The genetic diversity of brook lampreys genus *Lampetra* (Petromyzontidae) along the Pacific coast of North America

David Andrew Boguski

Submitted for the Master of Science Degree

Department of Biological Sciences
University of Manitoba

UNIVERSITY OF MANITOBA

Faculty of Graduate Studies

Master's Thesis/Practicum Final Report

The undersigned certify that they have read the Master's Thesis/Practicum entitled:

The genetic diversity of brook lampreys genus Lampetra (Petromyzontidae) along the Pacific coast of North America

submitted by

David Boguski

in partial fulfillment of the requirements for the degree of

MSc.

The Thesis/Practicum Examining Committee certifies that the thesis/practicum (and oral examination if required) is:

Approved

(Approved or Not Approved)

☑ Thesis ☐ Practicum

	Name/Unit:	Signature:
	Dr. M. Docker (advisor)	[redacted]
	Dr. B. Ford (Advisory Committee member)	[redacted]
	Dr. R. Mooi (Advisory Committee member)	[redacted]
	Dr. R. Roughley (Advisory Committee member)	[redacted]

Date: July 20, 2009

fgs ADMIN

Abstract

The number of non-parasitic (brook) lamprey species in the genus *Lampetra* is underestimated since isolated populations are generally considered one species due to their relatively conserved body form. The phylogeographic and phylogenetic structure was estimated among and within *Lampetra* species along the Pacific coast of North America (presumed to represent *Lampetra richardsoni*; *L. pacifica* – which is currently regarded as a junior synonym of *L. richardsoni*; *L. ayresii*; and *L. hubbsi*) using up to three mitochondrial and three nuclear genetic markers. These data show that *L. richardsoni* as currently recognized is polyphyletic when lampreys (some of which show up to 8 K2P% sequence divergence) from Siuslaw River and Fourmile Creek (Oregon) and Mark West, Paynes, and Kelsey creeks (California) are included; *Lampetra pacifica* is a valid species; the population from Kelsey Creek almost certainly represents a new species; and those from Siuslaw, Fourmile, and Mark West may also be distinct species.

Acknowledgements

I express my profound respect and thanks to my graduate advisor Dr. Margaret Docker who has played an instrumental role in my growth as a student at the University of Manitoba. I am privileged to have been guided by Margaret – a leader at the forefront of lamprey biology. Many thanks to Dr. Stewart Reid (Western Fishes, Ashland, Oregon) and Damon Goodman (U.S. Fish & Wildlife Service, Arcata, California) who collected most of the lamprey samples and, when collaborating on an earlier phylogenetic project with Margaret Docker, were responsible for recognizing that there were potentially some very distinct lamprey populations along the Pacific coast of North America. I thank my advisory committee: Dr. Bruce Ford, Dr. Randy Mooi, and Dr. Rob Roughley for their diligence and meticulous comments contributing to the final written report. I also thank all those people responsible for the interesting discussion and for all comments significantly improving the manuscript, specifically Dr. Michele Piercey-Normore. I thank Dr. H.J. Walker, Jr. (SCRIPPS Institution of Oceanography), Steve Johnson (Oregon Department of Fish and Wildlife) and Dr. Douglas Markle (Oregon State University), Kim Hastings (U.S. Fish & Wildlife Service, Juneau, Alaska), Patricia Woodruff and Dr. Eric Taylor (University of British Columbia), Aaron Jackson (Confederated Tribes of the Umatilla Indian Reservation), and Christina Luzier (U.S. Fish & Wildlife Service, Vancouver, Washington) for providing additional lamprey specimens. I thank my family and labmates for their continual support and patience. I thank Dr. Georg Hausner and Dr. Dana Schroeder, and their students for technical laboratory assistance.

I gratefully acknowledge the financial assistance provided to me through the Barrett-Hamilton Scholarship (endowed by Mr. Michael Nesbitt), Manitoba Graduate Scholarship

(Province of Manitoba), and Natural Sciences and Engineering Research Council (NSERC) Postgraduate Scholarship Program (PGS-M) award. In addition, I am thankful for the various travel awards I received from the University of Manitoba (U of M) – from the Faculty of Graduate Studies, the Faculty of Science, and the Department of Biological Sciences – that allowed me to present my research at two international conferences. Financial support for the project was provided through the U of M University Research Grant Program (project #30938 and #33838) and the NSERC Discovery Grant program.

Preface

This thesis is written in manuscript format, meaning that the central chapters of the thesis are meant to be independent units. *Pro forma* each chapter contains its own abstract, introduction, methods, and discussion. Moreover, information reviewed in each section is not necessarily exclusive to that section, but may be reviewed or readdressed in other sections, where appropriate. However, each section exhibits its own purpose using its own dataset – the only overlap is cytochrome *b* sequences from representatives of the major clades. The thesis introduction is a review of works leading up to the current study, and an introduction to the phenomena investigated with this research. Each subsequent chapter is a manuscript prepared for submission to a peer-reviewed journal. Collaborators in addition to the author of this thesis contributed logistics to the overall project (particularly in terms of specimen collection), but all data collected, analyses, and composition were completed by the author. The thesis discussion is a synthesis of information explored in the two central chapters and provides insight into future direction and the larger significance of my research. Finally, a compilation of literature cited for the entire thesis is included.

Table of Contents

List of Tables

Chapter 1

Chapter 2

Table

List of Figures

Chapter 1

Chapter 2

List of Appendices

General Introduction

Lamprey biology and non-parasitic forms

Lampreys, together with the hagfishes, are the sole extant representatives of the most primitive vertebrates, the "jawless fishes." This group, known as Agnatha (despite considerable controversy regarding whether the extant jawless fishes represent a monophyletic group; see Meyer and Zardoya, 2003), are thought to have evolved about 500 MYA, followed by evolution of the first gnathostomes (jawed vertebrates) about 460 MYA (Sansom et al.,1996). There are up to 10 genera of extant lampreys, depending on the classification (Nelson et al., 2004), and 38 described species of lamprey worldwide (Potter and Gill, 2003). They are classified under the order Petromyzontiformes (Regan, 1911), which includes all 34 northern hemisphere species in the family Petromyzontidae (Renaud, 1997), and four southern hemisphere species allocated to either the monospecific Geotriidae or the monogeneric Mordaciidae (Hubbs and Potter, 1971; Potter, 1980a).

All lampreys begin life as blind filter-feeding larvae, known as ammocoetes, which burrow into soft sediment at the bottom of freshwater rivers or lakes. They feed on microscopic organisms and detritus (Sutton and Bowen, 1994; Mundahl et al., 2005). Metamorphosis in lampreys is the precursor to a change in the animal's environment, corresponding to adjustments to its mode of life or feeding habits, and is accompanied by the appearance of a functional eye, development of tooth-bearing plates and horny tooth cusps, and the replacement of many larval organs with those of the adult (reviewed in Hardisty, 2006). Following metamorphosis, they may adopt either a non-trophic (non-parasitic) feeding type that will spawn and die within 6-9 months (Potter, 1980b), or they may migrate

to freshwater lakes or sea water, and become parasitic on teleost fish for several months to several years, returning to fresh water to spawn and die (Beamish, 1980; Farmer, 1980). Non-parasitic lampreys are referred to as "brook" lampreys because they spend their entire life in their natal stream. Approximately half of all lamprey species are non-parasitic (Renaud, 1997), and the repeated emergence of non-parasitic brook lamprey forms from a presumed parasitic ancestor is a phenomenon very well known from the Petromyzontiformes (Blank et al., 2008). Closely-related parasitic and non-parasitic lamprey species often cannot be distinguished as larvae or in the early stages of metamorphosis (see Docker, in press) but diverge in the later stages of metamorphosis (Hardisty and Potter, 1971; Hardisty, 2006). Given their divergent adult feeding types, one of the most conspicuous external differences between them is adult body size (see "Paired and satellite species," below).

Pacific coast brook lamprey species are widely distributed in North America from Alaska to Mexico (Renaud, 1997; Page and Burr, 1991; Mejía et al., 2004). There are two to three Pacific coast brook lampreys described from the genus *Entosphenus* (*E. lethophagus* Hubbs, 1971, *E. hubbsi* Vladykov and Kott, 1976a, and *E. folletti* Vladykov and Kott, 1976b), and up to two in the genus *Lampetra* (*L. richardsoni* Vladykov and Follett, 1965 and *L. pacifica* Vladykov, 1973a), although the validity of *E. folletti* and *L. pacifica* as distinct species and the generic status of *E. hubbsi* are disputed (see Appendix A). I will focus on brook lampreys of the genus *Lampetra* (including *E. hubbsi*) to investigate their genetic diversity and phylogeography. *Entosphenus* brook lamprey species were not examined in this thesis as the genus *Entosphenus,* excluding *E. hubbsi,* is a shallow clade based on small genetic differences in mtDNA (Docker et al., 1999; Lorion et al., 2000; Docker, 2006; Goodman et al. 2008). However, the western river lamprey (*Lampetra ayresii* Günther,

1870) and the Pacific lamprey (*Entosphenus tridentatus* Gairdner in Richardson, 1836) – that are not brook lampreys – are included; the former which is closely related to *L. richardsoni* (Docker et al., 1999) and the latter to facilitate comparisons of the level of genetic divergence within a genus distributed over often sympatric geographical distribution (Damon et al., 2008). *Lampetra* specimens could be discriminated from other taxa based on geographical distribution and morphological differences (e.g., Goodman et al., in press) – see below.

The western brook lamprey (*L. richardsoni*) is a non-parasitic species that inhabits freshwater streams along the Pacific coast of North America. It was initially considered to be the same species as the European brook lamprey (*L. planeri*), and was not described as a separate species until 1965 (Vladykov and Follett, 1965). *Lampetra richardsoni* is generally thought to be distributed from Alaska to California (Vladykov and Follett, 1965; Moyle, 2002), although some authors (Vladykov, 1973a; Page and Burr, 1991) consider those brook lampreys in California to be *L. pacifica* (see below). *Lampetra richardsoni* can be identified as adults by several relevant morphological characteristics including a supraoral (SO) lamina with 2 cusps; 3 inner laterals with a 2-3-2 (at times 2-2-2) cusp arrangement; an infraoral (IO) lamina of 7-8 cusps; and the absence of posterial circumorals (see Appendix B; Vladykov and Follett, 1965; Vladykov and Kott, 1976a). *Lampetra richardsoni* is believed to have evolved from the parasitic western river lamprey (*L. ayresii*) or a parasitic lamprey that is closely related to it (see below).

The Pacific brook lamprey, *L. pacifica*, was described by Vladykov (1973a) as another, independently derived brook lamprey presumed to be limited to streams of California and Oregon. In California it has been collected in three tributaries of the Sacramento River (Mill Creek, Big Chico Creek, and Putah Creek) as well as the San

Joaquin River (Vladykov, 1973a). In Oregon it is presumed to occur south of the Columbia River basin; it has been extensively collected from Crystal Springs Creek, and some specimens were collected in the Willamette and Clackamas rivers (Vladykov, 1973a). *Lampetra pacifica* can be distinguished from *L. richardsoni* by typically having fewer trunk myomeres (53-58 with an average of 55.5; compared to 60-67 in *L. richardsoni* with an average of 63.2), fewer teeth on the anterior field that are less strongly developed, a smaller oral disc and lower dorsal fins, as well as an abundance of dark pigmentation in the mouth cavity (Vladykov, 1973a). However, Robins et al. (1991) considered this species to be synonymous with *L. richardsoni* on the basis of a statement in Bond and Kan (1986) that the morphological distinction between the two appears to be slight. To date, there have been no studies examining whether populations previously considered *L. pacifica* are genetically distinct.

Additionally, the Kern brook lamprey, *Entosphenus hubbsi* or *Lampetra hubbsi* (see below*)*, may be a more ancient non-parasitic derivative of this lineage. This brook lamprey is believed to be endemic to the Kern River system, California, which now connects with the San Joaquin River through artificial Millerton Lake (Vladykov and Kott, 1976a). This species appears to demonstrate an intergradation of morphological characteristics among three lamprey genera (*Lethenteron, Lampetra,* and *Entosphenus*; Vladykov and Kott, 1976a; Docker et al., 1999), but was placed within the genus *Entosphenus* (Vladykov and Kott, 1976a) and has not been reassigned (Nelson et al., 2004). The Kern brook lamprey has a single row of unicuspid posterials, which is consistent with *Lethenteron* (Hubbs and Potter, 1971); four inner laterals and an IO lamina with 5 cusps, which is consistent with *Entosphenus* (Vladykov and Kott, 1976a; Hubbs and Potter, 1971; Docker et al., 1999); and

an SO lamina with two cusps, which is characteristic of *Lampetra* and *Lethenteron*; however one specimen had a third cusp, consistent with the genus *Entosphenus* (Vladykov and Kott, 1976a). However, mitochondrial DNA sequence data suggest *E. hubbsi* should be recognized as *Lampetra hubbsi* (Docker et al., 1999). For the remainder of this study, the Kern brook lamprey is referred to as *Lampetra hubbsi*. Docker et al. (1999) used small gene fragments (a total of 735 bp) and a phenetic method for reconstructing the phylogeny based on relevant morphological characteristics identified in the original species descriptions. I will test the phylogeny of these and other brook lampreys using a multigene approach (complete cytochrome *b* gene in Chapter 1; other mtDNA and nuclear markers in Chapter 2) and cladistics methods based on parsimony and Bayesian inference.

Paired and satellite species

The evolution of brook lampreys and their relationship to parasitic lampreys have been long-discussed in lamprey research (Zanandrea, 1959; Vladykov and Kott, 1979; reviewed in Docker, in press). For example, Loman (1912) recognized the European river lamprey (*Lampetra fluviatilis*) and the European brook lamprey (*Lampetra planeri*) to be morphologically very similar, but differed in their timing of metamorphosis and sexual maturation. In many genera, the ammocoetes of non-parasitic and parasitic lamprey species are morphologically very similar, yet as adults, these lampreys adopt different migratory and feeding habits as well as shifts in morphological, histological, and behavioural characteristics (Vladykov and Kott, 1979; Potter, 1980a). Additionally, these species often largely overlap in geographical distribution (Hubbs, 1925). It is believed that these 'paired species' or multiple 'satellite species' (Zanandrea, 1959 and Vladykov and Kott, 1979, respectively) are

closely related, with the non-parasitic species originating from a parasitic ancestor similar to that of the extant parasitic species (Zanandrea, 1959; Hubbs and Potter, 1971). This premise is based on the distribution of paired species and on differences in structures related to feeding (see Docker, in press). For example, rudimentary development of intestinal mucosal folds common to parasitic lampreys has been observed in non-parasitic lampreys during metamorphosis (Hilliard et al., 1983; Youson and Beamish, 1991). Additionally, buccal glands, which produce an anticoaguant necessary for parasitic feeding, develop during metamorphosis in non-parasitic as well as parasitic species (Baxter, 1956). Since it is generally thought that size-assortative mating between large parasitic and small non-parasitic lampreys would result in reproductive isolation between these lamprey forms (e.g., Beamish and Neville, 1992), most lamprey taxonomists recognize life-history type as a species-specific characteristic. This would be consistent with the biological species concept (Mayr, 1942), supporting a species as a group of actually or potentially interbreeding natural populations, which is reproductively isolated from other such groups. Not all authorities, however, consider life history type as warranting species designation (McPhail and Lindsey, 1970).

'Paired' or 'satellite' species occur in seven of the 10 lamprey genera (Hubbs and Potter, 1971). Therefore, non-parasitism appears to have evolved independently in different taxa (Hubbs and Potter, 1971; Vladykov and Kott, 1979). There are also suggestions that non-parasitism has evolved multiple times within recognized species, that is, different populations of the same species may have evolved independently such that a given brook lamprey species is polyphyletic (i.e., a taxon that is derived from two of more ancestral sources; see Docker, in press).

Mitochondrial (mt)DNA has also suggested that several species pairs are genetically indistinguishable over sympatric distributions (Docker et al., 1999; Lorion et al., 2000). For example, *L. richardsoni* (see above) is 'paired' with *L. ayresii*. *Lampetra ayresii* was redescribed by Vladykov and Follett (1958) after several earlier classifications (Ayres, 1855; Günther, 1870; Regan, 1911). It is an anadromous species with a more restricted distribution than *L. richardsoni*, and apparently exists only as widely scattered, isolated populations (Moyle et al., 1995) distributed from Tee Harbor, AK, to the Sacramento-San Joaquin drainage (Page and Burr, 1991). Molecular analysis of this species 'pair' in regions of southern British Columbia have yet to reveal any genetic differences using mtDNA (Docker et al., 1999) or diagnostic differences using allozyme data (Beamish and Withler, 1986), but to what extent this is true across the rest of their ranges is not known. Similarly, the degree of intraspecific genetic variation in both these species is unknown. Freshwater-resident *L. richardsoni* populations are generally isolated from one another, and *L. ayresii* – although anadromous – undergoes limited migration (Beamish, 1980); therefore, genetic differentiation might be expected among disjunct locations – unlike highly migratory anadromous Pacific lampreys (*Entosphenus tridentatus*) distributed from Alaska to California (Goodman et al., 2008).

Not all non-parasitic species have a parasitic counterpart. Several lamprey species distributed at or near their most southern limits have not been paired with an extant parasitic species (Hubbs and Potter, 1971; Docker et al., 1999). Relict species include the Po brook lamprey (*Lethenteron zanandreai*), the least brook lamprey (*Lampetra aepyptera*), and the Kern brook lamprey (*L. hubbsi*), that presumably represent more ancient non-parasitic

derivatives. *Lampetra hubbsi*, distributed in south-central California, is included in this study.

Taxonomy

Conventional lamprey taxonomy is problematic because lampreys do not possess many of the characters used in traditional fish taxonomy (e.g., bony elements, scales, paired fins) and are therefore difficult to classify (Appendix A; Hubbs and Potter, 1971). Furthermore, most of the characters that are useful for lamprey classification are limited to adult specimens (e.g., dentition) and cannot be used in their larval life-stage. In ammocoetes, identification to genus is often limited to such characters as pigmentation, the number of trunk myomeres, and the arrangement of the dorsal fins (Vladykov and Kott, 1980; Richards et al., 1982), whereas the species status can be identified in adults using those characters formerly mentioned as well as dentition, body proportions, and the number and morphology of velar tentacles (Hubbs and Potter, 1971; Gill et al., 2003). Adult body size, life history type, and habitat are also commonly used to distinguish between closely-related taxa (Docker et al., 1999). Nonetheless, degenerate (in terms of number of teeth and cusps), intermediate, and homoplastic characteristics often confound lamprey taxonomy. The variable degree of degeneracy in the dentition of various non-parasitic species has been hard to classify; however, it is believed to provide support for their repeated evolution over a long period of time – with more ancient non-parasitic species possessing more degenerate characters (Hubbs and Potter, 1971). *Lampetra hubbsi* illustrates intermediate characters between different genera (see above), whereas other species such as *Lampetra aepyptera* and *Lethenteron zanandreai* possess morphological characters (such as variable dentition) that

may be construed as homologous (reviewed in Docker et al., 1999), despite an independent origin.

The uncertainty surrounding lamprey taxonomy has thus been the attention of much debate – in particular, generic and subgeneric rankings (Appendix A; Vladykov and Kott, 1979; Potter, 1980a) and the generic placement of various species. Vladykov and Kott (1979) and Renaud (1997), for example, have classified *Lethenteron, Lampetra,* and *Entosphenus* as distinct genera. Alternatively, Potter (1980) has classified these taxa as subgenera within the genus *Lampetra*. Similarly, Bailey (1980) also classified these taxa as subgenera within the genus *Lampetra* and further suggested that *Eudontomyzon* and *Tetrapleurodon* also be recognized as subgenera within *Lampetra*. Species names in this thesis will use the generic designations of Vladykov and Kott (1979) and Renaud (1997) that are supported by Gill et al. (2003). Despite the most recent American Fisheries Society (AFS) list of species names (Nelson et al., 2004) still using *Lampetra* collectively to describe *Lampetra, Entosphenus, Lethenteron*, and *Tetrapleurodon* subgenera (*Eudontomyzon* does not occur in North America and is not explicitly dealt with by the AFS), AFS is currently reconsidering this decision and will recognize each as distinct genera in its next list of names (see Renaud et al., in press).

Due to the paucity of morphological characters used to discriminate species, lampreys showing even subtle morphological differences may be distinct species. For example, the European and western river lampreys (*L. fluviatilis* and *L. ayresii*, respectively) were originally considered as a single species and can be distinguished morphologically based on relatively small differences in body proportions (Vladykov and Follett, 1958); however, they are genetically very distinct (Docker et al., 1999). This may particularly be the case with

brook lampreys. Synonymy of lamprey species – such as whether *Ichthyomyzon hubbsi* Raney, 1952 should be synonymized with *I. greeleyi* Hubbs and Trautman, 1937 (Vladykov and Kott, 1979) – is often a contentious issue regarding brook lampreys. Other examples of where subtle morphological differences have led to taxonomic reconfiguration include a proposal by Vladykov and Kott (1976b) indicating that there was sufficient regional diversity in *Entosphenus lethophagus* from the Klamath River system to warrant the new species designation of Northern Californian brook lamprey (*E. folletti*), but it has since been synonymized (Robins et al., 1980). Similarly, a population of the Gulf lamprey (*Lethenteron meridionale* Vladykov et al., 1975) has been synonymized with *Lampetra aepyptera* (Etnier and Starnes, 1993). Alternatively, the Drin brook lamprey (*Eudontomyzon stankokaramani* Karaman, 1974) that had been synonymized with the Carpathian lamprey (*Eudontomyzon danfordi* Regan, 1911) has since been redescribed as a new species (Holčík and Šorić, 2004). Furthermore, Hubbs (1971) identified regional variation within the Pit-Klamath brook lamprey (*Entosphenus lethophagus*), though he did not consider it to warrant specific or subspecific designation. Regional variation within this species included the retention or loss of the nuptial metamorphosis, inferred to be a form of paedomorphosis (Hardisty, 1963). Hardisty (2006) draws attention to the possibility of geographical or ecological influence on trunk myomere count, where northern populations of non-parasitic lampreys have on average higher trunk myomere counts compared to those conspecifics in their more southerly ranges. Similar parallels are apparent in the average number of vertebrae and gill rakers found in other fish species (Beacham, 1985). Such evidence in lamprey research highlights a downfall of relying on this type of information alone to assess relationships between populations or as a method of classification.

The biological species concept (BSC) seems to have developed from observed relationships between breeding and morphology in some groups of organisms, and subsequently breeding tests ensued to test species status, despite difficult cases (Donoghue, 1985). The morphological species concept (MSC) is presented as a logical argument (Mayr, 1963): "Natural populations considered by general consent to be species are morphologically distinct. Morphological distinctness is thus the decisive criterion of species rank. Consequently, any natural population that is morphologically distinct must be recognized as a separate species." However, with the rise of genetics as a formal discipline, it became appropriate to assume that the exchange of genes was of prime importance in evolution (Mayr and Provine, 1980). Currently, there are at least three versions of the phylogenetic species concept (PSC), and all define species as "the smallest biological entities that are diagnosable and/or monophyletic" (Mayden, 1997).

The PSC appears to be the most applicable to lamprey research since, as previously shown, classifications and phylogenies based mainly on morphological data may result in some taxonomic problems. A phylogeny of lampreys distributed worldwide is currently being researched using complete cyt *b* sequence (N.J. Lang, Field Museum of Natural History, Chicago, IL, unpublished data). These data may help resolve debates regarding phylogeny and will lead to appropriate methods of classifying lampreys by eliminating ambiguous characters (i.e., eliminating morphological characters that may be homoplastic). It is always essential to discriminate between homologous or convergent (homoplastic) similarities of the characters. Only homologous characters are considered to indicate true phylogenetic relationships (Rieppel, 1980). Indeed, mtDNA sequence has proven useful in clarifying taxonomic relationships among many taxa where homoplasy or conservative

morphologies may otherwise have obscured the relationships (Docker et al., 1999; Yamazaki et al., 2006; Blank et al. 2008). For example, populations described as a single species based on morphology have been shown to be genetically very diverse (e.g., Martin and White, 2008). Several genetically distinct populations appear to be distinct species based on the PSC, and in at least one case, subsequent studies have also shown them to be species according to the BSC. In this case, two as-yet undescribed species of lampreys previously described as the Far Eastern brook lamprey (*Lethenteron reissneri*) from northern Japan (currently called *L.* sp. N) and from southern Japan and Korea (*L.* sp. S) were found to be genetically very distinct from each other and from other *Lethenteron* species (*Lethenteron japonicum*[1] [von Martens, 1868], *Lethenteron kessleri* [Anikin, 1905] and *L. reissneri* [Dybowski, 1869] from Russia; Yamazaki et al., 2003). Despite morphological similarity, there is no evidence for hybridization where they co-occur. Although spawning seasons and sizes at maturity overlapped (*L.* sp. S spawned later on average and was significantly larger), nesting assemblages nevertheless comprised only males and females of same form (Yamazaki and Goto, 2000).

