
-0.36133 0.11551
0.42190 0.17907
1.18854 0.17407
1.10050 -0.07462
1.12830 -0.17240
1.27027 -0.06706
1.40174 0.14685
1.55978 0.16689
1.64653 -0.00204
1.58265 -0.06720
1.48313 -0.07664
IMAGE=MALI7_1.JPG
ID=667
SCALE=0.038451
LM=20
1.78923 0.05945
1.66128 -0.04089
1.23963 -0.17979
0.96276 -0.22283
0.55115 -0.20521
-0.45730 -0.09252
-0.57975 -0.08089
-0.68452 0.00129
-0.57575 0.10098
-0.36611 0.11536
0.40668 0.26067
1.19907 0.26058
1.08636 -0.01995
1.13130 -0.11713
1.28671 0.00544
1.42075 0.22214
1.59480 0.22097
1.67963 0.02803
1.59971 -0.03179
1.49064 -0.03601
IMAGE=MALI8_1.JPG
ID=668
SCALE=0.038389
LM=20
1.77870 0.07156
1.66010 -0.04764
1.27015 -0.24965
1.04555 -0.31112
0.53425 -0.31006
-0.44388 -0.09620
-0.57054 -0.08741
-0.71836 0.00180
-0.57082 0.09732
-0.37473 0.11547
0.40933 0.18135
1.18455 0.19329
1.08686 -0.06161
1.13569 -0.16757
1.25853 -0.03288
1.35787 0.16449
1.55630 0.21677
1.65133 0.01734
1.58118 -0.04725
1.47911 -0.05112
IMAGE=MALI9_1.JPG
ID=669
SCALE=0.037577
LM=20
1.78365 0.06650
1.69064 -0.06066
1.30666 -0.28397
1.02477 -0.37101
0.47859 -0.37210
-0.47959 -0.09039
-0.56250 -0.08390
-0.71399 0.00503
-0.56642 0.09797
-0.40544 0.09241
0.41324 0.09291
1.13464 0.16119
1.06123 -0.10448
1.13918 -0.22985
1.22775 -0.07511
1.29289 0.15934
1.51128 0.20169
1.67637 0.01993
1.60765 -0.06018
1.50583 -0.07943
IMAGE=MALO10_1.JPG
ID=670
SCALE=0.038433
LM=20
1.77056 0.07788
1.67557 -0.06468
1.25634 -0.29039
0.94377 -0.36484
0.43750 -0.32509
-0.45665 -0.09789
-0.56701 -0.09103
-0.70523 0.00174
-0.58694 0.10495
-0.37037 0.11506
0.40116 0.16578
1.17705 0.15448
1.05967 -0.12735
1.12376 -0.23502
1.25035 -0.08899
1.33528 0.15970
1.50447 0.19974
1.66472 -0.00539
1.59236 -0.08169
1.49382 -0.10549
IMAGE=MALO11_1.JPG
ID=671
SCALE=0.038433
LM=20
1.77835 0.06759
1.65855 -0.04699
1.27185 -0.20880
0.96735 -0.27040
0.48138 -0.26313
-0.43875 -0.09798
-0.59231 -0.09084
-0.68679 0.00146
-0.58590 0.10955
-0.36130 0.12064
0.38030 0.22465
1.20610 0.23781
1.40162 0.23320
1.56077 0.22534
1.66231 0.03409
1.58870 -0.02928
1.49235 -0.03593
IMAGE=MALO12_1.JPG
PG
ID=672
SCALE=0.038406
LM=20
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1.64710 -0.05769
1.25480 -0.22306
0.95659 -0.26432
0.52033 -0.23073
-0.46592 -0.08604
-0.56694 -0.08519
-0.68982 0.00066
-0.56047 0.09615
-0.35464 0.11398
0.36975 0.23491
1.22372 0.23167
1.08701 -0.02590
1.11332 -0.15381
1.26646 -0.00658
1.37252 0.21669
1.54276 0.22746
1.64956 0.02972
1.56629 -0.05989
1.47251 -0.05968
IMAGE=MALO13_1.JPG
PG
ID=673
SCALE=0.038097
LM=20
1.78838 0.05522
1.64074 -0.03971
1.25527 -0.14369
0.96413 -0.17599
0.51679 -0.15296
-0.45630 -0.07282
-0.56644 -0.07318
-0.70158 0.00319
-0.58881 0.09257
-0.33810 0.12637
0.37603 0.30124
1.24761 0.30732
1.07701 0.07587
1.10714 -0.06249
1.25630 0.07759
1.39021 0.27456
1.57969 0.24215
1.66504 0.03853
1.57981 -0.01949
1.47290 -0.00359
IMAGE=MALO14_1.JPG
PG
ID=674
SCALE=0.038150
LM=20
1.77420 0.06407
1.64948 -0.05420
1.25166 -0.22921
1.00067 -0.28826
0.49714 -0.29448
-0.44961 -0.09108
-0.58045 -0.08170
-0.70574 0.00124
-0.58308 0.09929
-0.34716 0.11212
0.37304 0.17390
1.19738 0.22062
1.09233 -0.03149
1.11965 -0.16198
1.26107 -0.03471
1.37624 0.19499
1.53482 0.21153
1.66338 0.01076
1.57495 -0.06071
1.47114 -0.07762
IMAGE=MALO16_1.JPG
PG
ID=676
SCALE=0.037508
LM=20
1.77287 0.05336
1.65523 -0.05838
1.26463 -0.24891
0.97868 -0.29900
0.50679 -0.25362
-0.47250 -0.09445
-0.58108 -0.08466
-0.69320 0.00298
-0.57820 0.08924
-0.36620 0.11824
0.33676 0.19473
1.17679 0.20852
1.07862 -0.02209
1.11353 -0.16383
1.26410 -0.00621
1.36150 0.20345
1.52679 0.20501
1.66305 0.02415
1.58385 -0.04678
1.48084 -0.04920
IMAGE=MALO18_1.JPG
PG
ID=677
SCALE=0.038460
LM=20
1.78297 0.07946
1.66850 -0.05653
1.28548 -0.25053
1.00988 -0.31834
0.51691 -0.30884
-0.45924 -0.09579
-0.58393 -0.09015
-0.70636 0.00419
-0.58590 0.10672
-0.38042 0.11587
0.42425 0.18899
1.16811 0.19679
1.07474 -0.08658
1.13519 -0.19416
1.27998 -0.05885
1.38839 0.16503
1.55611 0.19373
1.65951 0.01183
1.58504 -0.06180
1.49483 -0.07589
IMAGE=MALO1_1.JPG
G
ID=678
SCALE=0.038731
LM=20
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1.65357 -0.05670
1.22876 -0.23138
0.95363 -0.26011
0.48425 -0.23225
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-0.56987 -0.09303
-0.68773 0.00199
-0.59575 0.09483
-0.40541 0.10410
0.33585 0.20853
1.20785 0.22844
1.09076 -0.02163
1.12109 -0.14168
1.27155 0.00371
1.38515 0.21252
1.55334 0.20753
1.66821 0.01280
1.58526 -0.04740
1.47270 -0.04687
IMAGE=MALO20_1.JPG
PG
ID=679
SCALE=0.037416
LM=20
1.77808 0.07633
1.64758 -0.05280
1.25106 -0.22247
0.87951 -0.28602
0.49714 -0.25921
-0.46899 -0.08318