Mitochondrial and nuclear DNA

The cytochrome *b* gene is one of the most widely used genes for phylogenetic studies of animals. Cytochrome *b* is one of the cytochromes involved in electron transport in the respiratory chain of mitochondria (Esposti et al., 1993). Although it evolves slowly in terms of non-synonymous substitutions, the rate of evolution in silent positions is relatively fast,

[1] The accepted name for this species is now *Lethenteron camtschaticum* (Nelson et al., 2004; Renaud et al., in press; see Appendix A)

making it appropriate for species-level comparisons and many population level questions (Irwin et al., 1991).

As a molecular marker, mtDNA has many advantages. For example, it generally evolves faster than single copy nuclear genes (Brown et al., 1982). Different regions of the mitochondrial genome evolve at different rates (Saccone et al., 1991), allowing suitable regions to be chosen for the question under study. Additionally, the conserved content and order of gene sequences allows primers to be designed for a variety of organisms without prior knowledge of their genomes. This has made mtDNA the genetic marker of choice for many molecular phylogenetic studies, particularly in animals (e.g., Bermingham and Avise, 1986; Avise et al., 1987; Irwin, et al., 1991).

One of the potential drawbacks to mitochondrial DNA is that it does not recombine (i.e., represents a single locus; Hayashi et al., 1985), although some evidence of recombination has recently been reported (Eyre-Walker et al., 1999; Hagelberg et al., 1999). Mitochondrial DNA is a presumed neutral marker (reviewed in William et al., 1995) that is maternally inherited in most species (exceptions include paternal leakage in mice, Gyllesten et al., 1991; biparental inheritance in marine mussels, Zouros et al., 1992). This being the case, phylogenies based solely on mtDNA may not be consistent with other phylogenies such as those based upon nuclear markers (e.g., Smith, 1992; Evans et al., 2004). Cases of hybridization, common among freshwater fishes (Schwartz, 1972), may go unrecognized if only mtDNA markers are employed as diagnostic tools. Furthermore, only the maternal half of the ancestry of many hybrid species would be reflected using a mtDNA phylogeny.

Nuclear DNA could subsequently be considered to alleviate problems associated with maternal inheritance when using mtDNA. Multi-copied ribosomal (r)RNA genes, located in

the nucleus of eukaryotic cells, are recombining, biparental markers, which have been used for phylogeny reconstruction (Hillis and Dixon, 1991). Due to their recombinant nature, these markers can reveal recent gene flow and hybridization events (Mayer and Soltis, 1999). Like mtDNA, because of the highly conserved nature of ribosomal 18S, 5.8S, and 28S genes, it is possible to amplify desired fragments for comparison across a wide range of taxa (Hillis and Dixon, 1991). A number of nuclear loci have been used to address the relationship among lampreys, hagfish, and gnathostomes (e.g., Mallatt and Sullivan, 1998; Zardova and Meyer, 2001; Yu et al., 2008) – or to reconstruct other deep divergences – but these loci appear not to provide sufficient resolution to infer phylogenies among closely-related lamprey taxa. However, the internal transcribed spacer (ITS) regions of rDNA are more variable and, because they are flanked by conserved regions from which primers can be designed in a variety of taxa, are very useful for making comparisons among more closely-related species. Lampreys, as do most eukaryotic organisms (with the exception of microsporideans, Huang et al., 2003), have two internal transcribed spacers; ITS1 is located between the 18S gene and the 5.8S gene, and ITS2 is located between the 5.8S and the 28S gene. The ITS regions have been used in molecular systematic studies mainly focusing on resolving interspecific relationships within genera and occasionally at higher taxonomic levels (Presa et al., 2002). These regions have been extensively used in plant systematics and have also been used to clarify phylogenies of a number of different fish species (Domanico et al., 1997; Presa et al., 2002), primates (Gonzales et al., 1990), and many invertebrates (Fritz et al., 1994; Rocha-Olivares et al., 2001).

Microsatellite markers are another form of nuclear marker that contain highly variable tandem repeats of one to six nucleotides found at high frequency in most taxa

(Selkoe and Toonen, 2006). Microsatellites are highly polymorphic and can provide estimates of migration, distinguish relatively high rates of migration from panmixia, and can estimate the relatedness of individuals (see Selkoe and Toonen, 2006). Other advantages to using microsatellites are that they are single-locus codominant markers that when used in conjunction with other microsatellites suggest variable mutation rates (Jin et al., 1996); this allows the recovery of multiple evolutionary events that span a much larger number of generations (Selkoe and Toonen, 2006) than when using only one single-locus marker.

Forthcoming chapters

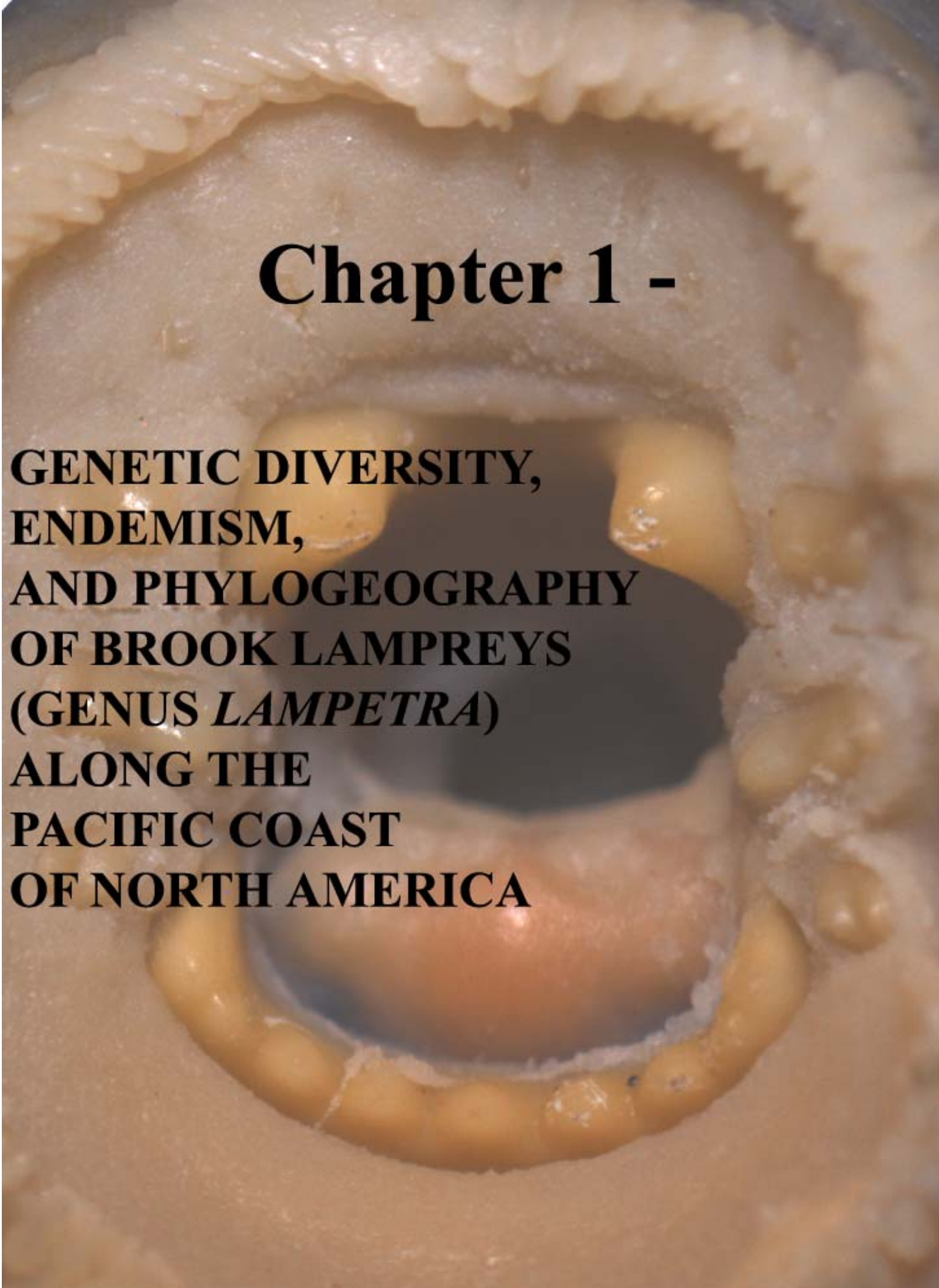
To investigate the genetic diversity of brook lampreys distributed along the Pacific coast of North America and explore the patterns of evolution that may exist within and among them, the phylogeny and phylogeography of west coast species within the genus *Lampetra* sensu stricto was inferred. This included a sampling scheme that focused largely on putative western brook lamprey (*L. richardsoni*) populations from throughout its range but that also involved relevant closely-related species: representatives of the Pacific and Kern brook lamprey (*L. pacifica* and *L. hubbsi*, respectively) and *L. richardsoni*'s parasitic counterpart, *L. ayresii*. The investigations spanned two evolutionary scales. At the lower level, observations were made for population-level diversification, genetic variation, and phylogeography. At the higher level, species-level diversification among congeners was more closely examined.

In the first chapter, a molecular phylogeny is constructed using mtDNA (complete cyt *b* gene) to examine the genetic diversity and phylogeography of Pacific coast *Lampetra*. A total of 136 *Lampetra* specimens collected from Alaska to California, including *L.*

richardsoni, *L. ayresii*, *L. pacifica,* and *L. hubbsi* from their type locales, were used in phylogenetic reconstructions.

Chapter 2 tests congruence of other DNA markers in reconstructing the phylogeny among major clades identified in Chapter 1. In addition to cyt *b*, mitochondrial NADH subunit 2 (ND2) and cytochrome oxidase subunit 1 (CO1) genes were sequenced to assess the congruence between mtDNA markers. Additionally, nuclear recombinant markers were also sequenced to contrast maternally inherited mtDNA, including multi-copy ITS1 (above), a single-copy nuclear intron from a transporter associated with antigen processing (TAP) gene (Uinuk-ool et al., 2003) used in phylogenetic application for the first time, as well as nine multi-locus microsatellite markers to survey across a larger region of the nuclear genome.

Conclusions stemming from the research are discussed following these chapters. Hypotheses pertaining to the evolution of non-parasitic and parasitic lampreys are presented to provoke future studies on *Lampetra* brook lampreys, their parasitic counterparts, and other brook lamprey taxa worldwide.



Chapter 1 -

GENETIC DIVERSITY, ENDEMISM, AND PHYLOGEOGRAPHY OF BROOK LAMPREYS (GENUS *LAMPETRA*) ALONG THE PACIFIC COAST OF NORTH AMERICA

Abstract

Brook lamprey is the collective term used to describe non-parasitic lampreys, lampreys that do not feed at all following metamorphosis, and this is a life history type that is presumed to have evolved repeatedly in seven of the 10 recognized lamprey genera. Several brook lamprey species have been described in the genus *Lampetra* but the number of brook lamprey species may be underestimated; isolated populations are often considered the same species due to their relatively conserved body form. Here, I estimate the phylogeographic structure among and within *Lampetra* species found throughout the Pacific drainage of North America (*Lampetra richardsoni*; *L. pacifica* – which is currently regarded as a junior synonym of *L. richardsoni*; *L. ayresii*; and *L. hubbsi*) using the mitochondrial cytochrome *b* gene. I present evidence that *L. richardsoni* is a polyphyletic species, that the level of genetic variation between *L. pacifica* and *L. richardsoni* (more than 2.3%) approximates levels found interspecifically in other fish species, and that *L. ayresii* (which is considered *L. richardsoni*'s parasitic 'paired species') is not reciprocally monophyletic with respect to *L. richardsoni*. The greatest sequence divergence (more than 8%) was observed among two putative *L. richardsoni* populations. The most divergent population was from Kelsey Creek, CA, and four additional populations from Siuslaw River (OR), Fourmile Creek (OR), Paynes Creek (CA), and Mark West Creek (CA) also showed deep genetic divergences compared to *L. richardsoni* from it type locale (Smith Creek, BC). The population from Kelsey Creek is almost certainly a new species. Based on their phylogenetic placement using both parsimony and Bayesian methods, the population from Mark West Creek is sister to *L. pacifica* and the population from Paynes Creek is sister to *L. hubbsi*. These populations may represent additional populations of these two species or one or both may be distinct species; Mark

West lampreys showed 4.3% sequence divergence relative to *L. pacifica* and Paynes Creek lamprey were 1.5% divergent from *L. hubbsi*. Siuslaw River and Fourmile Creek lampreys formed a clade with *L. pacifica* using parsimony analysis, but not using Bayesian methods and were 2.6 – 4.4% divergent from *L. pacifica*. All specimens examined from these populations were larvae, however, whereas most species-level characters in lamprey taxonomy are from the adult stage. Therefore, morphological examination of adult specimens will be required before one or more of these populations can be described as new species.

1. Introduction

The reconstruction of evolutionary histories is of primary importance when testing hypotheses regarding intricate and dynamic patterns of evolution. There are 38 described species of lampreys worldwide and approximately half are non-parasitic, non-migratory brook lampreys (Renaud, 1997; Potter and Gill, 2003). Brook lamprey species are found in seven of the 10 recognized lamprey genera, implying that non-parasitism has arisen independently multiple times (Hubbs and Potter 1971; Vladykov and Kott 1979). The repeated evolution of isolated non-parasitic brook lamprey forms within recognized species has also been proposed (e.g., Hubbs and Trautman 1937), but has not been well documented due to their rather conserved body form.

This is certainly the case along the Pacific coast of North America, where larval specimens – which can be collected more reliably than adults – predominate many collections. Brook lampreys remain as larvae for three to eight years (e.g., Schultz, 1930; Potter, 1980b), while the adult phase is relatively short lived; following metamorphosis, brook lampreys do not feed and spawn and die within 6-9 months (Hardisty and Potter, 1971). This proves problematic to taxonomists because most of the characters that are useful for lamprey classification (e.g., dentition, relative size of the eye and oral disc, number and morphology of velar tentacles; Hubbs and Potter, 1971; Gill et al., 2003) are limited to adult specimens. There are relatively few characters to distinguish among closely-related larvae found within a genus (e.g., Neave et al., 2007; Goodman et al., in press).

The Pacific west coast hosts a remarkable diversity of ichthyofauna, including several distinct lamprey species, but the number of lamprey species, their distribution, and their interrelationships are still disputed. The western brook lamprey (*Lampetra richardsoni*),

described by Vladykov and Follett (1965), is a purely freshwater form. *Lampetra richardsoni* is widely distributed, but there does not appear to be consensus among authors regarding its distributional range from Alaska to Oregon (Vladykov and Follett, 1965; Page and Burr, 1991) or extending into the Sacramento drainage in California (Moyle, 2002). This discrepancy is generally related to the fact that some authors (Vladykov, 1973a; Page and Burr, 1991) consider the more southerly populations of *Lampetra* brook lampreys to be *L. pacifica* (see below). *Lampetra richardsoni* is generally believed to have evolved from a form similar to that of the extant parasitic anadromous river lamprey, *Lampetra ayresii* (Hubbs and Potter, 1971). Although its range roughly overlaps with that of *L. richardsoni, L. ayresii* apparently occurs only as widely scattered, isolated populations (Moyle et al., 1995). These two species are generally considered 'paired' or 'satellite' species (Zanandrea, 1959; Vladykov and Kott, 1979). In most lamprey genera, there exist two or more species in which the larvae are morphologically similar or indistinguishable, yet at metamorphosis, these lampreys adopt different migratory and feeding habits as well as different morphological, histological, and behavioural characteristics (Vladykov and Kott, 1979; Potter, 1980a); it is believed that these species are closely related, with the non-parasitic species originating from the parasitic species. A close relationship between *L. richardsoni* and *L. ayresii* was supported by Docker et al. (1999), who found no differences in mitochondrial (mt)DNA sequence in a few specimens of each species collected in British Columbia. However, Beamish and Withler (1986) found that geographically isolated populations of *L. richardsoni* were as genetically distinct from one another as they were from *L. ayresii*, suggesting a polyphyletic origin of the freshwater-resident non-parasitic species. The Pacific brook lamprey, *L. pacifica*, was described by Vladykov (1973a) as another, independently derived

brook lamprey presumed to be limited to streams of California and Oregon. However, Robins et al. (1991) considered this species to be synonymous with *L. richardsoni* on the basis of a statement in Bond and Kan (1986) that the morphological distinction between the two appears to be slight. To date, there have been no studies examining whether populations previously considered *L. pacifica* are genetically distinct. Additionally, the Kern brook lamprey, *L. hubbsi*, may be a more ancient non-parasitic derivative of this lineage. This brook lamprey that is endemic to the Kern River system, California (Vladykov and Kott, 1976a), appears to demonstrate an intergradation of morphological characteristics among three lamprey genera (*Lethenteron*, *Lampetra*, and *Entosphenus*; Docker et al., 1999), but was originally placed within the genus *Entosphenus* (Vladykov and Kott, 1976a). However, mtDNA sequence data suggest *Entosphenus hubbsi* should be recognized as *Lampetra hubbsi* (Docker et al., 1999). For the remainder of this study, the Kern brook lamprey will be referred to as *Lampetra hubbsi* and this hypothesis will be tested herein with a larger molecular dataset.

Within the last two decades, the classical teachings of vertebrate evolution often have been challenged by new genetic results (Meyer and Zardoya, 2003). These data have helped resolve debates regarding phylogeny and have contributed to methods of classification. In lampreys, mtDNA sequence has proven useful in clarifying taxonomic relationships among many taxa where homoplasy or conservative body form may otherwise have obscured the relationships (Docker et al., 1999; Yamazaki et al., 2006; Blank et al., 2008). The conserved body form of brook lampreys has made classification based on traditional characters especially difficult. Populations described as a single species based on morphology have been shown to be genetically very diverse (e.g., Martin and White, 2008), and many may

represent distinct species based on phylogenetic species criteria (Wheeler, 1996). As with allozyme data (e.g., Schreiber and Engelhorn 1998), studies using mtDNA suggest that the European brook lamprey, *L. planeri*, has evolved independently at least twice from the European river lamprey, *L. fluviatilis* (Espanhol et al., 2007; Blank et al., 2008). Therefore, morphological and genetic differences between paired species, if any, probably reflect the time when each non-parasitic species evolved. While a number of nuclear loci have been used to address the relationship among lampreys, hagfish, and gnathostomes (e.g., Mallatt and Sullivan, 1998; Zardova and Meyer, 2001; Yu et al., 2008) – or reconstruct other deep divergences – these loci appear not to provide sufficient resolution to infer phylogenies among closely-related lamprey taxa.

Here, I investigate the phylogenetic and phylogeographic relationships of west coast brook lamprey species with the complete mitochondrial cytochrome *b* (cyt *b*) gene, including a sampling scheme that involves relevant closely-related species, in particular representatives of *L. richardsoni, L. ayresii, L. pacifica,* and *L. hubbsi*. Additionally, the hypothesis that *L. ayresii* independently gave rise – at different times and in different places – to different *L. richardsoni* populations is tested. Genetic sequence data used in phylogenetic analyses are almost exclusively from brook lampreys, with the exception of *L. ayresii* samples that were not extensively sampled across their distributional range. Implications of the phylogeny to lamprey classification and the repeated evolution of non-parasitism are discussed.

2. Materials and Methods

2.1 Taxon sampling

Lampreys were collected from river drainages and tributaries along the Pacific coast of North America from Alaska to California, USA (Table 1-1; Figs. 1-1, 1-2, 1-3) by Dr. Stewart Reid (Western Fishes, Ashland, OR) and Damon Goodman (Arcata Fish and Wildlife Service, Arcata, CA) and deposited in the Humboldt State University Fish Collection, or collected by myself and Dr. Stewart Reid and deposited at the University of Manitoba in the Stewart-Hay Museum. Additional samples were obtained from other individuals (see Acknowledgements) or were sent from museum collections (Table 1-1). Except where noted (Table 1-1), larval lampreys were collected due to their year-round availability and greater abundance; different size classes were collected to ensure that individuals were not all siblings. Larvae belonging to the genus *Lampetra* were identified (i.e., discriminated from *Entosphenus*) by the collectors in the field based on caudal pigmentation (Richards et al., 1982; Goodman et al., in press); identification to species was based on the distributional range of each species where congeners are not known to co-exist, or when present, adult morphology (Table 1-1). Single localities were usually sampled within each tributary, with the exception of Clackamas River, in which two localities were sampled. Samples obtained include the type locale of *L. richardsoni* (Smith Creek, BC), *L. pacifica* (Clackamas River, OR), *L. ayresii* (Sacramento Delta, CA), and *L. hubbsi* (Merced River, CA). Samples were collected by kicking substrate into a dipnet, or by using an ABP-2 backpack electroshocker (University of Wisconsin, Engineering Technical Services, Madison, Wisconsin). DNA was extracted from muscle tissue from each fish preserved in 95% ethanol.

2.2 DNA extraction, PCR amplification and sequencing

DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol with the exception of adding 10 µL of 20 mg/ml proteinase K (Invitrogen) to 600 µL of nuclear lysate. The cyt *b* gene was amplified via polymerase chain reaction (PCR). The complete 1191 bp cyt *b* gene and flanking regions were amplified using newly designed primers located in tRNA-Glutamine (Glu-F 5'-CACCGTTGTAGAATTCAACTATAAG-3') and tRNA-Proline (Pro-R 5'-TAATTTAATGTTAAGATRCTAGCTTTGG-3'). An alternative reverse primer found in the 12S rRNA gene was designed for use in samples collected in Mark West Creek (Cyt*b*-12S-R 5'-GTAAAACGACGGCCAGTGTGCGGAAACTTGCATGTG-3'), since the reverse primer Pro-R would not amplify the cyt *b* gene.

Each 30 µL PCR reaction contained 10x PCR manufacturer's buffer (20 mM Tris-HCl pH 8.4; 50 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 266 pmol of each primer, and 0.02 U of T*aq* DNA Polymerase (Invitrogen). Reactions were initially denatured at 94 °C for 2 min. Amplifications of all fragments were carried out in 30 cycles: denaturation at 94 °C for 2 min, primer annealing at 60 °C, 58 °C, and 55 °C for 10 cycles each, extension at 72 °C for 2 min, and additional extension at 72 °C for 5 min.

Purified PCR product was used as a template for the sequencing reaction using ABI PRISM® Big-Dye™ Terminator (v3.1, Applied Biosystems Inc.). Sequences were read on the ABI 3130 Genetic Analyzer (Appied Biosystems Inc.) automatic sequencer. Primer Pro-R as well as two internal sequencing primers (Cyt*b*-340R 5'-GACTCCAACGTTTCATGTYTCTTT-3' and Cyt*b*-196F

5'-GCCTTYTCTTCAGTTATACACATTTG-3') were used to sequence the complete cyt *b* gene. The reverse internal sequencing primer Cyt*b*-340R was used rather than the GLU-F primer found upstream of cyt *b* to avoid potential problems arising from heteroplasmy or PCR slippage in the non-coding region flanking the 5' end of cyt *b* (M.F. Docker, unpublished data). Primer Cyt*b*-12S-R was substituted for primer Pro-R when sequencing the Mark West Creek samples.

2.3 Phylogenetic analyses

Phylogenetic analyses included sequence data from 147 specimens including two sequences retrieved from Genbank: European river lamprey (*Lampetra fluviatilis*; GenBank No. Y18683) and sea lamprey (*Petromyzon marinus*; GenBank No. U11880). Cytochrome *b* sequences from 135 *Lampetra* specimens and 10 Pacific lamprey (*Entosphenus tridentatus*) derived from this study were submitted to GenBank (Table 1-1; Genbank Accession Nos. X-X). An effort was made to include at least four samples from each location. Preliminary sequence comparisons revealed few, if any, genetic differences within locations and therefore additional samples were not included (but see below regarding one population which was not monophyletic; Fig. 1-2). *Entosphenus tridentatus* samples were sequenced to provide a comparison to the levels of genetic variation within another lamprey species distributed over a similar geographic range. Analyses were rooted with *P. marinus* as the outgroup based on previous phylogenies (Hubbs and Potter, 1971; Docker et al., 1999; Gill et al., 2003).

Cytochrome *b* sequences were aligned using ClustalX v2.0 (Higgins and Sharp, 1989; Larkin et al., 2007). Parsimony analysis was conducted using a heuristic search and tree bisection and reconnection (TBR) branch swapping in PAUP* v4.0b10 (Swofford, 2002). A

routine parsimony analysis was computationally prohibitive due to the large number of haplotypes (53) observed. Therefore, I conducted parsimony tree searches for each dataset using the parsimony ratchet (PAUPRat; Nixon, 1999) and the MULTREES option in PAUP*. The ratchet was executed in PAUP* using 20 independent runs each with 200 iterations. For each iteration, 25% of the characters were perturbed to produce a parsimonious tree. The ratchet facilitates finding the globally optimum tree by weighting a portion of randomly selected characters in sequential tree searches. Node support for the 50% majority rule consensus tree was generated using bootstrap analyses (Felsenstein, 1985) with 1000 pseudoreplicates and 100 RAS.

Bayesian Metropolis coupled Markov chain Monte Carlo (B-MCMCMC) analyses were conducted with MrBayes v3.1 (Ronquist and Huelsenbeck, 2003; Ronquist and Huelsenbeck, 2005). Modeltest 3.6 (Posada and Crandall, 1998) suggested the GTR (Tavaré, 1986) model of evolution using an Akaike Information Criterion (AIC) test, with invariable sites (I = 0.448), and inclusion of the gamma shape parameter (Γ = 0.769) for the cyt *b* data. Additional final parameters included base frequencies where freqA = 0.2915, freqC = 0.2323, freqG = 0.1338, and freqT = 0.3423, and the rate matrix A/C = 4.78, A/G = 76.22, A/T = 2.10, C/G = 2.70, C/T = 38.95, and G/T = 1.00. Bayesian analysis was executed using two independent runs of 10,000,000 generations, sampling every 1000^{th} generation, resulting in 20 002 trees. The burnin for tree reconstruction was 9280 to incorporate only average standard deviation of split frequencies values (which is a measure of similarity between the tree samples in each independent run) of less than 0.006. The phylogeny resulted from a 50% majority rule consensus of 1442 trees. Node support for the Bayesian consensus tree was assessed through posterior probabilities.

The Kishino-Hasegawa (KH) test (Kishino and Hasegawa, 1989) was implemented in PAUP* comparing the consensus of trees generated by parsimony and Bayesian methods. The null hypothesis is no significant difference between methods. Two independent tests were performed using parsimony criterion and using likelihood criterion with the evolutionary model suggested from ModelTest.

2.4 Regression analyses

I hypothesize a significant correlation between genetic and geographic distance. Two independent analyses were executed based on results from the phylogenetic analyses (below). The regression analyses incorporated 64 specimens from 25 locations (Fig. 1-4; clade T) and 105 specimens from 40 locations (Fig. 1-4; clade RA including clade T). For each of these locations, the calculated average genetic difference between each population-pair and their corresponding geographic distance were imported into SigmaPlot v10 (Systat Software, Germany). The geographic distance between each population was inferred using Topo Canada v2 and Topo U.S. 2008 software in MapSource (Garmin International, Kansas) by creating routes along waterways to connect populations. Distances between populations are best approximations of dispersal routes to and from the Pacific Ocean and then along the coast based on contemporary landscape. Independent linear regression analyses were performed for geographic versus genetic distance within *L. richardsoni* clades (clades RA and T). The Kolmogorov-Smirnov goodness-of-fit test (Chakravarti and Roy, 1967) was used to test whether a sample comes from a population with a specific distribution. Linear regression analysis was used to determine the slope and *y*-intercept of a best-fit line through the data points and whether the points could be sufficiently described by linear analysis.

3. Results

3.1 Levels of sequence divergence

The complete cyt *b* gene (1191 bp) was sequenced from 145 lampreys, resulting in 53 haplotypes from 135 individuals from the genus *Lampetra* and six haplotypes from 10 individuals from the genus *Entosphenus* (Table 1-1; Fig. 1-4). The percent sequence divergence was calculated with the Kimura 2-parameter distance (K2P; Kimura, 1980) unless otherwise noted. *Lampetra hubbsi* differed genetically from the *L. richardsoni* populations (and all other populations excluding Paynes Creek) by more than 2.6%. A single sample that was collected from Paynes Creek revealed sequence divergence of 1.5% compared to *L. hubbsi*. Similar to *L. hubbsi*, *L. pacifica* differed from all the other populations by more than 2.3%. The greatest genetic variation, however, was discovered among the putative *L. richardsoni* populations with sequence comparisons that range from 0 to 8.0%. Twenty-four *Lampetra* populations were genetically indistinguishable or very similar (less than 0.3% divergent) from *L. richardsoni* samples collected from its type locale (Smith Creek; see Fig. 1-4; clade T). In the northern parts of its distribution (British Columbia), *L. ayresii* is genetically indistinguishable from *L. richardsoni* from Smith Creek, despite a 1.2% sequence divergence from conspecifics in the south (Oregon and California). In contrast, sequences from *E. tridentatus* collected over the same geographic range (more then 1600 km), showed a maximum divergence of 0.4% (Table 1-2).

3.2 Phylogenetic analysis - Parsimony

The PAUPRat analysis executed in PAUP* including all 1191 characters resulted in 4020 equally-parsimonious trees. A total of 250 characters were potentially parsimony

informative, 843 characters were constant, and 98 characters were parisomy-uninformative. A 50% majority rule tree was constructed from these topologies (Fig. 1-4), with a tree length of 566, a consistency index (CI) of 0.696, and retention index (RI) of 0.939. The frequency of equally-parsimonious trees that supported each branch within the consensus tree yielded similar or higher values than those of bootstrap (BS) support values (Fig. 1-4). The resulting tree supports North American *Lampetra* as a monophyletic group (BS = 96%) containing three main clades (Fig. 1-4, clades RA, HP, and K). Each main clade was determined based on the most encompassing monophyletic group that differed from *L. richardsoni* collected from its type locale by more than 2% sequence divergence. The genetic divergences between and among all major *Lampetra* clades are summarized in Table 1-2. The first (clade RA; BS = 77%) contains *L. richardsoni* and *L. ayresii* collected from their type locales (Smith Creek and Sacramento Delta, respectively; Fig. 1-4). This clade is characterized as having a large polytomy as well as increasingly more divergent populations. The polytomy consists of 22 populations of *L. richardsoni* and three populations of *L. ayresii* that differ by less than 0.1% from *L. richardsoni* from Smith Creek (Fig. 1-4, clade T, BS = 95%). In general, individuals in each locality shared the same cyt *b* haplotype. The most dramatic incongruence is the Nisqually River population that has five haplotypes, and groups with both *L. ayresii* and *L. richardsoni* species. The Nisqually River lampreys show intra-population genetic variation ranging from 0.1 to 0.8%. The range of genetic variation within the *richardsoni/ayresii* clade ranges from 0 to 2.3% (Fork Creek versus Navarro River; Table 1-2). This depth of divergence is much shallower than that observed within the second major clade, the *hubbsi/pacifica* clade (Fig. 1-4, clade HP), with genetic variation ranging from 0.1 to 5.7% (Crystal Springs Creek versus Clackamas River and Mark West Creek versus Paynes Creek,

respectively; Table 1-2). This clade contains *L. hubbsi* and *L. pacifica* collected from their type locales (Merced River and Clackamas River, respectively), as well as populations considered *L. richardsoni* based on presumed distribution records but where adult specimens remain to be collected (Fourmile Creek, Mark West Creek, Siuslaw River, and Paynes Creek). This mid-level clade is moderately supported as the sister group to the *richardsoni/ayresii* clade (BS = 65%), though the overall topology within this clade is poorly resolved as suggested by bootstrap values lower than 50% between many populations. However, the lamprey sequenced from Paynes Creek groups with *L. hubbsi* from Merced River (BS = 99%). Not only do *L. hubbsi* and *L. pacifica* differ genetically from *L. richardsoni* (clade T) and all other populations by more than 2.3%, but they differ from each other by more than 2.7%. The third main clade (K) is represented by a single population from Kelsey Creek, and is unequivocally supported as the sister group to both the *richardsoni/ayresii* and *hubbsi/pacifica* clades (Fig. 1-4, BS = 100%). Among *Lampetra* spp. from the west coast of North America, lampreys from Kelsey Creek show the most divergent cyt *b* sequence. Kelsey Creek lamprey sequences differ by 5.7 – 6.7% from the *richardsoni/ayresii* (RA) clade and 5.9 – 8.0% from the *hubbsi/pacifica* (HP) clade.

There were no species-specific genetic differences between *L. richardsoni* and *L. ayresii*, and these species were not reciprocally monophyletic (i.e., all lineages within each species do not share more recent common ancestors than any lineage from one species shares with any lineage from the other species).

3.3 Phylogenetic analysis - Bayesian

The implementation of the B-MCMCMC analysis in MrBayes resulted in 1442 trees (Harmonic mean of -ln likelihood = 4885.35, rooted with *P. marinus*; Fig. 1-4). The consensus of these Bayesian trees was incongruent with the parsimony consensus tree, and suggested a less structured phylogeny. Two Kishino-Hasegawa (KH) tests were used to compare the Bayesian consensus tree with the parsimony consensus tree. The first KH test was implemented using the parsimony criterion and suggested congruence between the consensus trees ($p = 0.8$). The second KH test implemented under the likelihood criterion suggested incongruence between trees ($p < 0.001$). A visual inspection of the consensus trees reveals obvious differences in the tree topologies. The Bayesian consensus tree supported fewer major clades. The *richardsoni/ayresii* clade (RA) and the polytomy within it (clade T) are unequivocally supported (PP = 100%; Fig. 1-4), and have nearly an identical topology to their parsimony counterpart. The most notable topological difference between the consensus trees is that the monophyletic structure exhibited by the *hubbsi/pacifica* clade (HP) constructed under the parsimony criterion was no longer achieved using Bayesian methods. Additionally, the Bayesian consensus tree supports lampreys from Fourmile Creek as being the sister group to the remaining *Lampetra* specimens (excluding *L. fluviatilis*), whereas the parsimony consensus tree supports lampreys collected from Kelsey Creek (K) as the sister group. The Bayesian analysis placed lampreys from Kelsey Creek as being sister to *L. hubbsi* from Merced River (and the specimen from Paynes Creek), which is incongruent with the parsimony analysis, but this clade was poorly resolved (PP = 51%).

Both parsimony and Bayesian analyses suggest that *L. ayresii* is polyphyletic in at least three clades (Fig. 1-4). *Lampetra ayresii* from California (Sacramento Delta and

Feather Creek) are strongly supported as a monophyletic group, based on high bootstrap (BS) and posterior probability (PP) values (BS = 100; PP = 100). The *L. ayresii* collected from Mill Creek (Oregon) forms a moderately well supported monophyletic group with lampreys collected from Klaskanine and Necanicum River, Oregon (BS = 56; PP = 85) and *L. ayresii* from British Columbia appear within the polytomy that includes Smith Creek (BC) *L. richardsoni* (clade T).

3.4 Phylogeography and regression analyses

In general, phylogenetic relationships among *Lampetra* populations were rarely related to geographic patterns. Populations situated in close proximity to one another were not necessarily closely related, and lampreys isolated by considerable distance likewise showed a range of genetic divergences. For example, *L. richardsoni* from within the polytomy (clade T) could be found at the northernmost collection site in the Farragut River, Alaska, south to the Navarro River in California; these two populations are separated by nearly 2700 km. While there was no relationship between genetic and geographic distances within the *richardsoni/ayresii* clade or within the polytomy (clades RA and T, respectively; Fig. 1-5), the other two main clades (clades HP and K) are restricted to the more southerly locations; they all occurred south of the Columbia River.

Despite various attempts at data transformations as well as the removal of outliers, genetic versus geographic distances did not pass the normality test. If this assumption is relaxed, indeed there is a statistically significant correlation between the geographic distance separating populations in clade RA and their genetic discrepancies, albeit small (R = 0.0004; Fig. 1-5).

4. Discussion

4.1 Phylogeny of brook lampreys

The phylogeny of *Lampetra* on the Pacific coast of North America is far more complex than previously thought. Both shallow and deep divergences were discovered among cyt *b* haplotypes sampled within the genus *Lampetra* that do not correspond with existing classification. One of the clearest and most notable findings was that *L. richardsoni* as currently recognized is not monophyletic; representatives of presumed *L. richardsoni* were embedded within each of the three major clades (clades RA, HP, and K) so that *L. richardsoni* is paraphyletic with respect to *L. ayresii*, *L. pacifica*, and *L. hubbsi*. The specimens from Fourmile Creek, Mark West Creek, Siuslaw River, and Paynes Creek (clade HP) and Kelsey Creek (clade K) would have to be removed from *L. richardsoni* for it to be monophyletic. These populations in Oregon and California, presumed to be *L. richardsoni* based on their distributional range (Moyle, 2002), were genetically very distinct from other *L. richardsoni* in the RA clade and from each other. These populations may therefore represent previously unidentified brook lamprey species (see below). Morphological analysis of adult specimens has not been conducted from these populations, although it should be mentioned that spawning-phase adults from Kelsey Creek have been collected recently for morphological analylsis by Stewart B. Reid but the results of these studies are not yet available.

Within each of the clades, the level of genetic variation between populations exceeds that found in *Entosphenus tridentatus*, which was at approximately 1% sequence divergence over a similar geographic range (this study). Docker et al. (2007) found a similar range of genetic variation (1%) using mtDNA ND2 and ND5 gene fragments. Conversely, some *L.*

richardsoni (clade T) in California vary by no more than seven nucleotide substitutions (up to 0.6%) from conspecifics found in the type locale in British Columbia. This suggests recent dispersal of lampreys within this clade. The nature of these movements is unknown, but it seems reasonable to suggest that dispersal via the Pacific Ocean (presumably by a lamprey with a parasitic anadromous life history type; see "Paraphyly of *Lampetra ayresii* and the evolution of non-parasitism" in the General Discussion) is the likely scenario since freshwater rivers and their respective tributaries remain interrupted over such a large distribution.

Also notable was the finding that the level of genetic variation shown between *L. richardsoni* and *L. pacifica*, which has been synonymized with *L. richardsoni* based on similar morphology (Robins et al., 1991), is at least equivalent to that of *L. richardsoni* compared to *L. hubbsi*. Both *L. pacifica* and *L. hubbsi* share a minimum sequence divergence of 2.3% from any other lamprey species. Despite *L. pacifica* and *L. hubbsi* being found within the same monophyletic group (clade HP) along with other genetically distinct populations assumed to be *L. richardsoni* – including Fourmile Creek, Mark West Creek, Siuslaw River, and Paynes Creek – adult *L. pacifica* and *L. hubbsi* from their type locales are clearly morphologically distinct. *Lampetra hubbsi* is discriminated morphologically from *L. pacifica* and *L. richardsoni* by a single row of unicuspid posterials and four inner laterals consistent with the genus *Entosphenus* and *Lethenteron*, and an infraoral lamina with 5 cusps consistent with *Entosphenus* (Vladykov and Kott, 1976a; Hubbs and Potter, 1971; Docker et al., 1999). Additionally, *L. pacifica* is discriminated morphologically from *L. richardsoni* by typically having fewer trunk myomeres (53-58 and averaging 55.5 in *L. pacifica*, compared to 60-67 and averaging 63.2 in *L. richardsoni*), having fewer teeth on the anterior field that

are less strongly developed, having a smaller oral disc and lower dorsal fins, as well as an abundance of dark pigmentation in the mouth cavity (Vladykov, 1973a). Based on these genetic data, *L. pacifica* from its type locale constitutes a tight monophyletic clade, but its relationship to some of these other brook lamprey populations is less clear. As the specimens from Fourmile Creek, Mark West Creek, and Siuslaw River form a monophyletic group with *L. pacifica* (according to the parsimony analysis; Fig. 1-4a), these specimens could be *L. pacifica.* Similarly, the single specimen collected from Paynes Creek could be *L. hubbsi* based on monophyly. However, given the observed levels of divergence between *L. pacifica* and these three populations (2.6 – 4.4%) and *L. hubbsi* and Paynes Creek (1.5%) – and the fact that Siuslaw and Fourmile did not form a monophyletic group with *L. pacifica* in the Bayesian analysis) – it is possible that one or more of these populations may be distinct species. On the other hand, the Kelsey Creek population – given its level of sequence divergence from other populationsis – is almost certainly a new species. However, all specimens examined from these populations were larvae, whereas most species-level characters in lamprey taxonomy are from the adult stage. Therefore, morphological examination of adult specimens will be required before one or more of these populations can be described as new species. Whether there are divergent adult body forms that are not evident in larvae or whether there are conserved body forms in both life history stages is not known.

Two alternative phylogenetic hypotheses of lamprey population origins were derived using parsimony and Bayesian methods. The most dramatic incongruence between parsimony and Bayesian analyses is the sister group that incorporates the most basal node found within the genus *Lampetra* (excluding *L. fluviatilis*). Lampreys from Kelsey Creek are

unequivocally supported in the parsimony analysis as being the sister group to both the *richardsoni/ayresii* and *hubbsi/pacifica* clades, whereas the topological placement of Kelsey Creek is relatively poorly supported in the Bayesian analysis. Paradoxically, lampreys from Fourmile Creek are unequivocally supported in the Bayesian analysis as being the sister group to all other ingroup clades, whereas the topological placement of Fourmile Creek is relatively poorly supported in the parsimony analysis. The emerging dichotomy is to provide the best rationalization of the data by means of a well structured phylogeny, or to provide the best statistical support based on an *a priori* model. The parsimony analysis provides clear structure of three major clades useful for representing evolutionary significant units.

At what point does sequence variation in cyt *b* for evolutionary significant units reflect species-level designation? This study by no means attempts to delineate species boundaries, but provides insight into lamprey phylogeny when compared with other vertebrates. The biological species concept (Mayr, 1942), incorporating reproductive isolation, and the phylogenetic species concept (Wheeler, 1996), incorporating monophyly, are both commonly used in species designation. Establishing threshold levels within and between genetically different groups is appealing, though arguably unattainable without a scientific consensus of the definition of a species concept. For example, 90% of putative sister species show mtDNA sequence divergences greater than 2% (Avise and Walker, 1999), based on nearly 2000 cyt *b* sequences recovered from GenBank spanning the major taxonomic classes (Johns and Avise, 1998). This study shows that eight lamprey populations are more than 2% genetically distinct from *L. richardsoni* (clade T) as well as any other lamprey (except where noted below due to their close phylogenetic placement; Fig. 1-4). These populations along with their minimum genetic distance from other populations include

Crystal Springs Creek (2.32% excluding Clackamas River), Clackamas River (2.32% excluding Crystal Springs Creek), Fourmile Creek (2.32%), Mark West Creek (4.09%), Siuslaw River (2.23%), Merced River (2.31% excluding Paynes Creek), Paynes Creek (2.84% excluding Merced River), and Kelsey Creek (5.65%). If 2% sequence divergence for the cyt *b* reflects species delineations, suggested above by Avise and Walker (1999), then the numbers of lamprey species currently recognized are underestimated. For example, the specimens from Fourmile Creek, Mark West Creek, Siuslaw River, and Paynes Creek all differ from *L. richardsoni* collected from its type locale and often from each other by more than 2%. A future hypothesis may test whether this level of genetic divergence is congruent with other species criteria according to the MSC and BSC (see General Introduction). However, without the corroboration of multiple analytical methods to achieve a robust phylogeny, evolutionary reconstruction based on a single genetic marker in the absence of morphological characters will remain only an initial means of exploring biotic diversity.

The cytochrome *c* oxidase subunit 1 (CO1) persists in the research of molecular phylogenetics as the gene of choice in many taxonomic studies and has been conceptualized in popular culture as a means of 'barcoding' biological diversity (Hebert et al., 2002). Species delineations based on CO1 have been suggested (Ward et al., 2005; Hubert et al., 2008), and the 2% sequence divergence among conspecific species based on cyt *b* proposed by Avise and Walker (1999) is markedly under the average in comparison (9.93% and 8.37%, respectively). Contrasting the rate of evolution between cyt *b* and CO1 in fishes has proved difficult since haplotypes of different mitochondrial genes are often not sampled for each individual. There have, however, been suggestions regarding the differences in rates of variation in both cyt *b* versus CO1 contrasted in other vertebrate taxa. For example, Lynch

and Jarrell (1993) stated that the estimated mean substitution rates (per billion years) for cyt *b* and CO1 in their analysis applied exclusively to mammals were 0.493 and 0.302 respectively, suggesting that cyt *b* evolves 1.63 fold faster than CO1. To facilitate comparisons in lampreys, a 645 bp fragment of the CO1 gene (coinciding with the fragment used in barcoding) was sequenced in a total of 22 individuals from one *Entosphenus tridentatus* population (Alsea River) and five *Lampetra* populations (including Smith Creek, Fourmile River, Clackamas River, Merced River, and Kelsey Creek; details presented in Chapter 2). The mean K2P substitution rates were calculated for each population in the cyt *b* and CO1 datasets, and pairwise comparisons between datasets reveal that, on average, cyt *b* evolves 1.43 fold faster than CO1, comparable to Lynch and Jarrell's (1993) report. Additionally, the estimated pairwise divergences among the populations above (averaging 2.5% within *Lampetra*; Table 2-4) reveal average values below the genetic divergences among other fish species within a genus – an average of 9.93% (ranging from 0 to 14.08%) was reported by Ward et al. (2005) and an average of 8.37% (ranging from 0 to 19.33%) was reported by Hubert et al. (2008) – but are still within the range reported to discriminate fish species. However, Hubert et al. (2008) and Ward et al. (2005) suggest that overlapping genetic distances at each taxonomic level imply that current classifications are subjective, there are inherent limitations in morphological-based identification systems, there is a need for a total evidence approach to taxon recognition, and that new cryptic species have likely gone unrecognized due to parallel evolution. This is not to belittle the profound accomplishments of classical taxonomists who have provided the foundation for systematics, nor does it suggest that contemporary approaches to classification will persist as the standard. The discovery of genetically distinct lamprey populations (Fourmile Creek, Mark West

Creek, Siuslaw River, and Paynes Creek) provides preliminary evidence for previously unrecognized *Lampetra* brook lamprey species on the Pacific coast of North America, but detailed morphological analysis of adult specimens as well as broad geographic sampling is required to test monopyly. Based on the PSC, with current data, specimens from Mark West Creek (and potentially Fourmile Creek and Siuslaw River) could be *L. pacifica*, and the single specimen from Paynes Creek could be *L. hubbsi*. Furthermore, the genetically distinct population from Kelsey Creek, CA, is a distinct species based on the PSC due to its monophyly and sister relationship to the rest of the ingroup (including *L. hubbsi*) as inferred from the parsimony analysis.

4.2 Paraphyly of L. ayresii and the evolution of non-parasitism

The most parsimonious explanation for the observed data is that parasitism has evolved multiple times, however previous evidence (see General Introduction) supports non-parasitism as derived. As such, the current molecular study supports the evolution of non-parasitism as independent and having occured multiple times. *Lampetra ayresii* from six locations distributed intermittently along the coast from northern British Columbia to California were not suggested to be monophyletic. Consistent with previous reports by Docker et al. (1999), there were no diagnostic genetic differences between *L. ayresii* and *L. richardsoni* found in British Columbia. Therefore, it is possible that parasitic lampreys nested within each of the three main clades have been misidentified since these populations were represented by larval collections in which there is a paucity of morphological data used for species identification. Despite this paucity of morphological data, specimens clearly belonged to the genus *Lampetra* and were not from an unrelated genus. Further south, one *L.*

ayresii specimen collected from Mill Creek, a tributary to Yaquina River in Oregon, appears to be closely related to larval lampreys collected from Klaskanine River and Necanicum River. This parasitic specimen is distinct from other conspecifics found further south in the Sacramento Delta as well as upstream into the Sacramento River reaching Feather Creek.