-0.56785 -0.07859
-0.70519 0.00124
-0.55375 0.09562
-0.35314 0.11803
0.37762 0.19287
1.17416 0.18372
1.06256 -0.07472
1.10832 -0.17532
1.24028 -0.05019
1.37050 0.18463
1.55021 0.19869
1.64643 -0.00384
1.56551 -0.05959
1.46346 -0.07105
IMAGE=MALO21_1.JPG
PG
ID=680
SCALE=0.037508
LM=20
1.78223 0.07350
1.66378 -0.05596
1.19964 -0.25842
0.97666 -0.30044
0.49894 -0.28116
-0.46417 -0.08565
-0.57277 -0.08355
-0.69241 0.00140
-0.57623 0.09799
-0.35621 0.12256
0.40541 0.19985
1.17247 0.19516
1.03955 -0.08127
1.09160 -0.19204
1.24390 -0.06247
1.35486 0.16837
1.54062 0.20169
1.65620 0.01209
1.56917 -0.07243
1.46219 -0.07526
IMAGE=MALO22_1.JPG
PG
ID=681
SCALE=0.037577
LM=20
1.78196 0.06905
1.66450 -0.05345
1.29315 -0.23521
0.92403 -0.31772
0.49642 -0.30482
-0.45208 -0.08942
-0.59463 -0.08736
-0.69640 0.00408
-0.58357 0.10050
-0.36234 0.11401
0.39681 0.17235
1.17679 0.16620
1.07852 -0.08437
1.13808 -0.18856
1.27296 -0.04166
1.37139 0.18351
1.55967 0.20520
1.65849 0.01552
1.59308 -0.05045
1.49288 -0.05385
IMAGE=MALO23_1.JPG
PG
ID=682
SCALE=0.038370
LM=20
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1.66435 -0.04846
1.20666 -0.25016
0.97456 -0.30378
0.47952 -0.29503
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-0.39660 0.10853
0.38402 0.18364
1.17318 0.19908
1.07520 -0.08856
1.13358 -0.19023
1.26994 -0.06780
1.36373 0.18411
1.55484 0.21414
1.65669 -0.00211
1.57840 -0.06848
1.48796 -0.05143
IMAGE=MALO24_1.JPG
PG
ID=683
SCALE=0.037861
LM=20
1.78639 0.07255
1.67581 -0.06279
1.22608 -0.25968
1.01451 -0.29390
0.52434 -0.26104
-0.49098 -0.09002
-0.57414 -0.08115
-0.70011 0.00325
-0.56170 0.09192
-0.37859 0.10749
0.46501 0.16438
1.16115 0.15495
1.07726 -0.12167
1.11831 -0.20802
1.27547 -0.09718
1.38508 0.12189
1.54957 0.15765
1.65435 -0.01322
1.58838 -0.08097
1.49721 -0.09689
IMAGE=MALO25_1.JPG
PG
ID=684
SCALE=0.037230
LM=20
1.78097 0.06000
1.65584 -0.04496
1.25504 -0.19747
0.98322 -0.25428
0.54114 -0.26871
-0.46054 -0.08512
-0.57259 -0.08596
-0.69800 0.00051
-0.56762 0.09817
-0.43158 0.10511
0.38250 0.21441
1.16931 0.21913
1.06850 -0.03976
1.09810 -0.15703
1.24036 -0.00085
1.36981 0.20451
1.53070 0.21303
1.65867 0.02124
1.56208 -0.05381
1.45227 -0.04426
IMAGE=MALO26_1.JPG
PG
ID=685
SCALE=0.037276
LM=20
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1.67611 -0.06027
1.28154 -0.28814
1.00111 -0.36183
0.49396 -0.34625
-0.48632 -0.09503
-0.56721 -0.08752
-0.70138 0.00393
-0.57608 0.09568
-0.36694 0.10593
0.38706 0.14510
1.17853 0.15367
1.08437 -0.11959
1.27098 -0.06448
1.36517 0.15691
1.52262 0.19456
1.66383 0.00956
1.58853 -0.06662
1.48609 -0.08089
IMAGE=MALO27_1.JPG
PG
ID=686
SCALE=0.037263
LM=20
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1.66095 -0.05309
1.27012 -0.25911
0.99325 -0.33330
0.51190 -0.31083
-0.48045 -0.08048
-0.57096 -0.08582
-0.68006 0.00093
-0.55918 0.10204
-0.36550 0.10859
0.39349 0.16282
1.15697 0.19534
1.06347 -0.06060
1.10726 -0.19752
1.24351 -0.01979
1.34725 0.18564
1.51601 0.21261
1.65612 0.03317
1.57448 -0.04453
1.48084 -0.04151
IMAGE=MALO28_1.JPG
PG
ID=687
SCALE=0.037276
LM=20
1.79057 0.06537
1.66716 -0.05104
1.23913 -0.22221
0.97576 -0.26128
0.48787 -0.24059
-0.46157 -0.08416
-0.58252 -0.08550
-0.69866 0.00275
-0.59566 0.09700
-0.37060 0.10727
0.39646 0.19399
1.17882 0.17497
1.08096 -0.08417
1.11848 -0.19521
1.24956 -0.04971
1.37658 0.17521
1.57601 0.18801
1.66925 0.00729
1.58846 -0.05604
1.49230 -0.06517
IMAGE=MALO29_1.JPG
PG
ID=688
SCALE=0.037416
LM=20
1.77380 0.07089
1.65536 -0.06284
1.26174 -0.26858
1.00842 -0.34787
0.48099 -0.32861
-0.45470 -0.09397
-0.58067 -0.08781
-0.70177 0.00173
-0.56652 0.09659
-0.31492 0.11089
0.39066 0.15506
1.13825 0.15384
1.04964 -0.08806
1.10573 -0.22218
1.23304 -0.07789
1.35694 0.18138
1.51153 0.18964
1.65738 0.02083
1.56377 -0.06971
1.46091 -0.07861
IMAGE=MALO2_1.JPG
G
ID=689
SCALE=0.038140
LM=20
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1.66145 -0.04751
1.27149 -0.20499
0.98919 -0.26322
0.45663 -0.25636
-0.45769 -0.08694
-0.56833 -0.08477
-0.69926 0.00179
-0.57583 0.09149
-0.39289 0.10184
0.35063 0.18142
1.19478 0.21760
1.08785 -0.01996
1.13419 -0.14645
1.29491 -0.01753
1.38781 0.20805
1.56071 0.21073
1.66906 0.02910
1.58943 -0.03809
1.47976 -0.04924
IMAGE=MALO30_1.JPG
PG
ID=690
SCALE=0.037263
LM=20
1.78397 0.05881
1.64779 -0.05449
1.28942 -0.23364
0.89396 -0.32282
0.53634 -0.29952
-0.46337 -0.09527
-0.57595 -0.08932
-0.69573 -0.00027
-0.57942 0.09664
-0.38260 0.11066
0.38875 0.19788
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1.27202 -0.05228
1.37082 0.19032
1.54513 0.21021
1.64700 0.01296
1.57203 -0.05453
1.48306 -0.05700
IMAGE=MALO31_1.JPG
PG
ID=691