It is uncertain to what extent the six collections of parasitic lampreys appearing in different parts of the *richardsoni/ayresii* clade have contributed to the speciation events in non-parasitic brook lampreys. It appears more complex than local parasitic lampreys giving rise to local non-parasitic lampreys, as brook lampreys can be genetically very similar over a vast geographic range and they do not consistently group with the local parasitic lampreys. It is possible that an ancestral parasitic species distributing itself along the coast acted as a conduit through which brook lampreys evolved and diversified (cf. Schreidber and Engelhorn 1998). As these anadromous ancestral parasitic river lampreys pervaded new coastal streams with the aid of ocean currents and active transport on prey species, they were able to colonize new coastal habitats. Non-parasitism may have subsequently been induced or evolved, through mechanism(s) that are not yet known (see "Paraphyly of *Lampetra ayresii* and the evolution of non-parasitism" in the General Discussion). Furthermore, the possibility of multiple ancestral parasitic lampreys cannot be ruled out, amplifying the complexity of this dynamic evolutionary system. Based on the relatively conserved body form of brook lampreys across their distribution and the fact that life-history type (parasitic versus non-parasitic) is currently used to discriminate between species pairs (see "Paired and satellite species" in the General Introduction), the independent evolution of brook lampreys and moreover, independent speciation events, appear to be occurring in parallel. Similar studies have been supported by Schluter et al. (2001) who have studied parallel evolution in

threespine sticklebacks. The successional events leading to the repeated emergence of morphologically conserved brook lampreys may seem improbable, but if these events are dependent on a common genetic control, closely-related paired and satellite species may represent the same evolutionary model.

4.3 Phylogeography and regions of endemism

This study's phylogeographic appraisal based on mtDNA of spatially isolated brook lampreys reveals that genetically similar or indistinguishable haplotypes are not always geographically localized. Therefore, isolation-by-distance models do not appear to explain the degree of genetic variation between different populations of *Lampetra* species. The regression analysis of genetic versus geographic distances between populations in clade T, though significant ($p < 0.0099$) when the assumptions of normality were relaxed, does not convey a strong relationship inferred from the slope (0.0004). Furthermore, there is no statistically significant genetic versus geographic relationship within the *richardsoni/ayresii* clade (clade RA). This is also evident as outlined on the collection site maps (Figs. 1-1, 1-2, 1-3) showing that populations over 2700 km of rather continuous space may be nearly genetically identical, whereas populations in close proximity to one another can vary substantially. Furthermore, Vladykov's (1973a) proposed distribution of *L. pacifica* from the Columbia River system to southern regions including the Sacramento and San Joaquin River system, are inconsistent with the genetic data supporting *L. pacifica* as being endemic to the Columbia River system. However, this geographic distribution may be underestimated if Fourmile Creek, Mark West Creek, and Siuslaw River – not sampled by Vladykov (1973a) – represent *L. pacifica*; these suggestions are somewhat premature without a formal taxonomic

revision or adult specimens. The Columbia River, separating the state of Washington to the north from Oregon to the south, acts as the northern limit of the most genetically divergent lampreys (clade HP), including *L. pacifica*, as previously proposed (Vladykov, 1973a). *Lampetra richardsoni* (clade T) spans both sides of the Columbia River where it is distributed as far south as the Navarro River, California. This is inconsistent with previous suggestions that *L. richardsoni* can be found in the Sacramento River drainage (Moyle, 2002). Additionally, this distribution extending into California is independent of where some authorities have recognized *L. pacifica* (Vladykov, 1973a; Page and Burr, 1991). The lamprey specimen from Paynes Creek could be *L. hubbsi* based on its phylogenetic placement, but it is still genetically distinct (1.5%) and adult specimens used for species-level identification were not collected. This could, however, suggest a range extension of *L. hubbsi* into the upper Sacramento River.

The restricted distributions of highly genetically different clades suggest that the evolution of several populations of lampreys have been occurring in isolation for a long time. It has become apparent that several of these regions support high biological diversity that is also common to lamprey taxa. For example, Clear Lake (CA) is renowned as one of the oldest lakes in North America (Sims, 1988; Sims et al., 1988) and may also represent an ancestral relict lake dating back to the early Pleistocene, making it 1.8 to 3.0 million years old (Casteel and Rymer, 1981; Hearn et al., 1988). Several fish species distributed within and/or surrounding Clear Lake are the focus of ecosystem-level coordinated management strategies (Moyle et al., 1995). Distinct classification has been given to several fish taxa endemic to Clear Lake and its surrounding area including, but not limited to, the Clear Lake hitch (*Lavinia exilicauda chi*). Based on the molecular data presented here, lampreys

collected from Kelsey Creek, a tributary to Clear Lake, almost certainly warrant recognition as a distinct species.

As ecosystems shift and landscapes change, allopatric and peripatric diversification may ensue with disruptive selective pressures. As inferred from the historical geological records of the central coast of California (Suchanek et al., 2003), both stochastic and large geological events may have had more of an influence on the *hubbsi/pacifica* clade of lampreys by isolating populations at an earlier time, whereas diversification from the *L. richardsoni* haplotype (clade T) to those that are more divergent in the *richardsoni/ayresii* clade (clade RA) may reflect relatively more recent forms of divergence. This divergence may be the result of colonization and subsequent isolation due to allopatry reinforced by the salinity gradient of the coast preventing secondary contact.

I have provided the first detailed molecular phylogeny of North American brook lampreys of the genus *Lampetra* from across their Pacific coastal range and have found significant diversification of lamprey populations comparable to other fish species. This study supports recognized and cryptic species, regions of endemism, and highlights the potential of parallel evolution and speciation. Furthermore, I offer a comparison and an alternative to the morphologically-based phylogenetic hypothesis of this *Lampetra* assemblage. This study should be taken into consideration for re-establishing the Pacific brook lamprey, *L. pacifica,* as a valid species. Moreover, lamprey specimens from Fourmile Creek, Mark West Creek, and Siuslaw River could represent *L. pacifica* based on their phylogenetic placement, despite each population displaying more than 2.3% sequence divergence from any other lamprey population, exceeding levels of genetic variation found interspecifically. The lamprey specimens from Paynes Creek could represent *L. hubbsi* based

on its phylogenetic placement, despite being genetically distinct (1.5%) from all other lamprey populations. All specimens examined from these populations above were larvae, however, whereas most species-level characters in lamprey taxonomy are from the adult stage. Therefore, morphological examination of adult specimens will be required before one or more of these populations can be described as new species. The greatest sequence divergence (more than 8%), however, was observed among two putative *L. richardsoni* populations (i.e., Kelsey Creek and Mark West Creek). The most divergent population was from Kelsey Creek, CA. Designating specimens from Kelsey Creek in any of the existing species would make them paraphyletic, and inconsistent with phylogenetic species criteria. Therefore, these specimens collected from Kelsey Creek, almost certainly represent a new species.

Table 1-1. Collection data for the lamprey species analyzed in this study; collection sites are presented from north to south for each species and map identification numbers correspond to locations on Figures 1-1, 1-2, and 1-3. Voucher numbers correspond to fish collections at Humbolt State University, the Scripps Institution of Oceanography, and the University of Manitoba (prefix HSU, STO, and MZF, respectively).

Species	Drainage	Collection Site	Province/State	Map Identification	*N*	Voucher #
Lampetra ayresii	Nass River	Quilgauw Creek	BC	2	1^{A}	_
	Fraser River	Fraser River	BC	6	1^{LH}	_
	Fraser River	Partington Creek	BC	7	1^{A}	_
	Yaquina River	Mill Creek	OR	34	1^{A}	_
	Sacramento River	Feather River	CA	52	4^{LH}	HSU3975
	San Francisco Bay	Sacramento Delta	CA	56	2^{A*}	STO169 STO171
Lampetra hubbsi	San Joaquin River	Merced River	CA	57	2^{L*}	HSU3969
Lampetra pacifica	Columbia River	Crystal Springs Creek	OR	29	3^{LH}	MZF3600 to MZF3602
	Columbia River	Clackamas River - N.F. Reservoir	OR	31	4^{L*}	MZF3603 to MZF3606
	Columbia River	Clackamas River - Big Eddy	OR	32	3^{L*}	MZF3607 to MZF3609
Lampetra richardsoni	Farragut River	Farragut River	AK	1	1^{A}	MZF3597
	Nass River	Ishkeenickh River	BC	3	2^{A}	_
	Vancouver Island	Arden Creek	BC	4	1^{L}	_
	Vancouver Island	Morrison Creek	BC	5	1^{L}	_
	Vancouver Island	Chase River	BC	8	2^{L}	_
	Fraser River	Smith Creek	BC	9	2^{A*}	MZF3598 MZF3599
	Nooksack River	Nooksack River	WA	10	3^{L}	HSU3933
	Skagit River	Nookachamps Creek	WA	11	4^{L}	HSU3931
	Lake Ozette	Big River	WA	12	4^{L}	HSU3951
	Cedar Creek	Cedar Creek	WA	14	2^{L}	HSU3955
	Queets River	Salmon River	WA	16	1^{L}	HSU3958
	Quinault Lake	Quinault River	WA	17	2^{L}	HSU3959
	Quinault Lake	Ziegler Creek	WA	18	1^{A}	HSU3937
	Lake Washington	Cedar River	WA	19	3^{L}	HSU3927
	Green River	Green River	WA	20	3^{L}	HSU3926
	Chehalis River	Satsop River	WA	21	2^{L}	HSU3962
	Nisqually River	Nisqually	WA	22	5^{L}	HSU3923
	Willapa River	Fork Creek	WA	23	5^{L}	HSU3964
	Columbia River	Klaskanine River	OR	24	5^{L}	HSU3859
	Columbia River	Mill Creek	WA	25	4^{L}	_
	Nehalem River	Fishawk Creek	OR	26	2^{L}	HSU3849
	Necanicum River	Necanicum River	OR	27	5^{L}	HSU3862
	Columbia River	Gibbons Creek	WA	28	2^{L}	_
	Yaquina River	Yaquina River	OR	35	2^{L}	HSU3875
	Columbia River	Owens Creek	OR	37	2^{L}	MZF3610 MZF3611

Table 1-1 continued

	Siuslaw River	Siuslaw River	OR	38	5^{L}	HSU3882
	Coos River	Millicoma River	OR	39	2^{L}	HSU3886
	Fourmile Creek	Fourmile Creek	OR	40	4^{L}	HSU3889
	Rogue River	Cow Creek	OR	41	4^{L}	MZF3612 to MZF3615
	Rogue River	Euchre Creek	CA	43	2^{L}	HSU3896
	Klamath River	Hunter Creek	CA	44	5^{L}	_
	Klamath River	McGarvey Creek	CA	45	5^{L}	_
	Redwood Creek	Prairie Creek	CA	46	2^{L}	_
	Redwood Creek	Boyes Creek	CA	47	2^{L}	HSU3621
	Redwood Creek	Streelow Creek	CA	48	3^{L}	HSU3965
	Sacramento River	Paynes Creek	CA	50	1^{L}	HSU3971
	Navarro River	Navarro River	CA	51	4^{L}	HSU3906
	Clear Lake	Kelsey Creek	CA	53	4^{L}	HSU3944
	Russian River	Mark West Creek	CA	54	4^{L}	MZF3616 to MZF3619
Entosphenus tridentatus	Bogachiel River	Soleduck River	WA	13	2^{L}	_
	Duckabush River	Duckabush River	WA	15	2^{L}	HSU3935
	Wilson River	Wilson River	OR	30	1^{L}	HSU3866
	Columbia River	Luckiamute Creek	OR	33	1^{L}	HSU3856
	Alsea River	Alsea River	OR	36	1^{L}	HSU3878
	Floras Creek	Floras Creek	OR	42	1^{L}	HSU3892
	Eel River	Eel River	CA	49	1^{L}	HSU3903
	San Pablo Bay	Sonoma Creek	CA	55	1^{L}	HSU3912

Note: Sample size (*N*); ^{A} Adult specimens; ^{L} Larval specimens; * Holotype collection site; ^{H} Ammocoetes collected in historical adult collection site

Table 1-2. Summary of genetic divergences (K2P model used for computing distances) for the major *Lampetra* clades and the *Entosphenus tridentatus* clade resulting from the parsimony analysis (Fig. 1-4). Data are from 145 sequences from 57 locations. Clade T represents a sub-clade within the RA clade (Fig. 1-4).

Comparisons	**Clade**		**Samples (*N*)**	**Number of pairwise comparisons**	**Min**	**Mean**	**Max**	**SE**
Within clades, between locations*	Clade T		64	2016	0	0.2	0.6	0.017
	Clade RA		105	5460	0	0.8	2.3	0.062
	Clade HP		26	325	0.1	2.7	5.7	0.381
	Clade K		4	6	0	0	0	0.000
	Entosphenus tridentatus		10	45	0	0.2	0.4	0.034
Between clades	Clade T	Clade HP	90	4005	2.8	3.3	5.1	0.069
		Clade K	68	2278	6.3	6.5	6.8	0.014
	Clade RA	Clade HP	131	8515	2.2	3.3	5.1	0.059
		Clade K	109	5886	5.7	6.3	6.8	0.025
	Clade HP	Clade K	30	435	5.9	6.5	8.1	0.126

*Clackamas River locations were treated as a single location as they are genetically indistinguishable and are separated geographically by approximately 6.4 km along a continuous portion of the river

Figure captions

Fig. 1-1. *Lampetra* collection sites in Alaska and northern British Columbia. Site numbers are associated with populations outlined in Table 1-1, Figure 1-4, and Figure 1-5. Orange circles denote that the population is part of the polytomy that includes *L. richardsoni* from their type locale as well as *L. ayresii* representatives (Fig. 1-4; clade T).

Fig. 1-2. *Lampetra* spp. and *Entosphenus tridentatus* collection sites in British Columbia, Washington, Oregon, and California. Site numbers are associated with populations outlined in Table 1-1, Figure 1-4, and Figure 1-5. Coloured circles denote the population location and correspond to the coloured clades in Figure 1-4. The half-orange half-red circle represents a single population with individuals from each grouping. White circles denote that the population is *Entosphenus tridentatus*.

Fig. 1-3. *Lampetra* spp. and *Entosphenus tridentatus* collection sites in California. Site numbers are associated with populations outlined in Table 1-1, Figure 1-4, and Figure 1-5. Coloured circles denote the population location and correspond to the coloured clades in Figure 1-4. White circles denote that the population is *Entosphenus tridentatus*.

Fig. 1-4. Parsimony (A) and Bayesian (B) consensus trees. (A) The 50% majority rule consensus of 4020 most parsimonious trees resulting from heuristic searches executed using the Parsimony Ratchet. Bootstrap support above 50% is shown below branches in bold, and consensus frequency values are shown above branches. The three major clades are denoted in colour: *richardsoni/ayresii* (clade RA) in red, *hubbsi/pacifica* (clade HP) in green, and Kelsey Creek (clade K) in blue. Each main clade was determined based on the most encompassing monophyletic group that differed from *L. richardsoni* collected from its type locale by more than 2% sequence divergence. Clade T (orange) represents a polytomy within the RA clade; it is expanded in (C). The scale refers to the number of changes. (B) The Bayesian-MCMCMC tree resulting from a 50% majority rule consensus of 1442 trees. The resulting posterior probabilities (PP) are shown above internal branches. The scale refers to the number of substitutions per site. (C) Expanded polytomy (clade T, orange) which is a subclade within RA that includes *L. richardsoni* from its type locale and *L. ayresii* representatives. Bootstrap support above 50% is shown below branches in bold, and consensus frequency values are shown above branches. The PP values are shown beside internal branches in the phylogram. Taxon labels that give only the collection site (by name and number in Table 1-1, Fig. 1-1 to 1-3), apply to *L. richardsoni* sequences; for species other than *L. richardsoni*, species name is also denoted in italics (lower case letters indicate individuals).

Fig. 1-5. Average pairwise sequence differences (base pair; bp) between locations versus geographic distance between locations (km) within clades RA and T (see Fig. 1-4). Independent regression analyses revealed a non-significant correlation ($p = 0.3111$) of the non-transformed clade RA data (including clade T data), yielding the linear equation $y = 0.0004x + 7.9914$ and a coefficient of determination (R^2) of 0.001. The second regression analysis revealed a statistically significant correlation ($p = 0.0099$) of the non-transformed clade T data, yielding the linear equation $y = 0.0004x + 1.1060$ and a coefficient of determination (R^2) of 0.026. The level of significance was assessed at $\alpha = 0.05$.

Fig. 1-1

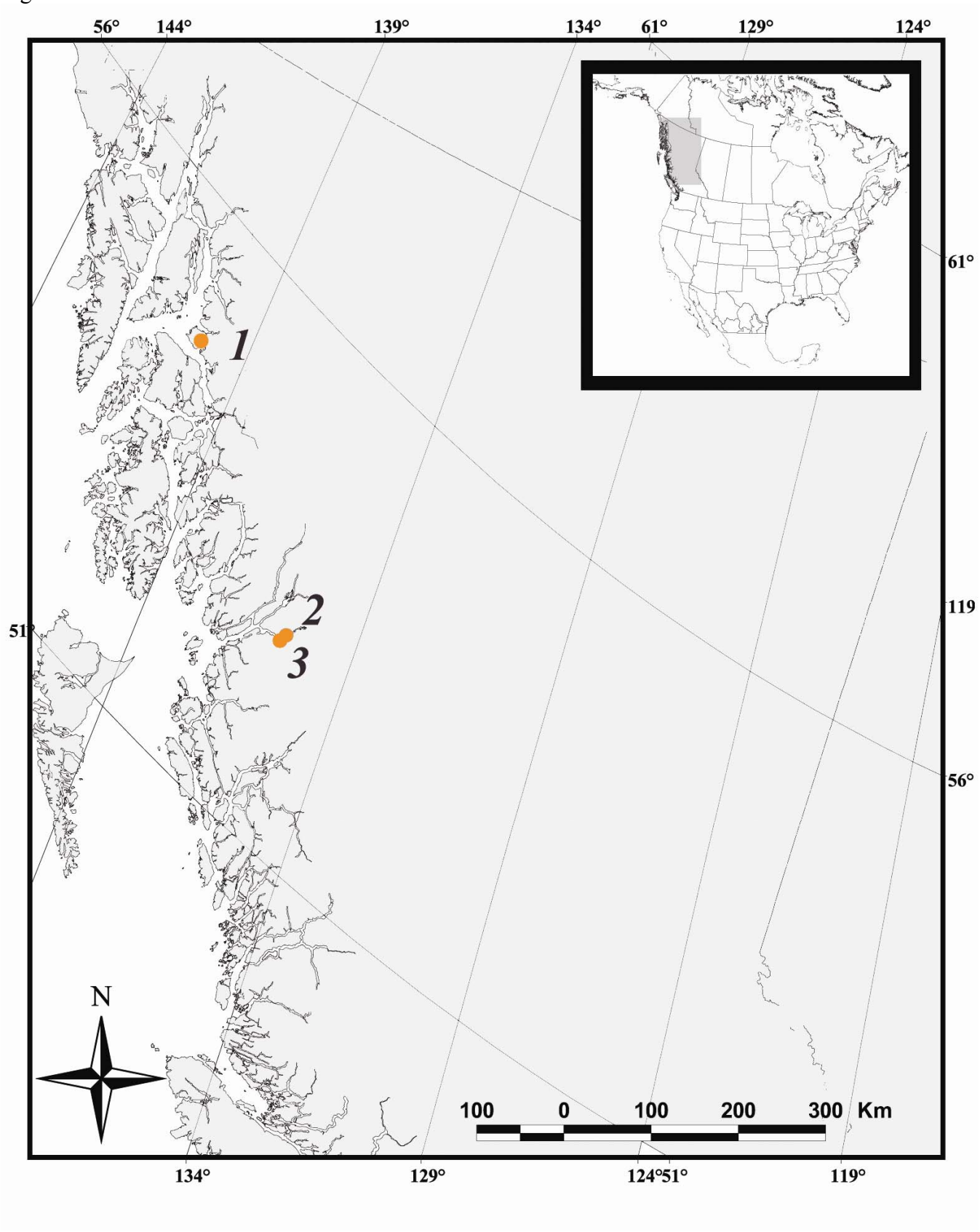

56°
144°
139°
134°
61°
129°
124°
61°
1
2
3
51°
119
56°
N
100 0 100 200 300 Km
134°
129°
124°
51°
119°

Fig. 1-2

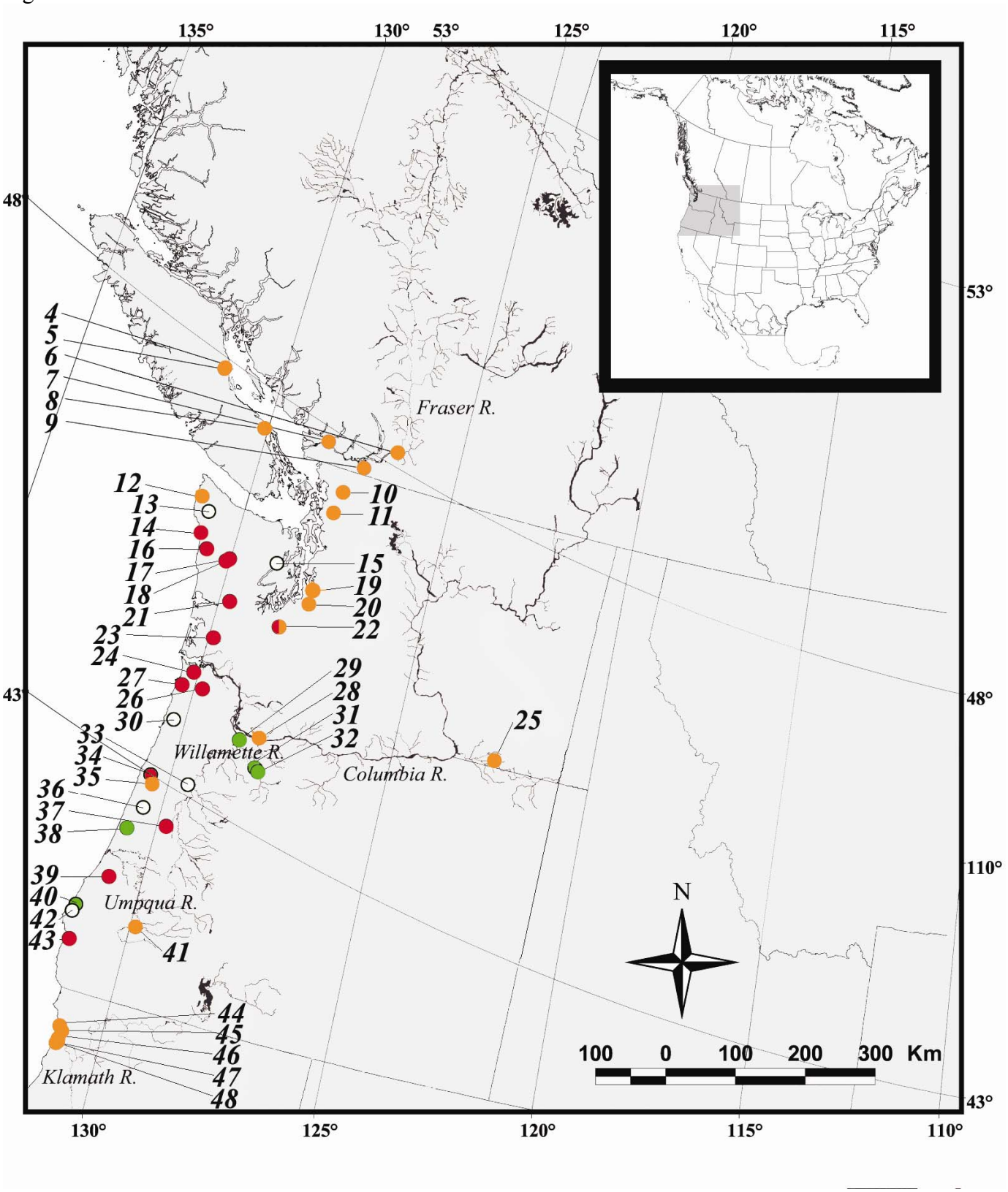

135°
130°
53°
125°
120°
115°
48°
43°
110°
Fraser R.
Willamette R.
Columbia R.
Umpqua R.
Klamath R.
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
N
100 0 100 200 300 Km