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0.51396 -0.33941
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-0.58620 -0.08558
-0.72260 0.00631
-0.59021 0.09462
-0.38993 0.10477
0.43512 0.12554
1.18777 0.14242
1.10392 -0.12330
1.16135 -0.23025
1.27808 -0.08863
1.36889 0.14119
1.55872 0.19322
1.68206 0.01557
1.60157 -0.07343
1.50750 -0.09529
IMAGE=MALO3_1.JP
G
ID=692
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LM=20
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1.27731 -0.26483
0.97896 -0.34049
0.47667 -0.33163
-0.48101 -0.09875
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1.17191 0.16156
1.06147 -0.12243
1.11252 -0.22848
1.25120 -0.06722
1.33943 0.17005
1.52329 0.19815
1.67052 0.01914
1.59229 -0.06917
1.49939 -0.08113
IMAGE=MALO4_1.JP
G
ID=693
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LM=20
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0.47038 0.14333
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1.13602 -0.10028
1.17007 -0.20411
1.31071 -0.06996
1.40327 0.14316
1.56110 0.18085
1.66190 0.01697
1.59811 -0.05713
1.51874 -0.06898
IMAGE=MALO6_1.JP
G
ID=694
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LM=20
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-0.57678 -0.08040
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0.32033 0.20484
1.18193 0.20427
1.07642 -0.05166
1.10037 -0.17606
1.23307 -0.04109
1.36663 0.17758
1.56784 0.19657
1.65925 0.01268
1.56960 -0.06945
1.47955 -0.08253
IMAGE=MALO7_1.JP
G
ID=695
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LM=20
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-0.38166 0.10925
0.37135 0.15374
1.20255 0.17915
1.06775 -0.11127
1.12026 -0.21997
1.26655 -0.07101
1.36405 0.16925
1.54263 0.20180
1.66854 0.00966
1.58678 -0.07317
1.47997 -0.08548
IMAGE=MALO9_1.JP
G
ID=696
SCALE=0.037577
LM=20
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1.64696 -0.05415
1.20921 -0.21417
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-0.58712 -0.08132
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-0.57589 0.09425
-0.37406 0.09578
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1.17801 0.20060
1.07512 -0.05534
1.09100 -0.16248
1.25172 -0.04198
1.37703 0.19156
1.56584 0.20216
1.65286 0.01367
1.56512 -0.05242
1.47493 -0.06171
IMAGE=MCL10_1.JP
G
ID=697
SCALE=0.036155
LM=20
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1.65109 -0.06559
1.21892 -0.23492
0.94596 -0.27479
0.44485 -0.22830
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-0.56312 -0.07351
-0.70610 0.00093
-0.56943 0.08822
-0.38140 0.10658
0.36908 0.20255
1.16168 0.19942
1.03156 -0.06060
1.03486 -0.17731
1.19834 -0.02167
1.32154 0.20626
1.53107 0.20498
1.64285 0.01030
1.55544 -0.05528
1.43773 -0.05744
IMAGE=MCL11_1.JP
G
ID=698
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LM=20
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0.49861 -0.27129
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-0.56013 -0.07858
-0.71243 -0.00028
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0.40857 0.16801
1.19760 0.20263
1.08436 -0.03772
1.09578 -0.15468
1.22078 -0.01687
1.34703 0.20685
1.53654 0.21149
1.63578 0.01930
1.56310 -0.04509
1.45459 -0.04541
IMAGE=MCL12_1.JP
G
ID=699
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LM=20
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1.66450 -0.06408
1.22885 -0.28866
1.00423 -0.33746
0.44819 -0.32209
-0.45001 -0.08634
-0.58172 -0.07588
-0.72542 -0.00265
-0.56461 0.09200
-0.33661 0.10312
0.38276 0.15433
1.18626 0.17857
1.08088 -0.07627
1.08919 -0.20760
1.23324 -0.04919
1.35034 0.18498
1.53876 0.19168
1.64613 0.02409
1.57014 -0.05709
1.45948 -0.07058
IMAGE=MCL13_1.JP
G
ID=700
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LM=20
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1.67409 -0.06649
1.24233 -0.29326
1.01233 -0.34370
0.51395 -0.32219
-0.43431 -0.08475
-0.57871 -0.07551
-0.72423 0.00293
-0.58172 0.09385
-0.32676 0.09699
0.42421 0.15325
1.19311 0.13614
1.11730 -0.11184
1.11977 -0.23351
1.24204 -0.12287
1.37661 0.12285
1.56582 0.15599
1.66650 -0.00714
1.59262 -0.07963
1.49695 -0.09006
IMAGE=MCL14_1.JP
G
ID=701
SCALE=0.035878
LM=20
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1.24685 -0.20842
0.99448 -0.25521
0.48602 -0.25388
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-0.59283 -0.07341
-0.69564 -0.00104
-0.56397 0.09322
-0.30731 0.09930
0.37236 0.18423
1.21937 0.21071
1.10416 -0.05919
1.11029 -0.16469
1.24677 -0.04283
1.38249 0.18662
1.57744 0.19446
1.66182 0.01101
1.77298 -0.05599
1.46260 -0.05883
IMAGE=MCL15_1.JP
G
ID=702
SCALE=0.036137
LM=20
1.78454 0.04496
1.64082 -0.04746
1.22155 -0.18783
0.93650 -0.22666
0.46120 -0.21544
-0.44215 -0.08771
-0.57306 -0.07771
-0.71776 0.00133
-0.56516 0.09814
-0.32051 0.12789
0.34160 0.24566
1.17726 0.26652
1.04811 0.02678
1.06581 -0.11372
1.20177 0.04180
1.35507 0.24600
1.55287 0.22694
1.65757 0.02571
1.55945 -0.02882
1.43943 -0.01536
IMAGE=MCL16_1.JP
G
ID=703
SCALE=0.036198
LM=20
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1.66246 -0.06080
1.23451 -0.24986
0.97208 -0.28838
0.51814 -0.27405
-0.42779 -0.08855
-0.59548 -0.07591
-0.72153 -0.00309
-0.58340 0.09980
-0.29246 0.10150
0.35727 0.16743
1.20520 0.22307
1.07563 -0.05648
1.09791 -0.17669
1.23581 -0.02252
1.36978 0.21301
1.56536 0.21314
1.67142 0.02941
1.59084 -0.05574
1.47795 -0.07126
IMAGE=MCL17_1.JP
G
ID=704
SCALE=0.036449
LM=20
1.78017 0.05696
1.66147 -0.04576
1.24101 -0.20634
1.00095 -0.23742
0.55242 -0.23687
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-0.59365 -0.07483
-0.70814 0.00044
-0.56026 0.08670
-0.35444 0.09415
0.43610 0.18824
1.21756 0.19842
1.10228 -0.04705
1.11143 -0.15643
1.23731 -0.04488
1.39943 0.18082
1.58453 0.18778
1.66356 0.03175
1.57172 -0.04491
1.47743 -0.05267
IMAGE=MCL18_1.JP
G
ID=705
SCALE=0.036208
LM=20
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1.01483 -0.26564
0.49616 -0.25207
-0.42550 -0.08283
-0.56686 -0.07103
-0.73048 0.00006
-0.57379 0.08368
-0.32670 0.08836
0.43836 0.15947
1.18947 0.19928
1.07757 -0.02734
1.10730 -0.15146
1.21751 -0.02156
1.34873 0.20127
1.53785 0.19622
1.65063 0.02504
1.55104 -0.03806
1.44499 -0.03022
IMAGE=MCL19_1.JP
G
ID=706
SCALE=0.035968
LM=20
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1.66861 -0.04945
1.24697 -0.25880
1.00107 -0.30670
0.48672 -0.29133
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-0.57644 -0.07381
-0.71284 0.00044
-0.55708 0.09027
-0.30242 0.09555
0.40352 0.13940
1.18425 0.18047
1.11900 -0.08757
1.14260 -0.19729
1.26242 -0.04911
1.36557 0.18182
1.54843 0.20488
1.66587 0.02641
1.57942 -0.06499
1.47836 -0.06993
IMAGE=MCL20_1.JP
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ID=708
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LM=20
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1.21886 -0.20945
0.99710 -0.26124
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0.39693 0.20412
1.16562 0.23053
1.04896 -0.01970
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1.21096 0.01828
1.35364 0.22188
1.56881 0.22026
1.63705 0.03063
1.54806 -0.02911
1.44492 -0.01981
IMAGE=MCL21_1.JP
G
ID=709
SCALE=0.035670
LM=20
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1.67382 -0.06021
1.18850 -0.28707
0.85859 -0.33387
0.46470 -0.32237
-0.43695 -0.09145
-0.62327 -0.08025
-0.72077 -0.00485
-0.58020 0.10589
-0.35547 0.09810
0.37009 0.13690
1.16860 0.21167
1.03804 -0.11295
1.07172 -0.22127
1.20602 -0.05677
1.32272 0.19150
1.51689 0.20082
1.66046 0.02313
1.58935 -0.06190
1.46097 -0.08227
IMAGE=MCL23_1.JP
G
ID=710
SCALE=0.036434
LM=20
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1.66112 -0.04517
1.22263 -0.17950
0.99158 -0.21157
0.51049 -0.22661
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-0.59121 -0.08450
-0.71361 -0.00172
-0.54794 0.08841
-0.36645 0.09396
0.38782 0.24043
1.23062 0.26068
1.10209 0.00890
1.11262 -0.10841
1.25461 0.00358
1.42028 0.22782
1.60585 0.21919
1.67419 0.03381
1.58251 -0.03435
1.47004 -0.03953
IMAGE=MCL24_1.JP
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ID=711
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LM=20
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1.26257 -0.19591
0.98960 -0.24839
0.51805 -0.24394
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-0.59966 -0.07726
-0.72104 0.00089
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-0.31276 0.10156
0.38893 0.20244
1.22086 0.24073
1.12696 -0.01656
1.14174 -0.12380
1.27427 0.01580
1.38903 0.23611
1.57169 0.21630
1.67518 0.01514
1.57950 -0.03188
1.46657 -0.02023
IMAGE=MCL25_1.JP
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ID=712
SCALE=0.036185
LM=20
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1.25916 -0.21048
0.99817 -0.25459
0.49569 -0.23385
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-0.55517 -0.07413
-0.71831 0.00047
-0.57440 0.09053
-0.33907 0.10960
0.41270 0.20190
1.24458 0.21818
1.13701 -0.01632
1.15163 -0.14159
1.30249 -0.02918
1.42752 0.21136
1.60003 0.20081
1.70140 0.05841
1.60918 -0.03102
1.50035 -0.04295
IMAGE=MCL26_1.JP
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ID=713
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LM=20
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1.23155 -0.22927
0.98228 -0.26431
0.47558 -0.23322
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1.20906 0.23611
1.09291 -0.05524
1.10296 -0.14666
1.27012 -0.00412
1.37238 0.21438
1.55638 0.21489
1.68209 0.03670
1.58286 -0.04335
1.47374 -0.05206
IMAGE=MCL27_1.JP
G
ID=714
SCALE=0.035711
LM=20
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1.23785 -0.25359
1.00484 -0.30843
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-0.57555 -0.08180
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-0.57502 0.08846
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1.05934 -0.07926
1.08147 -0.18088
1.20767 -0.05935
1.34763 0.17800
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1.66110 0.02754
1.57567 -0.05084
1.46773 -0.06664
IMAGE=MCL28_1.JP
G
ID=715
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LM=20
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1.20099 -0.25034
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1.06799 -0.04523
1.09314 -0.16571
1.20439 -0.02584
1.35130 0.20648
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1.65477 0.01222
1.57200 -0.05536
1.46303 -0.06142
IMAGE=MCL29_1.JP
G
ID=716
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0.93608 -0.21928
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1.35785 0.24195
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1.67919 0.04314
1.56882 -0.03765
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IMAGE=MCL2_1.JPG
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1.27739 -0.25784
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1.12332 -0.08879
1.14196 -0.19273
1.28583 -0.06796
1.39978 0.16452
1.58406 0.18310
1.66441 0.01319
1.59083 -0.06882
1.50080 -0.06825
IMAGE=MCL31_1.JP
G
ID=718
SCALE=0.036228
LM=20