Fig. 1-3

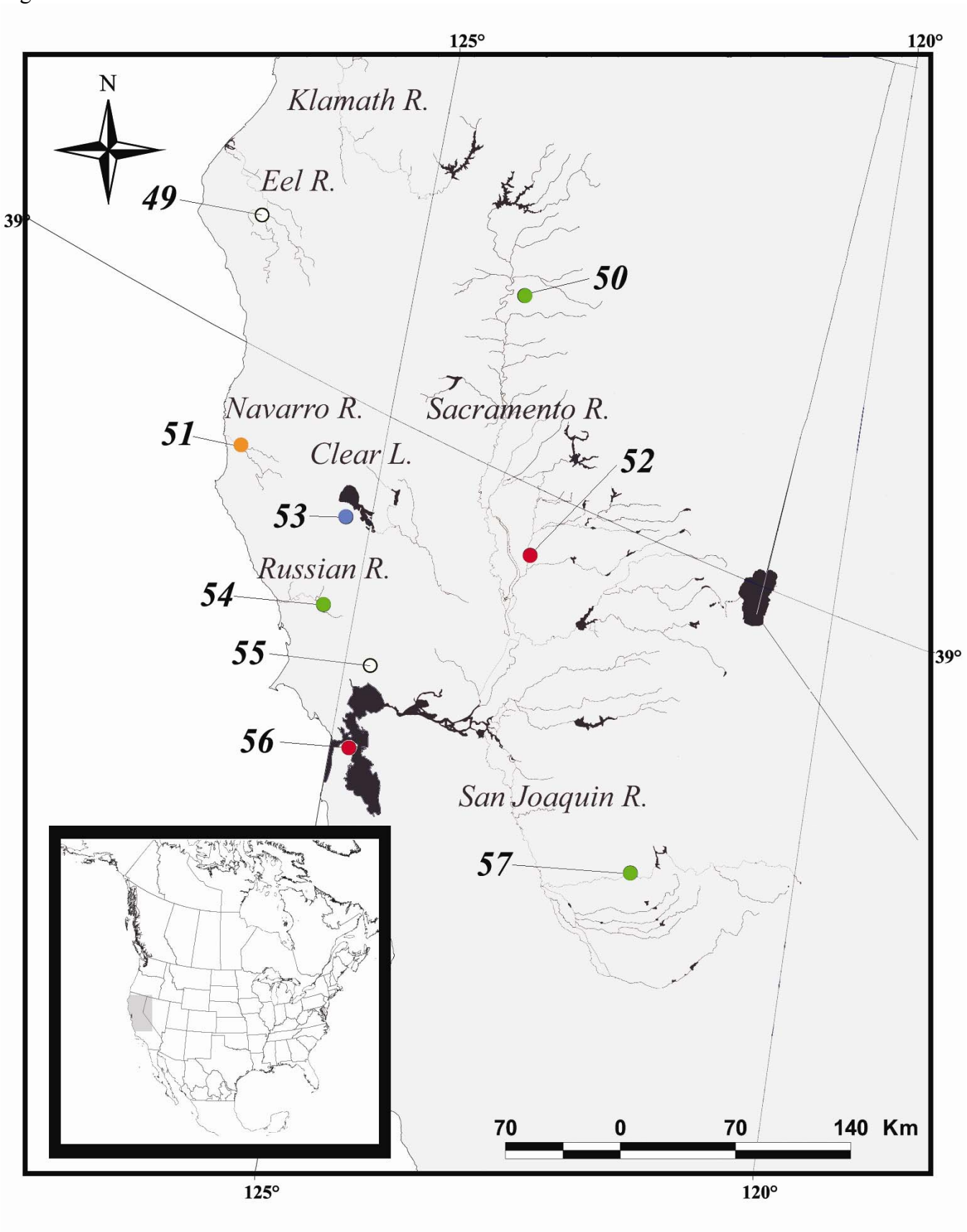

125°
120°
N
Klamath R.
Eel R.
49
39°
50
Navarro R.
Sacramento R.
51
Clear L.
52
53
Russian R.
54
55
39°
56
San Joaquin R.
57
70 0 70 140 Km
125°
120°

Fig. 1-4

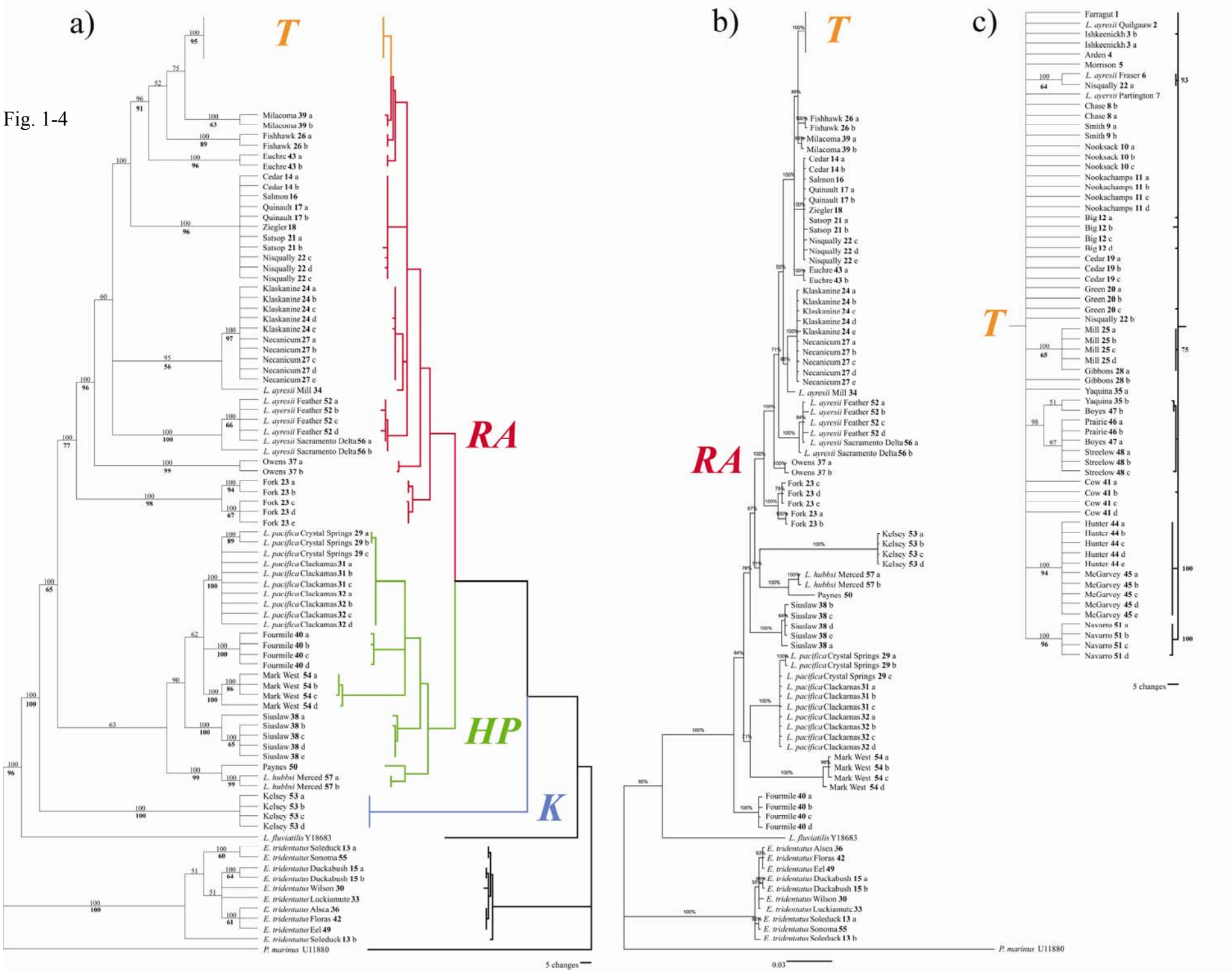
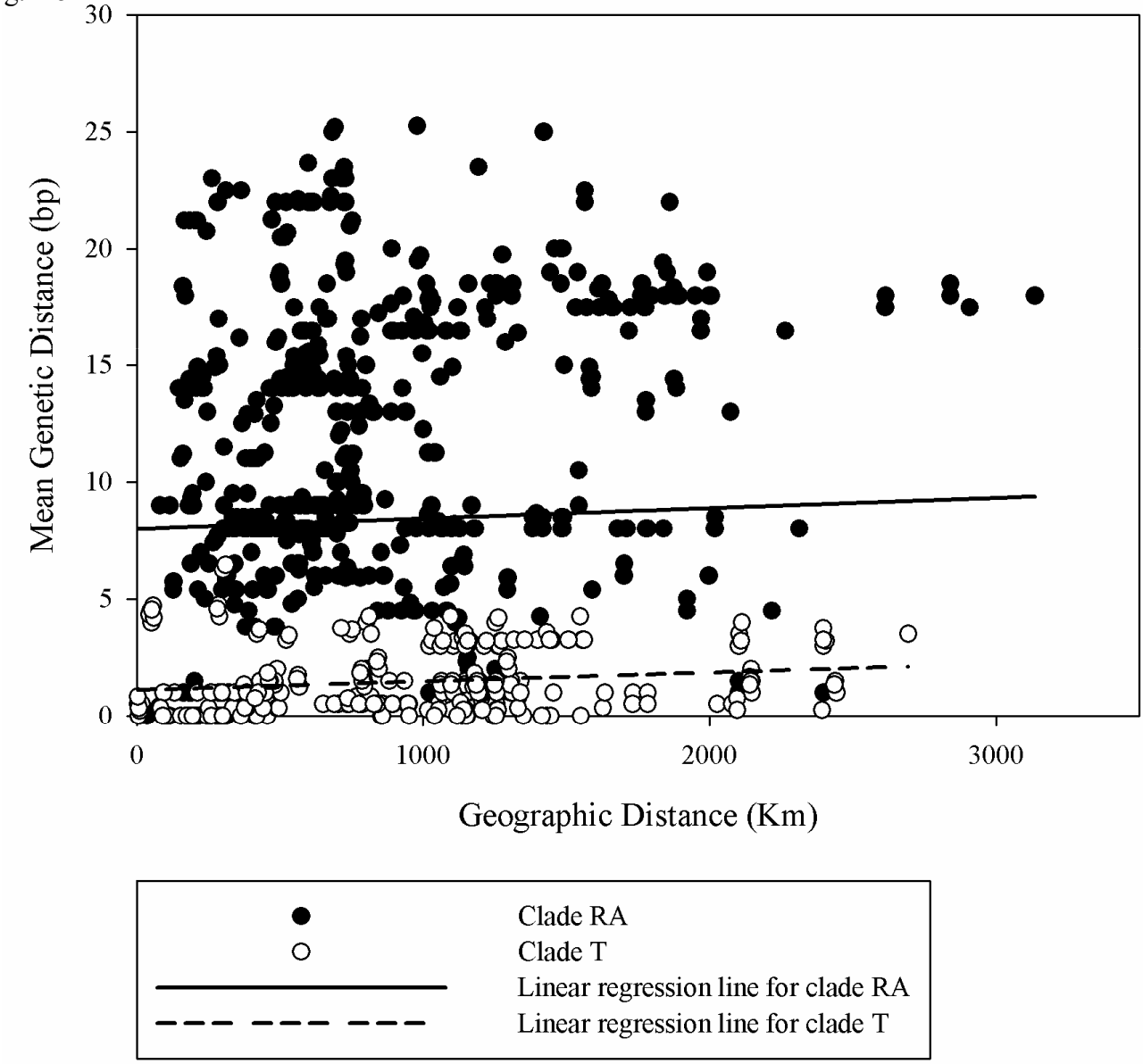
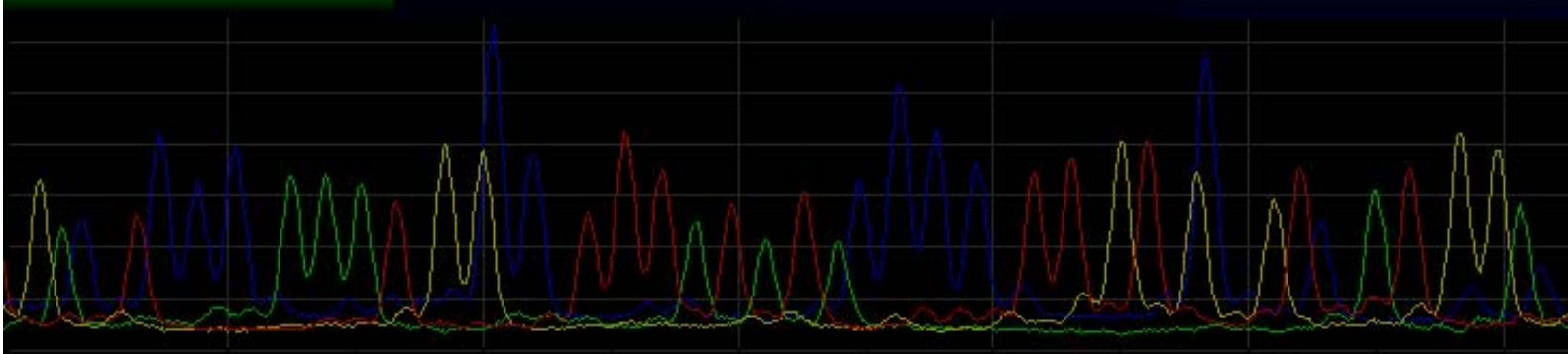

a)
T
RA
HP
K
Milacoma 39 a
Milacoma 39 b
Fishhawk 26 a
Fishhawk 26 b
Euchre 43 a
Euchre 43 b
Cedar 14 a
Cedar 14 b
Salmon 16
Quinault 17 a
Quinault 17 b
Ziegler 18
Satsop 21 a
Satsop 21 b
Nisqually 22 c
Nisqually 22 d
Nisqually 22 e
Klaskanine 24 a
Klaskanine 24 b
Klaskanine 24 c
Klaskanine 24 d
Klaskanine 24 e
Necanicum 27 a
Necanicum 27 b
Necanicum 27 c
Necanicum 27 d
Necanicum 27 e
L. ayresii Mill 34
L. ayresii Feather 52 a
L. ayersii Feather 52 b
L. ayresii Feather 52 c
L. ayresii Feather 52 d
L. ayresii Sacramento Delta 56 a
L. ayresii Sacramento Delta 56 b
Owens 37 a
Owens 37 b
Fork 23 a
Fork 23 b
Fork 23 c
Fork 23 d
Fork 23 e
L. pacifica Crystal Springs 29 a
L. pacifica Crystal Springs 29 b
L. pacifica Crystal Springs 29 c
L. pacifica Clackamas 31 a
L. pacifica Clackamas 31 b
L. pacifica Clackamas 31 c
L. pacifica Clackamas 32 a
L. pacifica Clackamas 32 b
L. pacifica Clackamas 32 c
L. pacifica Clackamas 32 d
Fourmile 40 a
Fourmile 40 b
Fourmile 40 c
Fourmile 40 d
Mark West 54 a
Mark West 54 b
Mark West 54 c
Mark West 54 d
Siuslaw 38 a
Siuslaw 38 b
Siuslaw 38 c
Siuslaw 38 d
Siuslaw 38 e
Paynes 50
L. hubbsi Merced 57 a
L. hubbsi Merced 57 b
Kelsey 53 a
Kelsey 53 b
Kelsey 53 c
Kelsey 53 d
L. fluviatilis Y18683
E. tridentatus Soleduck 13 a
E. tridentatus Sonoma 55
E. tridentatus Duckabush 15 a
E. tridentatus Duckabush 15 b
E. tridentatus Wilson 30
E. tridentatus Luckiamute 33
E. tridentatus Alsea 36
E. tridentatus Floras 42
E. tridentatus Eel 49
E. tridentatus Soleduck 13 b
P. marinus U11880
5 changes
b)
T
RA
Fishhawk 26 a
Fishawk 26 b
Milacoma 39 a
Milacoma 39 b
Cedar 14 a
Cedar 14 b
Salmon 16
Quinault 17 a
Quinault 17 b
Ziegler 18
Satsop 21 a
Satsop 21 b
Nisqually 22 c
Nisqually 22 d
Nisqually 22 e
Euchre 43 a
Euchre 43 b
Klaskanine 24 a
Klaskanine 24 b
Klaskanine 24 c
Klaskanine 24 d
Klaskanine 24 e
Necanicum 27 a
Necanicum 27 b
Necanicum 27 c
Necanicum 27 d
Necanicum 27 e
L. ayresii Mill 34
L. ayresii Feather 52 a
L. ayersii Feather 52 b
L. ayresii Feather 52 c
L. ayresii Feather 52 d
L. ayresii Sacramento Delta 56 a
L. ayresii Sacramento Delta 56 b
Owens 37 a
Owens 37 b
Fork 23 c
Fork 23 d
Fork 23 e
Fork 23 a
Fork 23 b
Kelsey 53 a
Kelsey 53 b
Kelsey 53 c
Kelsey 53 d
L. hubbsi Merced 57 a
L. hubbsi Merced 57 b
Paynes 50
Siuslaw 38 b
Siuslaw 38 c
Siuslaw 38 d
Siuslaw 38 e
Siuslaw 38 a
L. pacifica Crystal Springs 29 a
L. pacifica Crystal Springs 29 b
L. pacifica Crystal Springs 29 c
L. pacifica Clackamas 31 a
L. pacifica Clackamas 31 b
L. pacifica Clackamas 31 c
L. pacifica Clackamas 32 a
L. pacifica Clackamas 32 b
L. pacifica Clackamas 32 c
L. pacifica Clackamas 32 d
Mark West 54 a
Mark West 54 b
Mark West 54 c
Mark West 54 d
Fourmile 40 a
Fourmile 40 b
Fourmile 40 c
Fourmile 40 d
L. fluviatilis Y18683
E. tridentatus Alsea 36
E. tridentatus Floras 42
E. tridentatus Eel 49
E. tridentatus Duckabush 15 a
E. tridentatus Duckabush 15 b
E. tridentatus Wilson 30
E. tridentatus Luckiamutc 33
E. tridentatus Soleduck 13 a
E. tridentatus Sonoma 55
E. tridentatus Soleduck 13 b
P. marinus U11880
0.03
c)
T
Farragut 1
L. ayresii Quilgauw 2
Ishkeenickh 3 b
Ishkeenickh 3 a
Arden 4
Morrison 5
L. ayresii Fraser 6
Nisqually 22 a
L. ayersii Partington 7
Chase 8 b
Chase 8 a
Smith 9 a
Smith 9 b
Nooksack 10 a
Nooksack 10 b
Nooksack 10 c
Nookachamps 11 a
Nookachamps 11 b
Nookachamps 11 c
Nookachamps 11 d
Big 12 a
Big 12 b
Big 12 c
Big 12 d
Cedar 19 a
Cedar 19 b
Cedar 19 c
Green 20 a
Green 20 b
Green 20 c
Nisqually 22 b
Mill 25 a
Mill 25 b
Mill 25 c
Mill 25 d
Gibbons 28 a
Gibbons 28 b
Yaquina 35 a
Yaquina 35 b
Boyes 47 b
Prairie 46 a
Prairie 46 b
Boyes 47 a
Streelow 48 a
Streelow 48 b
Streelow 48 c
Cow 41 a
Cow 41 b
Cow 41 c
Cow 41 d
Hunter 44 a
Hunter 44 b
Hunter 44 c
Hunter 44 d
Hunter 44 e
McGarvey 45 a
McGarvey 45 b
McGarvey 45 c
McGarvey 45 d
McGarvey 45 e
Navarro 51 a
Navarro 51 b
Navarro 51 c
Navarro 51 d
5 changes

Fig. 1-5


30
25
20
15
10
5
0
Mean Genetic Distance (bp)
0
1000
2000
3000
Geographic Distance (Km)
Clade RA
Clade T
Linear regression line for clade RA
Linear regression line for clade T

Chapter 2 -

EVOLUTIONARY GENETIC MARKERS: TESTING THE CONGRUENCE OF PHYLOGENETICS AS INFERRED FROM THE GENUS *LAMPETRA*



Abstract

Molecular phylogenetic relationships among lamprey species have all been based on mitochondrial (mt)DNA data, as few variable nuclear loci are known in lampreys. This chapter compares mtDNA (cytochrome *b*, NADH 2, and cytochrome oxidase 1) and nuclear (transporter associated with antigen processing [TAP] intron 9 and internal transcribed spacer [ITS] region 1) sequence data, and nuclear microsatellite variability (nine loci) in brook lamprey populations along the Pacific coast of North America. Populations include recognized (*Lampetra richardsoni, L. pacifica,* and *L. hubbsi*) and proposed cryptic (*L.* cf. *richardsoni*) species. The six molecular markers each produced slightly different topologies. The mtDNA and microsatellite data support the distinctive genetic composition of six clades, and the polyphyly of *L. richardsoni*. *Lampetra* cf. *richardsoni* from Kelsey Creek is supported as the sister clade to the remaining *Lampetra* species for all mitochondrial phylogenies. The phylogeny based on nuclear TAP intron 9 sequence data contradicts that of mitochondrial phylogenies, placing *L. hubbsi* as the sister group to the remaining *Lampetra* species, and *L.* cf. *richardsoni* from Kelsey Creek in a polytomy with *L.* cf. *richardsoni* from Fourmile Creek and *L. pacifica* from Clackamas River. The microsatellite phylogeny has uncertain phylogenetic signal, recovering *L.* cf. *richardsoni* from Kelsey Creek as being distinct but in a polytomy that includes *Entosphenus tridentatus*. Large significant F_{st} values were recovered for all six clades, ranging from 0.21 to 0.75 and averaging 0.54. The ITS1 phylogeny supports *Lampetra* cf. *richardsoni* from Kelsey Creek as being a distinct clade within *Lampetra,* but does not provide sufficient resolution to discriminate between other congeneric species. All six markers support Kelsey Creek lampreys as being genetically distinct.

1. Introduction

With the advent of new genetic techniques, phylogenies generated with molecular data often conflict with traditional morphology (e.g., Sturmbauer and Meyer, 1992) but many of these earliest molecular phylogenies have used only a single locus (e.g., Kocher et al., 1989; Halanych et al., 1999). Many of these studies – particularly in animals – relied heavily on mitochondrial (mt)DNA or (r)DNA, the former being particularly useful for resolving relationships among closely-related species and the latter for deeper divergences (Hillis and Dixon, 1991). More recently, attempts to determine the evolutionary histories of organisms, especially those that are closely related, often yield discordant results depending on the gene or gene fragment used in the reconstruction (e.g., Shaw, 2002; Mattern, 2003). As a consequence, the evolutionary histories of many groups of organisms remain unresolved and those reconstructed from a single locus may include inherent bias.

With the proliferation of molecular tools used in phylogenetic studies, there has been growing awareness that the reliance on a single genetic marker may provide insufficient phylogenetic resolution. Accordingly, it has become an increasingly widespread practice to incorporate multiple datasets to substantiate phylogenetic inference. Complications arising from results based on the use of single genetic markers – including convergent evolution, incomplete lineage sorting, ancestral polymorphism, effective population size, variation in evolutionary rates between markers, hybridization, and the lack of neutrality subsequently affected by natural selection, none of which are mutually exclusive – may go unnoticed. As innovation continues to assist evolutionary biology's historic use of morphological characteristics to derive evolutionary events, it is important to utilize all available data to ensure that these complications are mitigated and reported as accurately as possible.

Therefore, this stresses the need to test the congruence between different genetic markers, and may reveal inherent biases in a genetic marker (e.g., maternal and biparental inheritance, or non-neutral versus neutral genetic markers; see below).

As a phylogenetic marker, mtDNA has many advantages. For example, it generally evolves faster than single-copy nuclear genes (Brown et al., 1982) and as a result can provide a strong high-level taxonomic signal (Avise, 2000). Depending on the mtDNA gene of choice, this taxonomic signal can vary as inferred from different regions of the mitochondrial genome evolving at different rates (Saccone et al., 1991), allowing suitable regions to be chosen for the particular taxonomic level to be studied. Additionally, the conserved content and order of gene sequences allow primers to be designed for a variety of organisms without prior knowledge of their genomes (Kocher et al., 1989). This has made mtDNA the genetic marker of choice for many molecular phylogenetic studies, particularly in animals (e.g., Bermingham and Avise, 1986; Avise et al., 1986; Irwin, et al., 1991). One of the potential drawbacks to mitochondrial DNA is that it does not recombine (i.e., represents a single locus; Hayashi et al., 1985), although some evidence of recombination has recently been reported (Eyre-Walker et al., 1999, Hagelberg et al., 1999). Mitochondrial DNA is a presumed neutral marker (reviewed in William and Kreitman, 1995) that is maternally inherited in most species (exceptions include paternal leakage in mice, Gyllesten et al., 1991; biparental inheritance in marine mussels, Zouros et al., 1992). This being the case, phylogenies based solely on mtDNA may not be consistent with phylogenies based upon nuclear markers (e.g., Smith, 1992; Evans et al., 2004). Cases of hybridization, common among freshwater fishes (Schwartz, 1972), may go unrecognized if only mtDNA markers are employed as diagnostic tools. Furthermore, only the maternal half of the ancestry of many hybrid species would be

reflected using a phylogeny inferred from mtDNA data. The reliability of studies based on a single-locus marker are often criticized due to inherent bias (see above; e.g., Will and Rubinoff, 2004; Ebach and Holdredge, 2005).

To circumvent biases that may occur with the exclusive use of mtDNA data, nuclear data have been used to compliment evolutionary reconstruction (e.g., Degnan, 1993; Palumbi and Baker, 1994). The nuclear ribosomal RNA (rRNA) array of a eukaryotic genome typically consists of several hundred tandemly repeated copies of the transcription unit and nontranscribed spacers. The variation within units and spacers tends to be homogenized in species and populations, whereas divergence is common among them (Hillis and Dixon, 1991). Furthermore, rRNA genes are recombining, biparental genetic markers that can reveal recent gene flow and hybridization events (Mayer and Soltis, 1999). The multi-copy internal transcribed spacer (ITS) regions of rRNA genes have mainly been used in phylogenetic studies to resolve interspecific relationships within genera due to their fast evolutionary rates (Pleyte et al., 1992; Phillips et al., 1999). While a number of nuclear loci have been used to address the relationship among lampreys, hagfish, and gnathostomes (e.g., Mallatt and Sullivan, 1998; Zardova and Meyer, 2001; Yu et al., 2008) – or reconstruct other deep divergences – these loci appear not to provide sufficient resolution to infer phylogenies among closely-related lamprey taxa.

Microsatellite markers are another form of nuclear marker that contains highly variable tandem repeats of one to six nucleotides found at high frequency in most taxa (Selkoe and Toonen, 2006). Microsatellites are highly polymorphic and can provide estimates of migration, distinguish relatively high rates of migration from panmixia, and can estimate the relatedness of individuals (e.g., Bowcock et al., 1994; reviewed in Selkoe and

Toonen, 2006). Other advantages to using microsatellites are that they are single-locus codominant markers that when used in conjunction with other microsatellites suggest variable mutation rates (Jin et al., 1996); this allows the recovery of multiple evolutionary events that span a much larger number of generations (Selkoe and Toonen, 2006) than when using one single-locus marker.

Lampreys, together with the hagfishes, are the sole extant representatives of the most primitive vertebrates, the "jawless fishes." There are 38 described species worldwide and approximately half are non-parasitic, non-migratory brook lampreys (Renaud, 1997; Potter and Gill, 2003). Brook lampreys are found in seven of the 10 recognized lamprey genera, implying that non-parasitism has arisen independently multiple times (Hubbs and Potter 1971; Vladykov and Kott 1979). The life cycle of brook lampreys lacks a parasitic feeding phase (i.e., they are non-trophic as adults), and the filter-feeding larval phase lasts for three to eight years prior to metamorphosis (Schultz, 1930; Potter, 1980b). All lampreys are semelparous – die after spawning (Hardisty and Potter, 1971).

There is a paucity of morphological characters used in conventional lamprey taxonomy, which is traditionally limited to adult specimens (e.g., dentition, relative size of the eye and oral disc, number and morphology of velar tentacles; Hubbs and Potter, 1971; Gill et al., 2003). There are even fewer characters available to distinguish among closely-related larvae found within a genus (e.g., Neave et al., 2007; Goodman et al., in press), and discriminating specimens to the species-level is rare (Richards et al., 1982). Molecular investigations based on mtDNA gene sequences thus have proven very useful in clarifying taxonomic relationships among many taxa where homoplasy or conservative morphologies may otherwise have obscured the relationships (Docker et al., 1999; Blank et al. 2008). The

conserved (and degenerate) morphology of brook lampreys has made classification based on traditional characters especially difficult (see Docker et al., 1999). Populations ascribed to a single widespread species based on morphology have been shown to be genetically very diverse (e.g., Yamazaki et al., 2006; Martin and White, 2008), and many poputions may represent distinct species. Likewise, phylogeography of brook lampreys along the Pacific coast of North America was recently assessed based on mitochondrial (mtDNA) variation of the cytochrome *b* (cyt *b*) gene (Chapter 1). Significant genetic divergence was reported between congeneric species including the western brook lamprey (*Lampetra richardsoni*), the Pacific brook lamprey (*L. pacifica*), and the Kern brook lamprey (*L. hubbsi*). Additionally, cryptic species tentatively described as *L.* cf. *richardsoni* were proposed. These potentially cryptic species (e.g., Mark West Creek and Kelsey Creek) were assumed to be *L. richardsoni* (Moyle, 2002), but Chapter 1 indicates that these populations do not form a monophyletic group with *L. richardsoni* from its type locale (see below). Consequently, it was concluded that *L. richardsoni* sensu lato represents a polyphyletic species based on a single mtDNA locus.

Chapter 1 examined relationships among 135 *Lampetra* populations and resolved three major clades (RA, HP, and K clade). The *richardsoni/ayresii* (RA clade) clade contains, but is not limited to, *L. richardsoni* and *L. ayresii* collected from each respective type locale. Similarly, the *hubbsi/pacifica* (HP clade) clade contains, but is not limited to, *L. hubbsi* and *L. pacifica* collected from each respective type locale. Finally, the Kelsey Creek clade (K clade) is represented by a single population of lamprey from Kelsey Creek, CA, presumed to be *L. richardsoni* (Moyle, 2002). In Chapter 2, the goal is to compare the congruence of different molecular markers in reconstructing the phylogeny among major

taxa represented in each of these clades. *Lampetra richardsoni* (from the polytomy within the RA clade), *L. pacifica,* and *L. hubbsi* (both from within the HP clade) each collected from their respective type locales, the sole representative from the K clade (Kelsey Creek; tentively described as *L.* cf. *richardsoni*), as well as one of the three *L.* cf. *richardsoni* from the HP clade (Fourmile Creek) are used in this study. Multiple genetic markers were used for phylogenetic analyses including three mtDNA markers – cyt *b,* NADH subunit 2 (ND2), and cytochrome oxidase subunit 1 (CO1). Variation in these three mtDNA markers will be used to assess congruence between maternally inherited genes commonly used in phylogenetic reconstruction. In comparison, a multi-copied nuclear marker (ITS1) and a presumed single-copy nuclear marker from the ninth intron of the transporter associated with antigen processing (TAP) gene (Uinuk-ool et al., 2003) will be used to test the congruence of recombinant biparental markers to mtDNA. Finally, microsatellite markers have recently been characterized for *L. richardsoni* (Luzier et al., in press). Nine of these microsatellite loci will be used for phylogenetic reconstruction.

2. Materials and methods

2.1 Taxon sampling for DNA sequence data

For analyses, a total of 18 *Lampetra* specimens representing five major taxa identified in Chapter 1 were used: specimens representing *L. richardsoni* (from the polytomy within the RA clade), *L. pacifica,* and *L. hubbsi* (both from within the HP clade), and two potentially cryptic species (designated here as *L.* cf. *richardsoni*; one within the HP clade and one from clade K; see Table 2-1 and Fig. 1-4); *L. richardsoni*, *L. pacifica,* and *L. hubbsi* were sampled from their type localities (Table 1-1; Fig. 2-1). *Lampetra* cf. *richardsoni* from Fourmile

Creek was chosen for this study because Bayesian and parsimony phylogenetic analyses were the most incongruent (see Chapter 1). Additional specimens from the genus *Lampetra* (e.g., *L. ayresii*) were not included since this chapter is intended to investigate relationships among clades, and not within clades. Four specimens of *Entosphenus tridentatus* were used as the outgroup (Table 2-1). Larval specimens were collected due to their year-round availability and greater abundance. Larvae belonging to the genus *Lampetra* were identified (i.e., discriminated from *Entosphenus*) based on caudal pigmentation (Richards et al., 1982; Goodman et al., in press); identification to species was based on the distributional range of each species where congeners are not known to co-exist, or when present, adult morphology (Table 2-1).

2.2 mtDNA

Three mitochondrial genes were sequenced for phylogenetic analyses: the complete cytochrome *b* gene (1191 bp; Chapter 1), the complete NADH subunit 2 gene (1044 bp), and a 645 bp fragment of cytochrome oxidase 1 corresponding to the DNA barcoding fragment (Folmer et al., 1994). Total DNA from muscle tissue was extracted using the Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol and proteinase K (Invitrogen). The mitochondrial genes were amplified via polymerase chain reaction (PCR). Target sequence was amplified using primers outlined in Table 2-2. Each 30 µL PCR reaction contained 10x PCR manufacturer's buffer (20 mM Tris-HCl pH 8.4; 50 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 266 pmol of each primer, and 0.02 U of *Taq* DNA Polymerase (Invitrogen). Reactions were initially denatured at 94 °C for 2 min. Amplifications of all fragments were carried out in 30 cycles: denaturation at 94 °C for 2

min, primer annealing at 60 °C, 58 °C, and 55 °C for 10 cycles each, extension at 72 °C for 2 min, and additional extension at 72 °C for 5 min.

PCR products were purified following precipitation with isopropanol and sodium acetate, and subsequently used as a template for the sequencing reaction using ABI PRISM® Big-Dye™ Terminator (v3.1, Applied Biosystems Inc.) and the sequencing primers outlined in Table 2-2. Sequences were read on the ABI 3130 Genetic Analyzer (Appied Biosystems Inc.) automatic sequencer. The resulting electropherograms (ABI files) were viewed in Chromas Lite v2.01 (By Conor McCarthy, Griffith University, Australia), exported to Microsoft® Word, and subsequently aligned by eye.

2.3 Nuclear DNA

Two nuclear fragments were sequenced for phylogenetic analyses: the ninth intron (1245 bp Merced River specimens; 1246 bp all other specimens; excluding indels) of the transporter associated with antigen processing (TAP) gene, and a fragment (280 bp in *Lampetra* spp.; 286 bp in *Entosphenus tridentatus*; excluding indels) of the the internal transcribed spacer (ITS) region 1 of the ribosomal RNA (rRNA) genes. The primers used for PCR and sequencing are outlined in Table 2-2. Processing of samples and sequencing followed the protocol for mtDNA with the following exceptions: Go*Taq*® DNA polymerase (Promega) was used instead of Invitrogen *Taq* DNA polymerase (but in the same concentration) and the PCR samples were denatured at 96 °C for 8 min and placed directly on ice prior to the addition of the polymerase (to remove any secondary structure of the template); subsequent denaturation steps for PCR occurred at 96 °C.

For TAP intron 9, the full 50 µL PCR reaction for TAP intron 9 was visualized on a 1.4% agarose gel made with 1x TAE buffer and then the band of appropriate length (approximately 1300 bp; Uinuk-ool et al., 2003) were gel excised for PCR purification using the Wizard® SV Gel and PCR Clean-Up System (Promega) prior to sequencing. Gel-extraction procedures were used because of the presence of multiple bands.

The complete ITS1 region could not be sequenced directly from PCR product since different amplicons varied in length at the 3' end. In addition, all specimens showed multiple double peaks on the PCR sequenced electropherograms (Fig. 2-2). Consequently, the ITS1 region was cloned prior to sequencing in two samples from each population: a second, independent amplification was conducted, using the same procedure as described above, and the resulting PCR products were cloned using a TOPO TA-Cloning kit (Invitrogen). Within 24 h of amplification, PCR products were inserted into the pCR4-Topo vector, which were used to transform One Shot® Top10 chemically competent *Escherichia coli* cells by heat shock at 42 °C, according to manufacturer's protocol. Cells were cultured in the provided SOC medium at 37 °C for 1 h in a shaking incubator, and then plated on LB medium supplemented with 75 µL of 60 mg/ml ampicillin and 40 µL of X-gal (2 mg/ml) on each plate. Plates were incubated overnight at 37 °C. White colonies (containing transformant cells) were screened for the presence of the ITS1 insert using the PCR primers. The selected clones were inoculated overnight in 5ml LB broth and 10 µL of ampicillin (60 mg/ml). Plasmid DNA was extracted using a QIAprep kit (Qiagen) following the manufacture's protocol. From each individual, 4 to 8 clones were sequenced for a total of 65 clones to identify the genetic variation found intra-individually. The sequence data were compared among clones from a given individual; the presence of more than one nucleotide at a given

site was denoted in the consensus sequence as a degenerate base. Details of the cloned ITS1 sequence data used to help generate a consensus sequence for phylogenetic analyses are provided (Table 2-3).

Preliminary alignment of nuclear sequences was performed using ClustalX v2.0 (Higgins and Sharp, 1989; Larkin et al., 2007), and alignments were subsequently edited using the software GeneDoc v2.6.002 (Nicholas and Nicholas, 1997). Size heterozygotes were detected in both nuclear sequence datasets. In TAP intron 9 degenerate bases were recorded based on the relative intensity of peaks on the eletropherogram. A series of A-repeats found to vary in length (9 to 11 repeats) was found beginning at position 884 in TAP intron 9 based on electropherograms.

2.5 Phylogenetic analyses for sequence data

Phylogenetic analyses included sequence data from 22 lamprey specimens (Table 2-1). Analyses were constrained with *E. tridentatus* from Alsea River as the outgroup based on previous phylogenies (Hubbs and Potter, 1971; Docker et al., 1999; Gill et al., 2003). Analyses of sequence data were performed on each independent dataset as well as a combined dataset.

Maximum parsimony (MP) analyses were executed in PAUP* v4.0b10 (Swofford, 2002). Independent heuristic searches were performed with 10,000 random taxon addition (RAS) replicates and TBR branch swapping. All characters were unordered, and all character transformations were equally weighted. Only the most parsimonious trees were retained to compute a consensus tree. Gaps were coded using GapCoder software (Young and Healy, 2003), to utilize the phylogenetic information contained in the indels. Indices

values for MP analyses were calculated in PAUP* v4.0b10 (Swofford, 2002). The consistency index (CI) is the relative (based on number of steps in the tree) amount of homoplasy in the dataset with larger values indicative of large amount of homoplasy. The retention index (RI) measures the amount of synapomorphic characters retained on a cladogram, with larger values indicative of more synapomorphic data. The homoplasy index (HI) explains the level of homoplasy in a dataset represent by the formula (1 – CI). Relative support for the internal nodes in each analysis was estimated using bootstrap (Felsenstein, 1985). Bootstrap values were estimated from 100 replicates, each employing 1000 random stepwise random addition sequences.

Bayesian analyses were conducted using MrBayes v3.1 (Ronquist and Huelsenbeck, 2003). A model of evolution particular to each dataset was generated with Modeltest 3.6 (Posada and Crandall, 1998) using hierarchical likelihood ratio tests (hLRTs). Four independent Bayesian analyses were run for 5,000,000 generations (10,000,000 generations for the combined dataset) until the standard deviation of split frequencies was below 0.01, and until the potential scale reduction factor (PSRF) was close to 1.0 for all parameters (Ronquist et al., 2005). The burnin period of 40% were discarded as a conservative measure to avoid the possibility of including random, sub-optimal trees. A majority rule Bayesian consensus tree was then calculated from the posterior distribution of trees, and the posterior probabilities calculated as the fraction of samples recovering any particular clade (Huelsenbeck and Ronquist, 2001).

2.6 Microsatellites

Additional specimens from each of the five *Lampetra* taxa mentioned above and *Entosphenus tridentatus* (for a total of 60) were used for microsatellite analyses to raise statistical support (Table 2-1). Ten individuals from each population were genotyped with nine microsatellite loci (Lri-1, Lri-2, Lri-4, Lri-5, Lri-6, Lri-7, Lri-8, Lri-9, and Lri-10; Table 2-2), and amplified with 5'-end-labeled primers with 6-FAM or HEX (Sigma Life Science) or NED or PET (Applied Biosystems Inc.) under the conditions outlined by Luzier et al. (in press). Only two *L. richardsoni* samples were available from Smith Creek; therefore an additional eight specimens from Big River were used since cyt *b* sequences were indistinguishable between these two populations (see Table 2-1 and Fig. 1-4). Samples were subsequently analyzed on an ABI 3130 automatic capillary sequencer. Fragment length was analyzed using GeneScan-600 LIZ size standard (Applied Biosystems Inc.). Fragment visualization and scoring were done using GeneMapper v.4.0 (Applied Biosystems Inc.).

Nei's (1978) unbiased estimate of expected heterozygosity (H_e), observed heterozygosity (H_o) and allele frequencies were calculated for each of the nine microsatellite loci for the six populations of lampreys using Tools for Populations Genetic Analyses (TFPGA) software (Miller, 1997). An exact test for goodness of fit to Hardy-Weinberg equiliblrium (HWE) was conducted for all loci within each group using the Monte Carlo method in TFPGA (20,000 permutations). The results of the HWE test were adjusted for significance using the Bonferroni correction procedure (Rice, 1989) to account for multiple, simultaneous tests.

The degree of genetic differentiation among lamprey populations was estimated using Weir and Cockerham's (1984) θ statistic used to calculate pairwise F_{st}. The θ statistic was

calculated with FSTAT software (Goudet, 2001). The significance of pairwise F_{st} values was tested with a G-statistic (Raymond and Rousset, 1995), and subsequently compared to a strict Bonferroni corrected level of significance (Rice, 1989). Individual lamprey samples collected from Big River were pooled with those from Smith Creek after the pairwise F_{st} value (0.0649) was found to be not significantly different ($p=0.0219$; $\alpha=0.002381$ after Bonferroni correction). A rooted neighbour-joining cluster analysis rooted with *E. tridentatus* was performed with Cavalli-Sforza and Edwards's (1967) chord distance using Populations, version 1.2.30 (O. Langella, Centre National de la Recherce Scientifique, Laboratoire Populations, Génétique et Evolution, Gif sur Yvette, France, available at http://bioinformatics.org/~tryphon/populations/) and visualized in TreeView (Page, 1996). Cavalli-Sforza and Edwards' (1967) chord distance makes no assumption regarding constant population size or mutation rates among loci, and this distance is generally used to provide an accurate tree topology among closely-related populations (Angers and Bernatchez, 1998). The resulting tree was bootstrapped among five genetic loci (Lri-1, Lri-2, Lri-5, Lri-6, and Lri-7) in which null alleles were absent (i.e., among loci which amplified in *E. tridentatus* as well as *Lampetra* spp.), with replacement for 2,000 permutations.

3. Results

3.1 Phylogenetic analyses of sequence data

For phylogenetic analyses based on sequence data, three mtDNA, two nuclear, and a combined molecular dataset were used. Sequence divergence within clades (between specimens) and among clades for each of dataset is summarized in Table 2-4.

Both maximum parsimony and Bayesian analyses resulted in five independent consensus trees and one combined mtDNA and nuclear sequence dataset consensus tree (Figs. 2-3 to 2-8). For MP analyses, the number of characters used to construct the consensus tree, the number of constant characters, the number of parsimony informative sites, the tree lengths, and index values (CI, RI, HI) for each dataset are summarized in Table 2-5. Mean likelihood values, as well as the model of evolution used to analyze each dataset, are summarized in Table 2-6.

The methods used to infer phylogeny (MP and Bayesian) produced very similar topologies for each of the five genes or gene fragments. With the exception of the ITS1 and combined dataset (Fig. 2-7 and 2-8), the MP method resulted in an increase in resolution over the Bayesian method (Fig. 2-3 to 2-6). All mtDNA phylogenetic analyses reveal that each population is monophyletic. This also suggests that *L. richardsoni* sensu lato is polyphyletic since its phylogenetic placement often neighbours several recognized species. The nuclear TAP intron 9 phylogeny supports the monophyly of *L. pacifica*, *L.* cf. *richardsoni* from Kelsey Creek, and *L. hubbsi*; however, it does not support the monophyly of *L.* cf. *richardsoni* from Fourmile Creek nor *L. richardsoni* from Smith Creek (Fig. 2-6). The ITS1 phylogeny supports *L.* cf. *richardsoni* from Kelsey Creek as a monophyletic group embedded within a polytomy encompassing all sampled *Lampetra* species (Fig. 2-7).

There are also noticeable discrepancies between the five independent molecular datasets in terms of the inferred relationships among the populations. The most obvious difference established is that of sister relationships. *Lampetra* cf. *richardsoni* from Kelsey Creek is strongly supported as the sister group to all other ingroup clades (BS = 100%; PP = 1.00) based on independent mtDNA data (Fig. 2-3 to 2-5), as well as with combined data

(Fig. 2-8). Conversely, *L. hubbsi* from Merced River is strongly supported as the sister group to all other ingroup clades (BS = 100%; PP = 1.00) in the TAP intron 9 dataset (Fig. 2-6). At higher taxonomic levels, the relationships between populations are commonly well supported with bootstrap and posterior probability values; however, they are inconsistent among molecular datasets.

3.2 Phylogenetic analysis using microsatellites

The nine microsatellite loci were also used for phylogenetic analysis. Ten of the 54 microsatellite tests showed significant deviation from HWE after Bonferroni correction owing to heterozygote deficiency (Table 2-7).

Significant F_{st} values were found between each population of *Lampetra* lampreys, suggesting each *Lampetra* population is genetically distinct from the others (Table 2-8). Bayesian support agrees with the F_{st} data matrix, corroborating the distinctive genetic composition of the six populations (Fig. 2-10). However, the microsatellite neighbour-joining (NJ) tree showed only five clades (Fig. 2-9); specimens from Fourmile Creek clustered with those of Smith Creek and Big River, despite significant genetic differentiation (Table 2-8; Fig. 2-10). Additionally, the monophyly of Pacific coast *Lampetra* is no longer supported because of a polytomy between *E. tridentatus, L.* cf. *richardsoni* from Kelsey Creek, and the rest of the *Lampetra* lampreys. Therefore, *L. hubbsi* from Merced River is the sister group to the remaining *Lampetra* specimens, excluding *L.* cf. *richardsoni* from Kelsey Creek.

4. Discussion

Incongruencies within and between mtDNA and nuclear genetic markers were discovered. Notably, the monophyly of each population was supported by all mtDNA markers, but only supported to various extents using nuclear markers (see below). In addition, the genealogical relationships among populations are somewhat inconsistent with previous findings (Chapter 1). In the discussion below, three questions that arise from this phylogenetic inconsistency are addressed: To what extent are the phylogenetic patterns of divergence congruent between independent genetic markers? Should distinct phylogenetic units (i.e., clades) be recognized as cryptic species (i.e., a group of organisms that may be morphologically indistinguishable but that satisfy the phylogenetic species criteria – and potentially criteria of the BSC)? How does this phylogenetic research impact conservation interest of regionally endemic brook lampreys?