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1.22494 -0.28219
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-0.58916 -0.07839
-0.71447 -0.00390
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-0.35236 0.10507
0.39559 0.13978
1.16144 0.18305
1.09192 -0.10596
1.11774 -0.21466
1.22134 -0.07567
1.35729 0.16807
1.55633 0.19411
1.66528 0.01283
1.59315 -0.06847
1.49192 -0.06741
IMAGE=MCL33_1.JPG
ID=720
SCALE=0.035968
LM=20
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1.28265 -0.27120
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0.53106 -0.33891
-0.44403 -0.08814
-0.57823 -0.08217
-0.72038 0.00156
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-0.31442 0.11052
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1.19121 0.15945
1.10800 -0.07698
1.12948 -0.18454
1.26406 -0.06425
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1.55765 0.18873
1.66099 0.03108
1.58351 -0.05432
1.48568 -0.06198
IMAGE=MCL35_1.JPG
ID=721
SCALE=0.035692
LM=20
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1.22075 -0.24110
1.02942 -0.26824
0.49879 -0.26139
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-0.57840 -0.08359
-0.72019 0.00178
-0.59166 0.08569
-0.37307 0.09765
0.38243 0.17491
1.18328 0.21034
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1.10644 -0.13434
1.25386 -0.00983
1.35022 0.19687
1.56908 0.19343
1.66115 0.04590
1.57707 -0.04403
1.47675 -0.03754
IMAGE=MCL36_1.JPG
ID=722
SCALE=0.034692
LM=20
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1.25783 -0.26882
0.99481 -0.31124
0.49375 -0.28343
-0.42565 -0.09267
-0.56675 -0.08177
-0.72803 -0.00377
-0.58145 0.09694
-0.34046 0.10162
0.42802 0.17217
1.21636 0.16159
1.09809 -0.10349
1.10860 -0.20966
1.25512 -0.08784
1.37891 0.15555
1.55252 0.19084
1.65951 0.02093
1.58323 -0.06210
1.48471 -0.07934
IMAGE=MCL37_1.JPG
ID=723
SCALE=0.034934
LM=20
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1.64928 -0.05909
1.21689 -0.24153
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0.45440 -0.26281
-0.44232 -0.10009
-0.57837 -0.08506
-0.70499 -0.00086
-0.57953 0.09361
-0.36783 0.08391
0.38812 0.17679
1.16644 0.21049
1.05718 -0.04440
1.08516 -0.15730
1.21113 0.00333
1.34307 0.21459
1.53906 0.21204
1.66114 0.03229
1.56304 -0.04973
1.44548 -0.03695
IMAGE=MCL38_1.JPG
ID=724
SCALE=0.035156
LM=20
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1.65505 -0.05189
1.22907 -0.20770
0.99130 -0.24759
0.52429 -0.25372
-0.43721 -0.08330
-0.58002 -0.07044
-0.72023 0.00091
-0.57493 0.08866
-0.37251 0.09870
0.37408 0.19225
1.21964 0.22792
1.10683 -0.02678
1.09473 -0.13741
1.25962 -0.01570
1.39698 0.20396
1.59481 0.21535
1.67590 0.02919
1.57492 -0.04782
1.45899 -0.04597
IMAGE=MCL3_1.JPG
ID=726
SCALE=0.037036
LM=20
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1.68005 -0.06053
1.21437 -0.27938
0.98188 -0.31481
0.49294 -0.28927
-0.42157 -0.08230
-0.58475 -0.07448
-0.73372 -0.00278
-0.58596 0.09289
-0.41247 0.09193
0.38636 0.14509
1.17099 0.15904
1.08971 -0.10801
1.10855 -0.22404
1.25115 -0.07704
1.36789 0.15595
1.55325 0.18868
1.67183 0.01065
1.59841 -0.07659
1.49731 -0.09080
IMAGE=MCL40_1.JPG
G
ID=727
SCALE=0.035692
LM=20
1.78387 0.06740
1.66731 -0.05026
1.24976 -0.22536
1.00526 -0.28108
0.51021 -0.26983
-0.41864 -0.08909
-0.58157 -0.08096
-0.73555 0.00235
-0.57960 0.09072
-0.34533 0.10878
0.42437 0.19905
1.19835 0.20169
1.09202 -0.06219
1.11976 -0.16406
1.26323 -0.04316
1.40713 0.18625
1.57436 0.20276
1.67406 0.02842
1.58870 -0.05559
1.47234 -0.05953
IMAGE=MCL41_1.JPG
G
ID=728
SCALE=0.034962
LM=20
1.78775 0.06180
1.64803 -0.05150
1.19606 -0.21681
0.93279 -0.24301
0.46548 -0.23016
-0.42618 -0.08124
-0.58743 -0.08304
-0.72899 0.00046
-0.54824 0.10139
-0.36321 0.11145
0.37756 0.22802
1.17656 0.22653
1.03033 -0.04034
1.05522 -0.14075
1.19711 0.00592
1.34672 0.21134
1.53756 0.20734
1.65843 0.01801
1.55960 -0.04997
1.43690 -0.04402
IMAGE=MCL42_1.JPG
G
ID=729
SCALE=0.034964
LM=20
1.79199 0.05829
1.65963 -0.04023
1.23305 -0.19811
1.01133 -0.22709
0.52390 -0.20156
-0.42631 -0.08009
-0.58545 -0.07612
-0.72641 -0.00146
-0.57268 0.09222