4.1 Phylogenetic patterns of divergence, congruence, and clade support

There is not complete congruence between genetic markers. The higher-level taxonomic relationships within *Lampetra* show significant population structure whereas the relationships between them vary depending on the genetic marker. For example, mtDNA phylogenies support combinations of *L. hubbsi* as the sister species to *L. pacifica, L. pacifica* as sister to *L.* cf. *richardsoni* from Fourmile Creek, and *L.* cf. *richardsoni* from Kelsey Creek as sister to *L. hubbsi*. Additionally the nuclear TAP intron 9 groups *L. richardsoni* with three of four *L.* cf. *richardsoni* from Fourmile Creek (although the fourth specimen from Fourmile Creek groups with *L.* cf. *richardsoni* from Kelsey Creek and *L. pacifica*). There are numerous possibilities for such incongruencies between phylogenies contructed using

independent markers. Since nuclear genes evolve at relatively slow rates (as discussed below), it is possible that TAP intron 9 has recovered an ancient hybridization event between two ancient northern and southern derivatives (as inferred from Fig. 2-6). In corroboration, the microsatellite neighbour-joining tree also supports introgression of *L. richardsoni* loci from Smith Creek (including Big River) into *L.* cf. *richardsoni* from Fourmile Creek despite the monophyletic support for independent clades using Bayesian methods. Pairwise θ among lamprey populations also revealed a high degree of distinctiveness based on F_{st} values. A general guideline for F_{st} values is as follows: $0< F_{st} <0.05$ indicates little genetic differentiation, $0.05< F_{st} <0.15$ indicates moderate differentiation and $0.15< F_{st} <0.25$ indicates great differentiation (Hedrick 2003). The resulting F_{st} values mostly suggested great differentiation averaging 0.54 (ranging from 0.21 to 0.75).

The independent evolutionary rates of different genetic markers may explain the observed incongruities. The use of three different mtDNA markers (cyt *b*, ND2, and CO1) allows various gene regions of the mtDNA genome to be explored. These different gene regions of the mtDNA genome are believed to display different evolutionary patterns as a result of varying substitution rates and different constraints on those substitutions (Attardi, 1985; Graur and Li, 2000). Accordingly, these different evolutionary rates are reflected in the level of divergence relative to a common ancestor, or alternatively, reflect the level of relatedness between taxa. For nuclear markers, single-copy nuclear markers are believed to generally evolve slower than mtDNA genes (Brown et al., 1982), whereas different regions of multi-copy nuclear genes can evolve at very different rates (Hillis and Dixon, 1991). Indeed in many groups of animals, the rate of nucleotide substitution among mitochondrial protein-coding genes is generally more rapid than the rate of nucleotide substitution among

protein-coding regions of nuclear genes (Vawter and Brown, 1986). Based on the mean K2P% genetic distance values, the mtDNA datasets appear to evolve faster than the nuclear datasets. Additionally, among mtDNA markers the majority of pairwise comparisons are consistently higher in the cyt *b* and ND2 dataset than the CO1 data, whereas comparisons between cyt *b* and ND2 are similar. Variation among nuclear datasets suggests TAP intron 9 evolves at a faster rate than ITS1. These rates of change become critical to phylogenetic studies when choosing a genetic marker appropriate for a particular systematic comparison. There are two reasons, according to Hillis and Dixon (1991), why choosing highly divergent markers may yield less robust results or incongruent results: 1) due to the level of homoplasy (parallelisms, convergences, and reversals) that increases as the substitution rate at each position increases; 2) the number of sequence alignments that are equally good become prohibitively large. Alternatively, choosing a genetic marker that is too conserved may yield insufficient phylogenetic signal to differentiate taxa, likely exemplified in the ITS1 phylogeny.

Additionally the vast majority of evolutionary studies employing mtDNA do not attempt to test the assumptions of the neutral model (Ballard and Kreitman, 1995). Mitochondrial DNA appears to be anything but a neutral marker (Ballard and Whitlock, 2004) and probably undergoes frequent adaptive evolution. For example, studies have suggested direct selection on the respiratory machinery (Grossman et al., 2004), nucleo-cytoplasmic coadaptation (Willett and Burton, 2004), two-level selection (Roze et al., 2005), or adaptive introgression, perhaps hitchhiking with a maternally transmitted parasite (Hurst and Jiggins, 2005). Therefore, the diversity of lampreys estimated from mtDNA may be biased due to effect of natural selection, time since last selective sweep, and demography.

Moreover, even when some relationships are supported by high bootstrap (BS) and posterior probability (PP) values in analyses using single-locus markers, such as the mtDNA and nuclear fragments used, the possibility of an alternative lamprey phylogeny remains. The consensus tree obtained is only a gene tree, which can differ from the species tree as a result of incomplete lineage sorting of ancestral polymorphisms during successive rounds of speciation (Nei 1987; Pamilo and Nei 1988; Takahata 1989; Avise, 2000b; Nichols, 2001) and/or interspecific hybridization (Avise 2000b). Any of the above explanations may be particularly more apparent where a recognized species is not monophyletic. For example, *Lampetra* cf. *richardsoni* from Fourmile Creek spans two distinct clades in the phylogeny inferred using TAP intron 9 (grouped both with *L. richardsoni* and as a polytomy with *L. pacifica* and *L. hubbsi*).

The interrelationships between populations appear ambiguous, but comparing the level of genetic divergence among populations can be used to delineate whether genetic differences correspond to species-level differences (see "*Cryptic speciation"*). In a study identifying Canadian freshwater fishes using CO1, the mean K2P distance of congeneric species ranged from 0 to 19.33%, which on average was 27-fold higher than variation found within a species (Hubert et al., 2008). Levels of CO1 variation among the divergent *Lampetra* populations studied here (1.89 to 3.84%) fall within the lower limits of this range. However, these values also overlap those found in comparisons between genera within a family; Hubert et al., 2008). In contrast, different species of vertebrates ordinarily show more than 2% sequence divergence for cyt *b* (Avise and Walker, 1999; see Chapter 1). All pairwise K2P distances between the divergent *Lampetra* populations studied here surpass this proposed threshold, ranging from 2.58 to 6.41%. The TAP intron 9 fragment was able to

reconstruct phylogenetic relationships among higher-level taxa, although its failure to recover monophyletic populations in Fourmile Creek and Smith Creek require that it be examined further. TAP intron 9 recovers genetic distances ranging from 0.08 to 1.08% between congeneric *Lampetra* species, 5-fold less pronounced than variation found between *Lampetr*a and *Entosphenus.*

Despite incongruence in interrelationships, all genetic data support the notion that *L. richardsoni*, as it is currently recognized, is polyphyletic and that these highlighted populations (see "*Cryptic speciation*" below) are all genetically distinct. *Lampetra* cf. *richardsoni* from Kelsey Creek is supported as the sister clade to the remaining *Lampetra* species for all mitochondrial phylogenies. The phylogeny based on nuclear TAP intron 9 sequence data contradicts that of mitochondrial phylogenies, placing *L. hubbsi* from the Merced River as the sister group to the remaining *Lampetra* species, and *L.* cf. *richardsoni* from Kelsey Creek in a polytomy with *L.* cf. *richardsoni* from Fourmile Creek and *L. pacifica* from Clackamas River. The microsatellite phylogeny has uncertain phylogenetic signal, recovering *L.* cf. *richardsoni* from Kelsey Creek as being distinct but in a polytomy that includes *Entosphenus tridentatus*. The ten of 54 microsatellite tests that suggest a significant deviation from HWE was not attributable to null alleles, but this deviation may reflect Wahlund effects among age classes (i.e., reduction of heterozygosity in a population caused by subpopulation structure; Waples and Teel, 1990), cryptic population structure, inbreeding effects, or unintentional group sampling (Castric et al., 2002). The ITS1 phylogeny does, however, support *Lampetra* cf. *richardsoni* from Kelsey Creek as being a distinct clade within *Lampetra,* but does not provide sufficient resolution to discriminate between congeneric species. In addition to the many suggestions influencing the congruence

of a phylogeny (mentioned above), the topology resulting from the use of a multi-copy rDNA fragment may be indicative of the effects of concerted evolution. Concerted evolution describes the molecular process of DNA sequence homogenization among different loci within multigene families (Arnheim et al., 1980). DNA sequence homogenization via concerted evolution is driven by two molecular processes, gene conversion and unequal crossing over. The relative contribution of each as a homogenizing agent, however, continues to be the subject of debate (Muir et al., 2001).

Phylogenies of closely-related species based on mtDNA and nuclear DNA markers can provide powerful tools for testing alternative hypotheses of species origins, but the incongruence between and within these markers can be confusing. However, discrepancies between mitochondrial and nuclear markers have been informative. For example, the different inheritance pattern of mtDNA (maternally inherited) versus nuclear DNA (biparentally inherited) have revealed sex-biased fidelity to particular groupings or reproductive locations (FitzSimmons et al., 1997; Gladden et al., 1999; Lyrholm et al., 1999).

This study found that each of the populations identified as being genetically distinct based upon cyt *b* sequences (Chapter 1) were distinct at the other markers as well (see below), but – in terms of the actual relationship among each population – it produced conflicting results. Fourmile Creek, Siuslaw River, and Mark West Creek were highlighted in Chapter 1 as potentially being cryptic species or possibily *L. pacifica*. *Lampetra* cf. *richardsoni* from Fourmile Creek was chosen here to investigate further since the results from Chapter 1 were not clear as parsimony and Bayesian phylogenies disagree. Further incongruence among markers and methods of analysis, here, indicate that no conclusions can be drawn as yet regarding Fourmile Creek (and other *L.* cf. *richardsoni* from Siuslaw River

and Mark West Creek). *Lampetra* cf. *richardsoni* from Fourmile Creek was supported as being distinct based on all mtDNA markers, a Bayesian population structure analysis using microsatellite DNA, as well as significant and high F_{st} values. Nuclear sequence data, however, failed to reveal Fourmile Creek as monophyletic. The Kelsey Creek population is almost certainly a distinct species (tentively identified as *Lampetra* sp.), but Fourmile Creek (as well as Siuslaw River and Mark West Creek) populations are best to continue as *L.* cf. *richardsoni* until further study.

4.2 Cryptic speciation

The theory of descent with modification (Darwin, 1859) set the foundation for phylogenetic studies. However, the process of species delimitation remains contentious but it is nevertheless a vitally important problem (Agapow et al., 2004). Based on the findings that each of the populations examined here were genetically distinct at the six molecular markers examined (and at levels on par with species-level differences; see above), it appears that Fourmile and Kelsey creeks (and perhaps additional locations from Chapter 1, specifically, Siuslaw River and Mark West Creek) each represent cryptic brook lamprey species. Independently segregating mtDNA and nuclear markers often recovered the same genetic clades, consistent with the genealogical congruence criterion (Avise and Ball, 1990; Baum and Shaw, 1995). The rationale for this approach is that such congruencies likely arise only when populations have been separated from one another for long periods leading to separate evolutionary trajectories. The major phylogenetic subdivisions in the gene genealogies become coincident with the major population-level subdivisions (Avise and Ball, 1990). These subdivisions within the context of this study coincide with species-level divergence.

However, similar congruence between separate genes has been shown for isolated populations within species (see example in Avise, 2000b) and molecular characters leading to species delimitations through phylogenetic methods should be used with caution as they are not inherently superior to non-molecular characters. Many valid species may be morphologically distinct in the absence of molecular divergence (Echelle and Dowling, 1992; Docker et al., 1999), whereas other species, as in this study, share conservative body forms but are characterized by large genetic discontinuities.

However, the lack of monophyly in *L. richardsoni* means that *L. richardsoni* sensu lato is not a valid species according to the phylogenetic species criteria, suggesting these populations are genetically distinct because each diverged from a different ancestor. Although this study cannot determine the cause of significant genetic differentiation for brook lampreys along the Pacific coast of North America based on the genetic sequence and microsatellite data, each has likely evolved in allopatry. Each population is confined to its freshwater environment due to an inability to survive in salt water (Hardisty, 2006), and long-distance dispersal via fresh water is prevented due to discontinuous waterways along the Pacific coast.

These genetic data strongly support the species status of *L. richardsoni*, *L. pacifica*, and *L. hubbsi* collected from their type locales. Additionally the data support the population from Kelsey Creek as a new cryptic species. However, the status of Fourmile Creek is not yet clear. Since the specimens collected were predominantly larvae, for which few morphological characters are useful for species-level identification, adult specimens are clearly required before formal taxonomic changes are recommended. It should be mentioned that spawning-phase adults from Kelsey Creek have been collected recently for

morphological analylsis by Stewart B. Reid but the results of these studies are not yet available.

4.3 Conservation implications

Although the best-known lamprey species is probably the parasitic sea lamprey (*Petromyzon marinus*), which is an invasive species in the Laurentian Great Lakes, most other lamprey species worldwide are of conservation concern (Renaud, 1997). Freshwater-resident species with restricted ranges are of particular concern (e.g., the Vancouver lamprey [*Entosphenus macrostomus*] and the Morrison Creek lamprey [*Lampetra richardsoni* var. *marifuga*], listed respectively as Threatened and Endangered by the Committee on the Status of Endangered Wildlife in Canada; COSEWIC, 2000). The conserved body form of *Lampetra* brook lampreys across their Pacific coast distribution (leading to their general recognition as a single species, *Lampetra richardsoni*) would thus imply that conservation attention is not warranted. However, the combined genetic data suggest that *L. richardsoni* sensu lato represents at least three or four independent *Lampetra* spp. (*L. pacifica*, *L.* cf. *richardsoni* from Kelsey Creek, and possibly *L.* cf. *richardsoni* from Fourmile Creek, in addition to *L. richardsoni* sensu stricto). Therefore, considering these genetically unique populations as a single, common, well-distributed species could underestimate west coast biodiversity and minimize their need for protection. *Lampetra pacifica, L.* cf. *richardsoni* from Fourmile Creek, and *L.* cf. *richardsoni* from Kelsey Creek each appear to be endemic to a relatively small geographic distribution. Although *L. richardsoni* remains a widely distributed taxon, each new cryptic *L.* cf. *richardsoni* species has now become rare. Based

on these new and robust genetic data, each of the genetically distinct species shoud be recognized in regional conservation decision making and action plans.

Special attention should be directed to the most distinct population within the brook lamprey phylogeny: *L.* cf. *richardsoni* from Kelsey Creek, California. Given its phylogenetic distinctiveness as the sister group to all other ingroup *Lampetra* species based on the combined sequence dataset, as well as its uncertain generic position based on microsatellite data, Kelsey Creek lampreys represent a key component of regional phylogenetic diversity, often an important criterion in conservation planning (Rodrigues and Gaston, 2002). Not exclusive to Kelsey Creek, lampreys worldwide are affected by habitat degradation through pollution and stream regulation (Renaud, 1997), which continues to threaten isolated populations of brook lampreys. Moreover, the reproductive capacity of non-parasitic species, contributing to its overall fitness and success as a species, is limited (Vladykov, 1973b). Indeed, in 2004 the general public petitioned the U.S. Fish and Wildlife Service to list lampreys including *L. richardsoni* (Federal Register/Vol.69, No. 247, 77158) as threatened or endangered, and *L. hubbsi* (Federal Register/Vol.69, No. 247, 77152) as endangered under the Endangered Species Act of 1973. However, the Department of the Interior reported that the petition did not present scientific or commercial information that would warrant such designation. I argue that this combination of phylogenetic distinctiveness, regional endemism, and rarity warrants conservation attention and action. I hope that this study will provoke future studies of cryptic *L.* cf. *richardsoni* species.

Table 2-1. List of the species used to generate DNA sequence and microsatellite data for molecular phylogenetic analyses, with collection site and identification number (which is consistent with the numbering system used in Chapter 1; numbers in parentheses correspond to the map identification in Table 1-1 and Figures 1-1 to 1-3). Voucher numbers correspond to fish collections at Humbolt State University and the University of Manitoba (prefix HSU and MZF, respectively). Specimens marked as "Chapter 1" in the "Source" column are those used for cyt *b* sequencing in Chapter 1; those marked "This chapter" were additional samples used in this chapter only.

Species	**Collection Site**	**Identification**	**Voucher Nos.**	**Source**
Entosphenus tridentatus	Alsea River, OR	Alsea (36) a	HSU3878	This chapter
		Alsea (36) b	HSU3878	This chapter
		Alsea (36) c	HSU3878	This chapter
		Alsea (36) d	HSU3878	This chapter
		Alsea (36) e^+	HSU3878	This chapter
		Alsea (36) f^+	HSU3878	This chapter
		Alsea (36) g^+	HSU3878	This chapter
		Alsea (36) h^+	HSU3878	Chapter 1
		Alsea (36) i^+	HSU3878	This chapter
		Alsea (36) j^+	HSU3878	This chapter
Lampetra cf. *richardsoni*	Fourmile Creek, OR	Fourmile (40) a	HSU3889	Chapter 1
		Fourmile (40) b	HSU3889	Chapter 1
		Fourmile (40) c	HSU3889	Chapter 1
		Fourmile (40) d	HSU3889	Chapter 1
		Fourmile (40) e^+	HSU3889	This chapter
		Fourmile (40) f^+	HSU3889	This chapter
		Fourmile (40) g^+	HSU3889	This chapter
		Fourmile (40) h^+	HSU3889	This chapter
		Fourmile (40) i^+	HSU3889	This chapter
		Fourmile (40) j^+	HSU3889	This chapter
Lampetra cf. *richardsoni*	Kelsey Creek, CA	Kelsey (53) a	HSU3944	Chapter 1
		Kelsey (53) b	HSU3944	Chapter 1
		Kelsey (53) c	HSU3944	Chapter 1
		Kelsey (53) d	HSU3944	Chapter 1
		Kelsey (53) e^+	HSU3944	This chapter
		Kelsey (53) f^+	HSU3944	This chapter
		Kelsey (53) g^+	HSU3944	This chapter
		Kelsey (53) h^+	HSU3944	This chapter
		Kelsey (53) i^+	HSU3944	This chapter
		Kelsey (53) j^+	HSU3944	This chapter
Lampetra hubbsi	Merced River*, CA	Merced (57) a	HSU3969	Chapter 1
		Merced (57) b	HSU3969	Chapter 1
		Merced (57) c	HSU3969	This chapter
		Merced (57) d	HSU3969	This chapter
		Merced (57) e^+	HSU3969	This chapter
		Merced (57) f^+	HSU3969	This chapter
		Merced (57) g^+	HSU3969	This chapter
		Merced (57) h^+	HSU3969	This chapter
		Merced (57) i^+	HSU3969	This chapter
		Merced (57) j^+	HSU3969	This chapter

Table 2-1 continued

Lampetra pacifica	Clackamas River*, OR	Clackamas (31) a	MZF3603	Chapter 1
		Clackamas (31) b	MZF3604	Chapter 1
		Clackamas (31) c	MZF3605	Chapter 1
		Clackamas (31) d	MZF3606	Chapter 1
		Clackamas (31) e[+]	MZF3620	This chapter
		Clackamas (32) f[+]	MZF3621	This chapter
		Clackamas (32) g[+]	MZF3622	This chapter
		Clackamas (32) h[+]	MZF3607	Chapter 1
		Clackamas (32) i[+]	MZF3608	Chapter 1
		Clackamas (32) j[+]	MZF3609	Chapter 1
Lampetra richardsoni	Smith Creek*, BC	Smith (9) a[A]	MZF3598	Chapter 1
		Smith (9) b[A]	MZF3599	Chapter 1
	Big River, WA	Big (12) c[+]	HSU3951	Chapter 1
		Big (12) d[+]	HSU3951	Chapter 1
		Big (12) e[+]	HSU3951	Chapter 1
		Big (12) f[+]	HSU3951	This chapter
		Big (12) g[+]	HSU3951	This chapter
		Big (12) h[+]	HSU3951	This chapter
		Big (12) i[+]	HSU3951	This chapter
		Big (12) j[+]	HSU3951	This chapter

[A]Adult specimen
*Holotype collection site
[+]Microsatellite data only

Table 2-2. Genetic markers used in this study, including locus information, primer names and sequences, and references.

Genome	Locus	Primer name	Primer sequence	Reference
mtDNA	Cyt *b*	[1]Glu-F	F - CACCGTTGTAGAATTCAACTATAAG	Chapter 1
		[1,2]Pro-R	R - TAATTTAATGTTAAGATRCTAGCTTTGG	M.F. Docker, unpublished
		[2]Cytb-196F	F - GCCTTYTCTTCAGTTATACACATTTG	Chapter 1
		[2]Cytb-340R	R - GACTCCAACGTTTCATGTYTCTTT	Chapter 1
	ND2	[1]Lamprey 679F	F - TTGGGCCCATACCCCAAATATGAT	M.F. Docker, unpublished
		[1,2]Lamprey 680R	R - CCTTACTGTAAGCTTTGAAGGCTTA	M.F. Docker, unpublished
		[2]Lamprey ND2-585F	F - CCCCTTTAACCCAACAATCAC	This chapter
		[2]Lampetra ND2-612F	F - AAGTATGGCCCCCTTTAACC	This chapter
	CO1	[1,2]Lamprey CO1-F 678F	F - TTTGGGGCCTGAGCAGGAATAGT	M.F. Docker, unpublished
		[1,2]Lamprey CO1-R 212R	R - TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al., 1994
Nuclear	TAP intron 9	[1,2]Lamprey TAPexon8-143F	F - ATGTCACCTTCGCGTACC	This chapter; from Uinuk-ool et al., 2003
		[1,2]Lamprey TAPexon9-29R	R - CCACCAGTGCCGTYACC	This chapter; from Uinuk-ool et al., 2003
		[2]Lampetra TAPintron9-612F	F - AGTCATGGGAGTGCAAAACA	This chapter
		[2]Lamprey TAPintron9-726R	R - GAAGTGGGAATGTGAGCAAG	This chapter
		[2]Lamprey TAPintron9-994R	R - AGTAGACCGCAGCACTTTCC	This chapter
	ITS 1	[1,2]Lamprey ITS1-1630F	F - GTTTAGTGAGGTCCTCGGATTG	This chapter
		[1,2]Lamprey ITS2-34R	R - TCCTCCGCTTAGTAATATGCTTAAAT	This chapter
Nuclear - Microsatellite	Lri-1	A120	F - ACCACGGAAGCCATAGTTTC	Luzier et al., in press
			R - GTGTGCCCTGTGTGTATATGC	Luzier et al., in press
	Lri-2	A111	F - GGCTCTTACCGAACACCTG	Luzier et al., in press
			R - CAGCGTGCTAACTGCTATCC	Luzier et al., in press
	Lri-4	A116a	F - TCACCGTGATGTTCTGGAG	Luzier et al., in press
			R - GCCAGTCATCTCGGTCATC	Luzier et al., in press
	Lri-5	E104	F - GCCGACAACAACCAACATC	Luzier et al., in press
			R - CACGCAGGTCACCCTCTAC	Luzier et al., in press
	Lri-6	E7	F - CGAGGCTCTGCCTGAGTA	Luzier et al., in press
			R - CCGTCGCTATTTCATTGC	Luzier et al., in press

Table 2-2 continued

Lri-7	C106	F - TGCCAAACATTCCAAGTG	Luzier et al., in press
		R - AGGTCTTCCTCCAACAGTG	Luzier et al., in press
Lri-8	A114	F - GCGAACGCCTATTAAGGC	Luzier et al., in press
		R - TCTCCCTTGGGTCGATTC	Luzier et al., in press
Lri-9	E126	F - GAGAGGAGCGAGGCTCTAC	Luzier et al., in press
		R - ACATCCACGCTTAAATACTGG	Luzier et al., in press
Lri-10	C102	F - GCCAATCCGTGAAAATGT	Luzier et al., in press
		R - CACGCACACACGTATTGTAG	Luzier et al., in press

[1] Denotes those primers used for PCR amplification
[2] Primer used for sequencing

Table 2-3. Summary of ITS1 cloned data used to create a consensus sequence for analyses.

Species	Specimen Identification	Number of clones	Length variation for complete ITS1 region (bp)
Entosphenus tridentatus	Alsea b	5	336 – 360
	Alsea c	4	330 – 342
Lampetra cf. *richardsoni*	Fourmile a	5	312 – 314
	Fourmile b	4	312
Lampetra cf. *richardsoni*	Kelsey b	7	312
	Kelsey c	5	312
Lampetra hubbsi	Merced b	8	312
	Merced c	5	312
Lampetra pacifica	Clackamas b	4	312
	Clackamas c	7	312
Lampetra richardsoni	Smith a	6	312
	Smith b	5	312

Table 2-4. Summary of percent Kimura 2-parameter (K2P%) distances within and among species of lampreys for five genetic markers. Sample size (*N*), mean K2P%, minimum and maximum K2P%, as well as the standard error are reported.