-0.33133 0.11189
0.40336 0.24324
1.22037 0.23204
1.11191 -0.00431
1.11830 -0.13643
1.25200 -0.03212
1.40611 0.18937
1.58509 0.19346
1.66666 0.02440
1.58062 -0.03915
1.48535 -0.03685
IMAGE=MCL44_1.JPG
G
ID=730
SCALE=0.034923
LM=20
1.77940 0.05344
1.65148 -0.05368
1.26020 -0.23015
0.95605 -0.28450
0.51701 -0.25954
-0.44200 -0.08673
-0.56870 -0.08794
-0.71214 0.00038
-0.57389 0.08878
-0.36247 0.10286
0.44260 0.20324
1.18932 0.18838
1.08155 -0.06067
1.09936 -0.16891
1.22483 -0.05017
1.36319 0.17709
1.56200 0.18798
1.65515 0.01129
1.56873 -0.05913
1.46691 -0.05127
IMAGE=MCL46_1.JPG
G
ID=732
SCALE=0.034951
LM=20
1.78234 0.05746
1.64655 -0.04595
1.23071 -0.21258
0.93155 -0.25920
0.50368 -0.24612
-0.43509 -0.08781
-0.57044 -0.07891
-0.72623 -0.00022
-0.57497 0.09315
-0.35778 0.09944
0.40962 0.20205
1.20030 0.23222
1.08381 -0.01365
1.08902 -0.12623
1.23852 0.00833
1.37110 0.20927
1.55875 0.20714
1.64025 0.02878
1.55178 -0.03358
1.44183 -0.03225
IMAGE=MCL47_1.JPG
G
ID=733
SCALE=0.035460
LM=20
1.76728 0.04410
1.64516 -0.05782
1.22259 -0.24239
0.97247 -0.27586
0.51390 -0.25289
-0.41703 -0.08312
-0.56634 -0.07553
-0.73874 0.00084
-0.57277 0.08536
-0.32661 0.09089
0.35290 0.17047
1.12153 0.21204
1.00774 -0.02553
1.05568 -0.15525
1.20056 -0.02061
1.31053 0.20048
1.50311 0.20137
1.64387 0.03123
1.55058 -0.03651
1.43812 -0.05090
IMAGE=MCL48_1.JPG
G
ID=734
SCALE=0.034934
LM=20
1.79382 0.06121
1.68029 -0.06313
1.23620 -0.26297
0.98162 -0.30093
0.49423 -0.26808
-0.43236 -0.08348
-0.59103 -0.08234
-0.72675 0.00601
-0.60396 0.08617
-0.34559 0.10770
0.41273 0.16731
1.17273 0.15294
1.05176 -0.10425
1.07108 -0.20603
1.22370 -0.09118
1.36643 0.12600
1.56261 0.16142
1.66836 0.01450
1.58838 -0.07795
1.48810 -0.08035
IMAGE=MCL49_1.JPG
G
ID=735
SCALE=0.034715
LM=20
1.77629 0.06521
1.66382 -0.04962
1.25388 -0.26395
0.97364 -0.32271
0.49408 -0.31072
-0.43314 -0.08722
-0.58116 -0.07635
-0.72162 0.00255
-0.58480 0.09184
-0.34595 0.09723
0.37570 0.16004
1.19092 0.19650
1.07253 -0.06881
1.09489 -0.17875
1.22429 -0.02310
1.34655 0.19445
1.52781 0.19022
1.64618 0.03345
1.56662 -0.04731
1.46785 -0.05134
IMAGE=MCL4_1.JPG
ID=736
SCALE=0.036756
LM=20
1.78274 0.05166
1.66335 -0.07095
1.23822 -0.25291
0.97830 -0.29520
0.49558 -0.28302
-0.45222 -0.08756
-0.58301 -0.08069
-0.71971 0.00570
-0.58656 0.09140
-0.32956 0.10097
0.37700 0.16681
1.18269 0.20401
1.08946 -0.04052
1.09464 -0.16445
1.22654 -0.02649
1.32556 0.18250
1.53825 0.19670
1.65760 0.02658
1.58090 -0.05162
1.47495 -0.06046
IMAGE=MCL50_1.JPG
G
ID=737
SCALE=0.034962
LM=20
1.77041 0.05669
1.64803 -0.05672
1.21709 -0.23893
0.95303 -0.29733
0.47848 -0.26232
-0.44599 -0.08868
-0.57770 -0.08635
-0.69438 -0.00032
-0.61270 0.09640
-0.34697 0.09795
0.34687 0.17618
1.14509 0.21779
1.04668 -0.04505
1.06471 -0.14685
1.19579 -0.01193
1.32962 0.20249
1.52703 0.20908
1.64106 0.02457
1.55689 -0.05217
1.44577 -0.04642
IMAGE=MCL5_1.JPG
ID=738
SCALE=0.036740
LM=20
1.78332 0.05606
1.65707 -0.05935
1.23773 -0.25485
0.98322 -0.30149
0.49589 -0.28754
-0.45578 -0.08904
-0.58571 -0.07896
-0.71211 -0.00229
-0.55687 0.09477
-0.35245 0.09977
0.41062 0.18514
1.18127 0.20287
1.06977 -0.05978
1.09628 -0.16058
1.20122 -0.03437
1.34349 0.18335
1.55320 0.20339
1.66958 0.02161
1.57115 -0.06170
1.46472 -0.06842
IMAGE=MCL6_1.JPG
ID=739
SCALE=0.036449
LM=20
1.77476 0.04883
1.64590 -0.05832
1.23208 -0.22329
0.98701 -0.27005
0.49253 -0.25734
-0.45513 -0.08243
-0.58029 -0.07311
-0.70895 0.00050
-0.55449 0.08855
-0.32273 0.10442
0.42639 0.19452
1.17579 0.20641
1.06840 -0.03813
1.09071 -0.15785
1.20658 -0.02272
1.34676 0.19638
1.54728 0.19042
1.63812 0.02322
1.55021 -0.04758
1.44520 -0.04656
IMAGE=MCL7_1.JPG
ID=740
SCALE=0.036208
LM=20
1.78893 0.06807
1.65467 -0.05842
1.12804 -0.23489
0.96515 -0.24705
0.45775 -0.23161
-0.43334 -0.06687
-0.58026 -0.06494
-0.71843 0.00250
-0.56572 0.08967
-0.38527 0.09086
0.38284 0.18158
1.17765 0.18396
1.07684 -0.09403
1.08094 -0.19156
1.22703 -0.09943
1.38778 0.16034
1.56176 0.17281
1.65504 0.00380
1.56692 -0.07790
1.45800 -0.08437
IMAGE=MCL8_1.JPG
ID=741
SCALE=0.035713
LM=20
1.64803 -0.05672
1.21709 -0.23893
0.95303 -0.29733
0.47848 -0.26232
-0.44599 -0.08868
-0.57770 -0.08635
-0.69438 -0.00032
-0.61270 0.09640
-0.34697 0.09795
0.34687 0.17618
1.14509 0.21779
1.04668 -0.04505
1.06471 -0.14685
1.19579 -0.01193
1.32962 0.20249
1.52703 0.20908
1.64106 0.02457
1.55689 -0.05217
1.44577 -0.04642
IMAGE=MCL5_1.JPG
ID=738
SCALE=0.036740
LM=20
1.78332 0.05606
1.65707 -0.05935
1.23773 -0.25485
0.98322 -0.30149
0.49589 -0.28754
-0.45578 -0.08904
-0.58571 -0.07896
-0.71211 -0.00229
-0.55687 0.09477
-0.35245 0.09977
0.41062 0.18514
1.18127 0.20287
1.06977 -0.05978
1.09628 -0.16058
1.20122 -0.03437
1.34349 0.18335
1.55320 0.20339
1.66958 0.02161
1.57115 -0.06170
1.46472 -0.06842
IMAGE=MCL6_1.JPG
ID=739
SCALE=0.036449
LM=20
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1.64590 -0.05832
1.23208 -0.22329
0.98701 -0.27005
0.49253 -0.25734
-0.45513 -0.08243
-0.58029 -0.07311
-0.70895 0.00050
-0.55449 0.08855
-0.32273 0.10442
0.42639 0.19452
1.17579 0.20641
1.06840 -0.03813
1.09071 -0.15785
1.20658 -0.02272
1.34676 0.19638
1.54728 0.19042
1.63812 0.02322
1.55021 -0.04758
1.44520 -0.04656
IMAGE=MCL7_1.JPG
ID=740
SCALE=0.036208
LM=20
1.78893 0.06807
1.65467 -0.05842
1.12804 -0.23489
0.96515 -0.24705
0.45775 -0.23161
-0.43334 -0.06687
-0.58026 -0.06494
-0.71843 0.00250
-0.56572 0.08967
-0.38527 0.09086
0.38284 0.18158
1.17765 0.18396
1.07684 -0.09403
1.08094 -0.19156
1.22703 -0.09943
1.38778 0.16034
1.56176 0.17281
1.65504 0.00380
1.56692 -0.07790
1.45800 -0.08437
IMAGE=MCL8_1.JPG
ID=741
SCALE=0.035713
LM=20
1.77743 0.07078
1.66137 -0.05540
1.26581 -0.25683
1.02479 -0.33033
0.51334 -0.34095
-0.41658 -0.10065
-0.57006 -0.08508
-0.72538 -0.00093
-0.57663 0.09990
-0.34645 0.10036
0.45485 0.15614
1.15707 0.16100
1.07898 -0.09813
1.11726 -0.21869
1.26517 -0.10014
1.39095 0.13412
1.56541 0.17925
1.65213 -0.00109
1.57308 -0.07176
1.47683 -0.09031
IMAGE=NIL11_1.JPG
ID=746
SCALE=0.027618
LM=20
1.77416 0.07068
1.66018 -0.06541
1.26607 -0.27647
1.03480 -0.35229
0.53015 -0.34653
-0.45627 -0.09244
-0.58217 -0.08724
-0.72931 -0.00128
-0.55104 0.08195
-0.38295 0.09757
0.43878 0.14532
1.15307 0.17439
1.05365 -0.04647
1.09533 -0.16210
1.22454 -0.00720
1.37075 0.19017
1.55238 0.19305
1.64410 0.03057
1.55658 -0.04056
1.44748 -0.04579
IMAGE=NIL1_1.JPG
ID=747
SCALE=0.027604
LM=20
1.78687 0.07692
1.66912 -0.03931
1.22903 -0.22963
1.03554 -0.28480
0.57834 -0.30239
-0.44478 -0.08969
-0.57328 -0.07984
-0.73972 -0.00196
-0.54060 0.09831
-0.31257 0.11561
0.46683 0.17260
1.16878 0.19059
1.09886 -0.01726
1.62829 0.22641
1.67150 0.02755
1.59381 -0.02384
1.50637 -0.01454
IMAGE=NAR6_1.JPG
ID=744
SCALE=0.034962
LM=20
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1.67832 -0.06081
1.22801 -0.24865
1.03130 -0.28977
0.56025 -0.27453
-0.47991 -0.08249
-0.56506 -0.07854
-0.72709 0.00387
-0.55242 0.09153
-0.28401 0.12373
0.43187 0.21427
1.15665 0.18398
1.05423 -0.04124
1.08156 -0.16282
1.20620 -0.03918
1.35979 0.17569
1.55847 0.18727
1.63750 0.01601
1.55117 -0.06210
1.43378 -0.04592
IMAGE=NIL10_1.JPG
ID=745
SCALE=0.027403
LM=20
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1.66137 -0.05540
1.26581 -0.25683
1.02479 -0.33033
0.51334 -0.34095
-0.41658 -0.10065
-0.57006 -0.08508
-0.72538 -0.00093
-0.57663 0.09990
-0.34645 0.10036
0.45485 0.15614
1.15707 0.16100
1.07898 -0.09813
1.11726 -0.21869
1.26517 -0.10014
1.39095 0.13412
1.56541 0.17925
1.65213 -0.00109
1.57308 -0.07176
1.47683 -0.09031
IMAGE=NIL11_1.JPG
ID=746
SCALE=0.027618
LM=20
1.77416 0.07068
1.66018 -0.06541
1.26607 -0.27647
1.03480 -0.35229
0.53015 -0.34653
-0.45627 -0.09244
-0.58217 -0.08724
-0.72931 -0.00128
-0.55104 0.08195
-0.38295 0.09757
0.43878 0.14532
1.15307 0.17439
1.05365 -0.04647
1.09533 -0.16210
1.22454 -0.00720
1.37075 0.19017
1.55238 0.19305
1.64410 0.03057
1.55658 -0.04056
1.44748 -0.04579
IMAGE=NIL1_1.JPG
ID=747
SCALE=0.027604
LM=20
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1.66912 -0.03931
1.22903 -0.22963
1.03554 -0.28480
0.57834 -0.30239
-0.44478 -0.08969
-0.57328 -0.07984
-0.73972 -0.00196
-0.54060 0.09831
-0.31257 0.11561
0.46683 0.17260
1.16878 0.19059
1.09886 -0.01726

1.12574 -0.13175 0.44682 0.18029 -0.59117 -0.07832 LM=20 1.60280 -0.08073 1.22319 0.16439 -0.45650 -0.08127
1.26430 -0.00332 1.16849 0.15658 -0.72929 0.01140 1.77761 0.06521 1.52445 -0.08371 1.12329 -0.09841 -0.58818 -0.07557
1.39626 0.21881 1.09600 -0.08163 -0.60573 0.08770 1.68470 -0.05510 IMAGE=NKL22_1.JP 1.14729 -0.20047 -0.71927 0.00191
1.59319 0.22132 1.12747 -0.17587 -0.37367 0.09104 1.30787 -0.28764 G 1.27808 -0.06859 -0.56410 0.08660
1.67499 0.03958 1.24789 -0.05484 0.41930 0.09982 1.02075 -0.35608 ID=765 1.37921 0.14018 -0.30547 0.10643
1.58150 -0.03030 1.37743 0.15538 1.25661 0.11841 0.54682 -0.33244 SCALE=0.033094 1.56300 0.16136 0.45632 0.16784
1.50124 0.01169 1.56046 0.18495 1.15967 -0.14698 -0.42683 -0.08539 LM=20 1.65622 0.00699 1.23512 0.17917
IMAGE=NIL2_1.JPG 1.64379 0.01894 1.19106 -0.26945 -0.57197 -0.06882 1.78554 0.06796 1.58073 -0.07326 1.12417 -0.05995
ID=748 1.56728 -0.06370 1.33327 -0.12482 -0.73340 -0.00321 1.66973 -0.05679 1.48915 -0.08410 1.15575 -0.17746
SCALE=0.027564 1.46489 -0.06455 1.43250 0.10889 -0.59138 0.08146 1.24691 -0.24045 IMAGE=NKL29_1.JP 1.27324 -0.05510
LM=20 IMAGE=NIL6_1.JPG 1.59968 0.15504 -0.31016 0.09074 1.00032 -0.28386 G 1.39636 0.16396
1.78185 0.07430 ID=752 1.70118 0.02721 0.46253 0.12214 0.53867 -0.27417 ID=771 1.57339 0.17572
1.68594 -0.04757 SCALE=0.027624 1.63919 -0.09879 1.23487 0.14126 -0.43993 -0.08567 SCALE=0.034701 1.66526 0.02115
1.28370 -0.25466 LM=20 1.54934 -0.11179 1.13550 -0.09745 -0.58504 -0.08434 LM=20 1.57734 -0.05777
1.06080 -0.32527 1.77120 0.07862 IMAGE=NKL13_1.JP 1.17542 -0.19409 -0.72578 0.00402 1.77771 0.06810 1.48995 -0.07131
0.54510 -0.35216 1.66810 -0.04815 G 1.30428 -0.05263 -0.57147 0.10204 1.66669 -0.04782 IMAGE=NKL32_1.JP
-0.47314 -0.09710 1.25983 -0.25550 ID=757 1.39945 0.14918 -0.33270 0.12369 1.25013 -0.25685 G
-0.56772 -0.08393 1.02404 -0.31499 SCALE=0.033321 1.58268 0.16492 0.45118 0.22914 1.00478 -0.32064 ID=775
-0.72151 -0.00199 0.54668 -0.28303 LM=20 1.66510 0.01322 1.19641 0.20214 0.53688 -0.31649 SCALE=0.034669
-0.55265 0.09484 -0.44112 -0.08378 1.77481 0.06278 1.59780 -0.06929 1.09438 -0.06532 -0.43412 -0.09076 LM=20
-0.35302 0.10665 -0.58230 -0.08243 1.65935 -0.05653 1.50549 -0.06637 1.12443 -0.17717 -0.56716 -0.08165 1.78963 0.06879
0.45229 0.16693 -0.72615 -0.00020 1.23445 -0.26166 IMAGE=NKL19_1.JP 1.26305 -0.04617 -0.72607 0.00108 1.66867 -0.04728
1.19778 0.14619 -0.55245 0.08859 1.00445 -0.28982 G 1.39582 0.19671 -0.57315 0.09245 1.26160 -0.22993
1.14020 -0.10996 -0.32167 0.11565 0.55055 -0.25070 ID=762 1.57725 0.19622 -0.34597 0.10802 0.99157 -0.26165
1.15834 -0.19950 0.46614 0.19162 -0.43735 -0.08209 SCALE=0.033286 1.65710 0.02893 0.45412 0.16815 0.51136 -0.23490
1.29848 -0.08666 1.16879 0.17375 -0.58160 -0.08117 LM=20 1.58226 -0.05156 1.18346 0.17932 -0.45435 -0.07529
1.43966 0.13423 1.08976 -0.05175 -0.71993 0.00208 1.78748 0.07644 1.48637 -0.05785 1.10302 -0.11246 -0.58351 -0.07301
1.60021 0.16017 1.11949 -0.16760 -0.56002 0.09585 1.66203 -0.04034 IMAGE=NKL23_1.JP 1.12736 -0.19654 -0.71525 -0.00302
1.66952 -0.00893 1.32751 -0.00579 -0.35216 0.09356 1.26710 -0.22257 G 1.27113 -0.07645 -0.57031 0.08539
1.59289 -0.07417 1.39702 0.16089 0.41640 0.19866 1.02172 -0.27750 ID=766 1.38260 0.16141 -0.34267 0.10450
1.50327 -0.08045 1.57078 0.18707 1.17490 0.19310 0.55631 -0.26625 SCALE=0.033307 1.56969 0.18672 0.41154 0.20553
IMAGE=NIL3_1.JPG 1.65186 0.02017 1.06609 -0.06853 -0.44968 -0.08101 LM=20 1.65094 0.01363 1.24516 0.19409
ID=749 1.57226 -0.06024 1.10213 -0.17738 -0.55874 -0.07442 1.76467 0.06374 1.57212 -0.07239 1.09826 -0.06929
SCALE=0.027614 1.47487 -0.05368 1.22226 -0.03055 -0.71067 -0.00015 1.65928 -0.06311 1.48096 -0.07925 1.12772 -0.18990
LM=20 IMAGE=NIL8_1.JPG 1.36675 0.19350 -0.56340 0.09059 1.27509 -0.27368 IMAGE=NKL2_1.JPG 1.23477 -0.06783
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0.51504 -0.36900 1.66154 -0.04991 IMAGE=NKL14_1.JP 1.14610 -0.14658 -0.72890 0.00077 1.67622 -0.06549 1.48053 -0.06377
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1.10512 -0.19971 0.43760 0.17745 0.51995 -0.28002 SCALE=0.034001 1.55226 0.17068 0.46450 0.17652 1.03058 -0.30426
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-0.42925 -0.09533 1.26453 -0.24768 IMAGE=NKL16_1.JP 1.20547 -0.07080 -0.72466 -0.00116 1.68680 -0.07492 1.46698 -0.06644
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SCALE=0.027618 1.48028 -0.07503 1.12274 -0.09459 -0.59027 -0.08715 LM=20 1.58692 -0.09060 1.22377 0.14452
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1.33649 -0.33247
1.07472 -0.41129
0.48168 -0.38956
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-0.62890 -0.08006
-0.73592 0.01143
-0.59849 0.08074
-0.33172 0.08681
0.40785 0.06652
1.24106 0.10745
1.16342 -0.13722
1.20013 -0.25607
1.30998 -0.09258
1.39894 0.12885
1.57517 0.15993
1.69057 0.02645
1.62916 -0.08548
1.53593 -0.10555
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-0.73287 -0.00072
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1.14650 -0.21682
1.26966 -0.08203
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1.55786 0.17689
1.65712 0.00857
1.58561 -0.07037
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1.03227 -0.29211
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-0.58952 -0.08083
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1.14974 -0.03139
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1.41718 0.17573
1.59704 0.18408
1.65930 0.03278
1.59405 -0.04491
1.51231 -0.05010
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1.14560 -0.22185
1.28871 -0.10230
1.40305 0.12439
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1.66214 0.00414
1.59625 -0.07380
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0.44390 0.21293
1.26481 0.18012
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1.16382 -0.17846
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1.59721 0.18265
1.67375 0.01641
1.58533 -0.05610
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1.57206 0.16860
1.66085 0.01385
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1.65835 0.01206
1.58991 -0.07533
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0.42681 0.14712
1.21213 0.15591
1.12401 -0.09758
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1.29138 -0.05321
1.40166 0.15834
1.58834 0.17101
1.67211 0.02822
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1.27442 -0.08027
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-0.60028 -0.07306
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1.12401 -0.09758
1.16475 -0.20423
1.29138 -0.05321
1.40166 0.15834
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1.60471 -0.05902
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1.26111 -0.25744
0.98289 -0.32630
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0.43040 0.17634
1.19670 0.14116
1.10436 -0.08936
1.13991 -0.18913
1.27442 -0.08027
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-0.58891 -0.08019
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-0.56152 0.09042
-0.31612 0.10222
0.46005 0.18323
1.14514 0.19379
1.03817 -0.07290
1.06777 -0.18452
1.16976 -0.05431
1.32418 0.17043
1.52855 0.18590
1.63214 0.01535
1.54463 -0.06699
1.44212 -0.06318
IMAGE=NL10_l.JPG
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-0.58840 -0.08121
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IMAGE=NL9_l.JPG
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UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Phylogeography and local adaption in prickly sculpin, *Cottus asper*" submitted by Stefan Dennenmoser in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

_____

Supervisor, S. Vamosi

_____

Co-Supervisor, S. Rogers

_____

Supervisory Committee Member, J. Theodor

_____

Internal/External, P. Kooyman

_____

External Examiner, D. Ruzzante, Dalhousie University

_____

Date