Comparisons	Species		[1]*N*	Mean (Min, Max) ± SE				
				Cyt *b*	ND2	CO1	*TAP intron 9	ITS 1
Within species, between congeners	*E. tridentatus*		4 (2)	0.18 (0.08, 0.34) ± 0.05	0.19 (0.10, 0.29) ± 0.04	0.31 (0, 0.62) ± 0.16	0 (0, 0) ± 0	0 (0, 0) ± 0
	L. cf. *richardsoni* – Fourmile Creek		4	0.13 (0.08, 0.17) ± 0.02	0 (0, 0) ± 0	0 (0, 0) ± 0	0.12 (0, 0.25) ± 0.06	0 (0, 0) ± 0
	L. cf. *richardsoni* – Kelsey Creek		4	0 (0, 0) ± 0	0.10 (0, 0.19) ± 0.05	0 (0, 0) ± 0	0 (0, 0) ± 0	0 (0, 0) ± 0
	L. hubbsi		4	0.13 (0, 0.25) ± 0.04	0 (0, 0) ± 0	0 (0, 0) ± 0	0 (0, 0) ± 0	0 (0, 0) ± 0
	L. pacifica		4	0 (0, 0) ± 0	0.05 (0, 0.10) ± 0.03	0 (0, 0) ± 0	0 (0, 0) ± 0	0 (0, 0) ± 0
	L. richardsoni		2	0 (0, 0) ± 0	0 (0, 0) ± 0	0 (0, 0) ± 0	0.08 (0.08, 0.08) ± 0	0 (0, 0) ± 0
Between species	*E. tridentatus*	*L.* cf. *richardsoni* – Fourmile Creek	8 (6)	10.00 (9.79, 10.20) ± 0.04	13.00 (12.82, 13.18) ± 0.05	8.27 (8.09, 8.80) ± 0.11	2.83 (2.76, 2.85) ± 0.02	5.35 (5.35, 5.35) ± 0
		L. cf. *richardsoni* – Kelsey Creek	8 (6)	12.73 (12.65, 12.85) ± 0.03	14.39 (14.15, 14.77) ± 0.06	8.63 (8.45, 9.18) ± 0.11	2.85 (2.85, 2.85) ± 0	5.74 (5.74, 5.74) ± 0
		L. hubbsi	8 (6)	10.49 (10.39, 10.70) ± 0.03	13.80 (13.62, 13.98) ± 0.05	9.81 (9.72, 10.09) ± 0.06	3.28 (3.28, 3.28) ± 0	5.35 (5.35, 5.35) ± 0
		L. pacifica	8 (6)	10.68 (10.60, 10.81) ± 0.03	13.26 (13.05, 13.54) ± 0.05	9.16 (8.98, 9.71) ± 0.12	3.02 (3.02, 3.02) ± 0	5.35 (5.35, 5.35) ± 0
		L. richardsoni	6 (4)	10.37 (10.29, 10.40) ± 0.02	13.47 (13.28, 13.65) ± 0.06	8.27 (8.09, 8.81) ± 0.14	2.72 (2.68, 2.76) ± 0.02	5.35 (5.35, 5.35) ± 0
	L. cf. *richardsoni* – Fourmile Creek	*L.* cf. *richardsoni* – Kelsey Creek	8	6.27 (6.23, 6.32) ± 0.02	5.37 (5.32, 5.53) ± 0.03	2.70 (2.70, 2.70) ± 0	0.27 (0.08, 0.33) ±0.04	0.37 (0.37, 0.37) ± 0
		L. hubbsi	8	3.04 (2.93, 3.11) ± 0.02	3.56 (3.56, 3.56) ± 0	2.37 (2.37, 2.37) ± 0	1.01 (0.83, 1.07) ± 0.04	0 (0, 0) ± 0
		L. pacifica	8	2.74 (2.67, 2.76) ± 0.01	2.00 (1.95, 2.05) ± 0.02	2.05 (2.05, 2.05) ± 0	0.43 (0.25, 0.49) ± 0.04	0 (0, 0) ± 0
		L. richardsoni	6	3.09 (3.03, 3.12) ± 0.02	3.05 (3.05, 3.05) ± 0	1.89 (1.89, 1.89) ± 0	0.19 (0.08, 0.25) ± 0.02	0 (0, 0) ± 0
	L. cf. *richardsoni* – Kelsey Creek	*L. hubbsi*	8	5.97 (5.93, 6.02) ± 0.02	6.85 (6.80, 7.01) ± 0.03	3.84 (3.84, 3.84) ± 0	0.91 (0.91, 0.91) ± 0	0.37 (0.37, 0.37) ± 0
		L. pacifica	8	6.03 (6.03, 6.03) ± 0.00	5.62 (5.54, 5.86) ± 0.04	3.18 (3.18, 3.18) ± 0	0.33 (0.33, 0.33) ± 0	0.37 (0.37, 0.37) ± 0
		L. richardsoni	6	6.41 (6.41, 6.41) ± 0.00	6.65 (6.59, 6.81) ± 0.04	3.03 (3.03, 3.03) ± 0	0.21 (0.16, 0.25) ± 0.02	0.37 (0.37, 0.37) ± 0
	L. hubbsi	*L. pacifica*	8	2.66 (2.58, 2.75) ± 0.02	4.00 (3.97, 4.07) ± 0.02	3.18 (3.18, 3.18) ± 0	1.08 (1.08, 1.08) ± 0	0 (0, 0) ± 0
		L. richardsoni	6	3.06 (3.02, 3.11) ± 0.02	4.89 (4.89, 4.89) ± 0	3.35 (3.35, 3.35) ± 0	0.87 (0.83, 0.91) ± 0.02	0 (0, 0) ± 0
	L. pacifica	*L. richardsoni*	6	2.85 (2.85, 2.85) ± 0.00	3.28 (3.25, 3.35) ± 0.02	2.05 (2.05, 2.05) ± 0	0.37 (0.33, 0.41) ± 0.02	0 (0, 0) ± 0

[1]Values in parentheses are adjusted for the TAP intron 9 dataset

*Adjusted values for the TAP intron 9 dataset incorporating *N* = 2 for Alsea

Table 2-5. Summary of information corresponding to the parsimony analysis of each dataset.

Dataset	Total of characters	Number of constant versus parsimony informative characters	Length of tree (steps)	Consistency Index (CI)	Retention Index (RI)	Homoplasy Index (HI)
Cyt *b*	1191	1001 vs. 185	228	0.864	0.955	0.136
ND2	1044	848 vs. 190	225	0.929	0.977	0.071
CO1	645	566 vs. 76	93	0.86	0.955	0.14
TAP intron 9	1256	1206 vs. 57	60	0.967	0.98	0.033
ITS 1	286	270 vs. 18	18	1	1	0
Combined	4422	3891 vs. 526	633	0.888	0.961	0.112

Table 2-6. Models of evolution, arithmetic mean likelihood values, and parameters chosen for each dataset by Modeltest 3.6 (Posada and Crandall, 1998).

Dataset	Model	Mean -ln likelihood	Invariable site (I)	Gamma shape (Γ)	Transition/transversion ratio	Base frequencies (freqA, freqC, freqG, freqT)	Rate matix (A/C, A/G, A/T, C/G, C/T, G/T)
Cyt *b*	[1]HKY+G	2717.31	0	0.19	11.47	0.29,0.23,0.15,0.33	N/A
ND2	[2]TrN+G	2498.03	0	0.29	All rates equal	0.32,0.26,0.12,0.30	1,20.96,1,1,7.63,1
CO1	[1]HKY+G	1388.40	0	0.12	4.43	0.27,0.25,0.16,0.32	N/A
TAP intron 9	[1]HKY	2106.39	0	Equal rates for all sites	2.10	0.28,0.21,0.22,0.29	N/A
ITS 1	[3]F81	488.74	0	Equal rates for all sites	All rates equal	0.12,0.32,0.39,0.17	N/A
Combined	[1]HKY+I+G	9212.00	0.56	0.76	5.64	0.28,0.24,0.18,0.30	N/A

References [1]Hasegawa et al., 1985; [2]Tamura and Nei, 1993; [3]Felsenstein, 1981

Table 2-7. Observed and expected heterozygosities (H_o and H_e) and number of alleles (A) with sample sizes (N) for nine microsatellite loci from six populations of lampreys along the the Pacific coast of North America. The size range of each locus (number of base pairs) is indicated by values in parentheses. Significant departures from HWE (after strict Bonferroni correction) are indicated by values in bold italics.

Locus	Statistic	Alsea R. *E. tridentatus*	Fourmile Cr. *L.* cf. *richardsoni*	Kelsey Cr. *L.* cf. *richardsoni*	Merced R. *L. hubbsi*	Clackamas R. *L. pacifica*	Smith Cr. *L. richardsoni*
Lri-1	H_o	0.82	0.10	0.19	0.00	0.00	0.00
(89 – 111 bp)	H_e	0.60	0.10	0.20	0.00	0.00	0.00
	A	6	2	2	1	1	1
	N	10	10	10	10	10	10
Lri-2	H_o	0.53	0.42	***0.53***	0.27	0.00	0.36
(135 – 163 bp)	H_e	0.40	0.10	1.00	0.30	0.00	0.40
	A	2	3	2	2	1	3
	N	10	10	10	10	10	10
Lri-4	H_o		0.00	0.00	0.00	0.00	0.28
(253 – 271 bp)	H_e		0.00	0.00	0.00	0.00	0.10
	A		1	1	1	1	3
	N		10	10	6	10	10
Lri-5	H_o	0.49	0.10	0.00	0.27	0.59	0.43
(258 – 274 bp)	H_e	0.50	0.10	0.00	0.30	0.70	0.50
	A	3	2	1	2	3	4
	N	10	10	10	10	10	10
Lri-6	H_o	0.50	***0.53***	0.00	***0.53***	***0.53***	***0.53***
(276 – 291 bp)	H_e	0.50	1.00	0.00	1.00	1.00	1.00
	A	4	2	1	2	2	2
	N	10	10	10	10	10	10
Lri-7	H_o	0.65	0.57	0.00	0.00	0.68	0.53
(139 – 157 bp)	H_e	0.60	0.70	0.00	0.00	0.80	0.50
	A	5	3	1	1	3	3
	N	10	10	10	10	10	10
Lri-8	H_o		0.00	0.00	0.00	0.39	0.67
(249 – 257 bp)	H_e		0.00	0.00	0.00	0.30	0.80
	A		1	1	1	2	4
	N		10	10	10	10	10
Lri-9	H_o		***0.53***	***0.53***	***0.53***	***0.53***	***0.53***
(223 – 232 bp)	H_e		1.00	1.00	1.00	1.00	1.00
	A		2	2	2	2	2
	N		10	10	10	10	10
Lri-10	H_o		0.53	0.00	0.19	0.51	0.79
(274 – 328 bp)	H_e		0.40	0.00	0.20	0.40	0.70
	A		2	1	2	2	5
	N		10	10	10	10	10

Table 2-8. Pairwise θ among 6 populations of lampreys distributed along the Pacific coast of North America. Values were calculated with FSTAT (Goudet, 2001) and significant population differentiation was tested after a strict Bonferroni correction (Rice, 1989); $p<0.05$*; $p<0.01$**; $p<0.001$***. Populations where significance was not able to be tested are also indicated (a).

	Fourmile Cr.	Kelsey Cr.	Merced R.	Clackamas R.	Smith Cr.
Alsea R. – *E. tridentatus*	0.4962[a]	0.6183[a]	0.5838[a]	0.4118[a]	0.4666[a]
Fourmile Cr. – *L.* cf. *richardsoni*		0.7330***	0.4934**	0.5102**	0.2107***
Kelsey Cr. – *L.* cf. *richardsoni*			0.7458**	0.7295***	0.6325***
Merced R. – *L. hubbsi*				0.6274**	0.4567**
Clackamas R. – *L. pacifica*					0.3445***
Smith Cr. – *L. richardsoni*					

Figure captions

Fig. 2-1. *Lampetra* spp. and *Entosphenus tridentatus* collection sites in British Columbia, Washington, Oregon, and California. The population name is located adjacent to collection site denoted with a circle. Open circles denote the type locale. Population names correspond to species names in parentheses.

Fig. 2-2. An example electropherogram of an ITS1 sequence derived from PCR product (top) versus that of two cloned plasmids (bottom). All sequence data were derived from the same specimen (Clackamas b). Arrows indicate where both C and T were observed in direct PCR sequencing.

Fig. 2-3. Parsimony (left) and Bayesian (right) consensus trees for the cytochrome *b* dataset. (Left) The 50% majority rule consensus of 10 most parsimonious trees resulting from a heuristic search. Bootstrap support above 50% is shown above branches. (Right) The Bayesian-MCMCMC tree resulting from a 50% majority rule consensus of 6000 trees. The resulting posterior probabilities (PP) are shown above internal branches. Taxon labels correspond to the collection site and an individual identifier (a, b, c, and d). The scale refers to the number of substitutions per site.

Fig. 2-4. Parsimony (left) and Bayesian (right) consensus trees for the ND2 dataset. (Left) The most parsimonious tree retained resulting from a heuristic search. Bootstrap support above 50% is shown above branches. (Right) The Bayesian-MCMCMC tree resulting from a 50% majority rule consensus of 6000 trees. The resulting posterior probabilities (PP) are shown above internal branches. Taxon labels correspond to the collection site and an individual identifier (a, b, c, and d). The scale refers to the number of substitutions per site.

Fig. 2-5. Parsimony (left) and Bayesian (right) consensus trees for the CO1 dataset. (Left) The 50% majority rule consensus of the three most parsimonious trees resulting from a heuristic search. Bootstrap support above 50% is shown above branches. (Right) The Bayesian-MCMCMC tree resulting from a 50% majority rule consensus of 6000 trees. The resulting posterior probabilities (PP) are shown above internal branches. Taxon labels correspond to the collection site and an individual identifier (a, b, c, and d). The scale refers to the number of substitutions per site.

Fig. 2-6. Parsimony (left) and Bayesian (right) consensus trees for the TAP intron 9 dataset. (Left) The 50% majority rule consensus of 36 most parsimonious trees resulting from a heuristic search. Bootstrap support above 50% is shown above branches. (Right) The Bayesian-MCMCMC tree resulting from a 50% majority rule consensus of 6000 trees. The resulting posterior probabilities (PP) are shown above internal branches. Taxon labels correspond to the collection site and an individual identifier (a, b, c, and d). The scale refers to the number of substitutions per site.

Fig. 2-7. Parsimony (left) and Bayesian (right) consensus trees for the ITS1 dataset. (Left) The 50% majority rule consensus of 2928797 most parsimonious trees resulting from a heuristic search. Bootstrap support above 50% is shown above branches. (Right) The Bayesian-MCMCMC tree resulting from a 50% majority rule consensus of 6000 trees. The resulting posterior probabilities (PP) are shown above internal branches. Taxon labels correspond to the collection site and an individual identifier (a, b, c, and d). The scale refers to the number of substitutions per site.

Fig. 2-8. Parsimony (left) and Bayesian (right) consensus trees of the concatenated datasets (cyt *b*, ND2, CO1, TAP intron 9, and ITS1). (Left) The 50% majority rule consensus of 144 most parsimonious trees resulting from a heuristic search. Bootstrap support above 50% is shown above branches. (Right) The Bayesian-MCMCMC tree resulting from a 50% majority rule consensus of 12000 trees. The resulting posterior probabilities (PP) are shown above internal branches. Taxon labels correspond to the collection site and an individual identifier (a, b, c, and d). The scale refers to the number of substitutions per site.

Fig. 2-9. Consensus neighbour-joining tree of the relationships among six populations of congeneric *Lampetra* species. Genetic distance was measured with Cavalli-Sforza and Edwards (1967) chord distance. Two-thousand bootstrap replicates were performed and *Entosphenus tridentatus* individuals were rooted as the outgroup. Bootstrap support above 50% is shown above branches.

Fig. 2-10. Bayesian population assignment test with the software Structure based on nine microsatellite loci uncovered six distinct populations (*E. tridentatus* – Alsea River; *L.* cf. *richardsoni* – Fourmile Creek; *L.* cf. *richardsoni* – Kelsey Creek; *L. hubbsi* – Merced River; *L. pacifica* – Clackamas River; *L. richardsoni* – Smith Creek and Big River). The length of the burnin period is 500000 and the number of MCMC repetitions after burin is 10000. Ln likelihood was -599.3 for the six groups.

Fig. 2-1

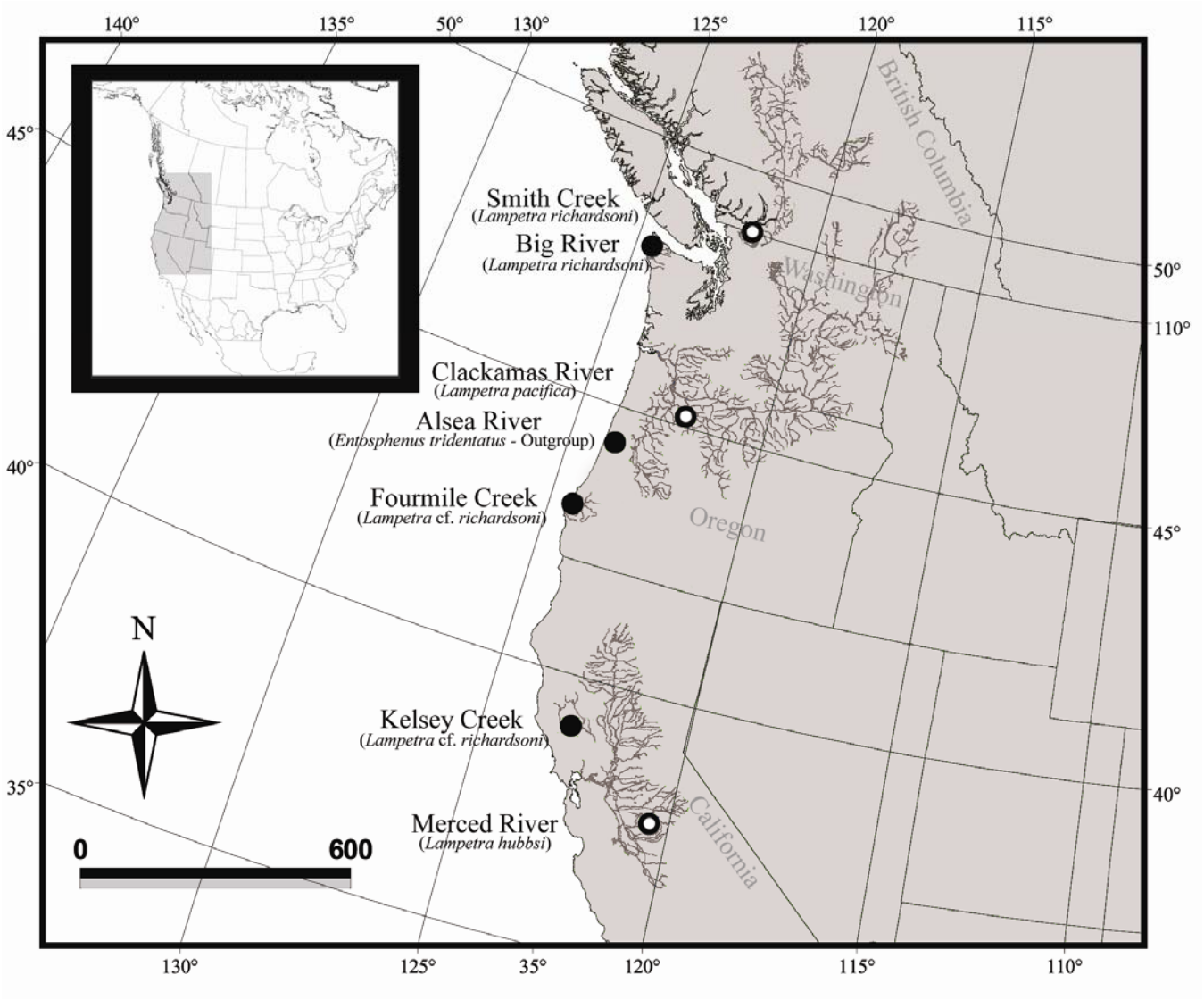

140°
135°
50°
130°
125°
120°
115°
45°
40°
35°
50°
110°
45°
40°
130°
125°
35°
120°
115°
110°
Smith Creek
(Lampetra richardsoni)
Big River
(Lampetra richardsoni)
Clackamas River
(Lampetra pacifica)
Alsea River
(Entosphenus tridentatus - Outgroup)
Fourmile Creek
(Lampetra cf. richardsoni)
Kelsey Creek
(Lampetra cf. richardsoni)
Merced River
(Lampetra hubbsi)
British Columbia
Washington
Oregon
California
N
0
600

Fig. 2-2

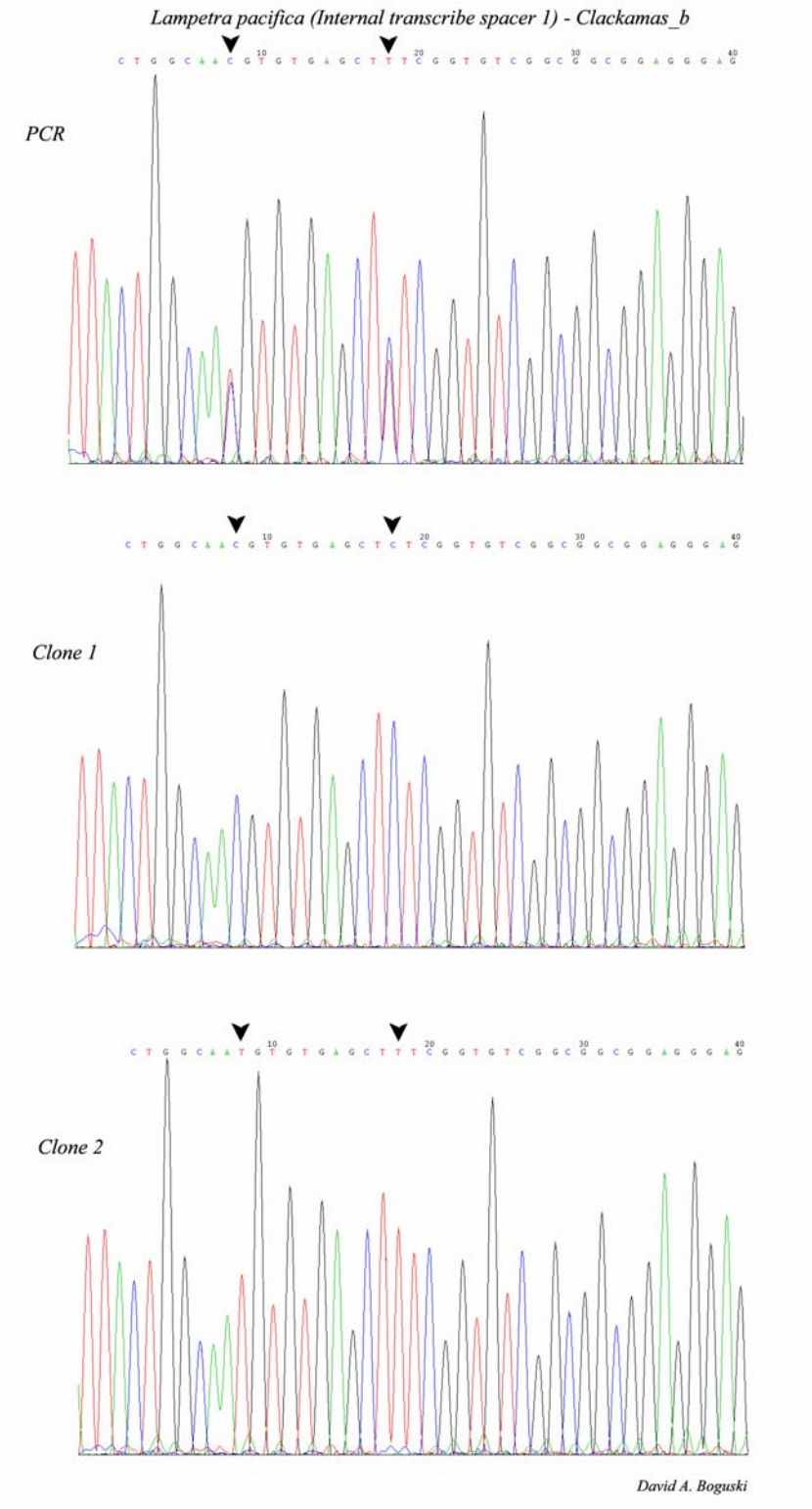

Lampetra pacifica (Internal transcribe spacer 1) - Clackamas_b
PCR
Clone 1
Clone 2
David A. Boguski

Fig. 2-3

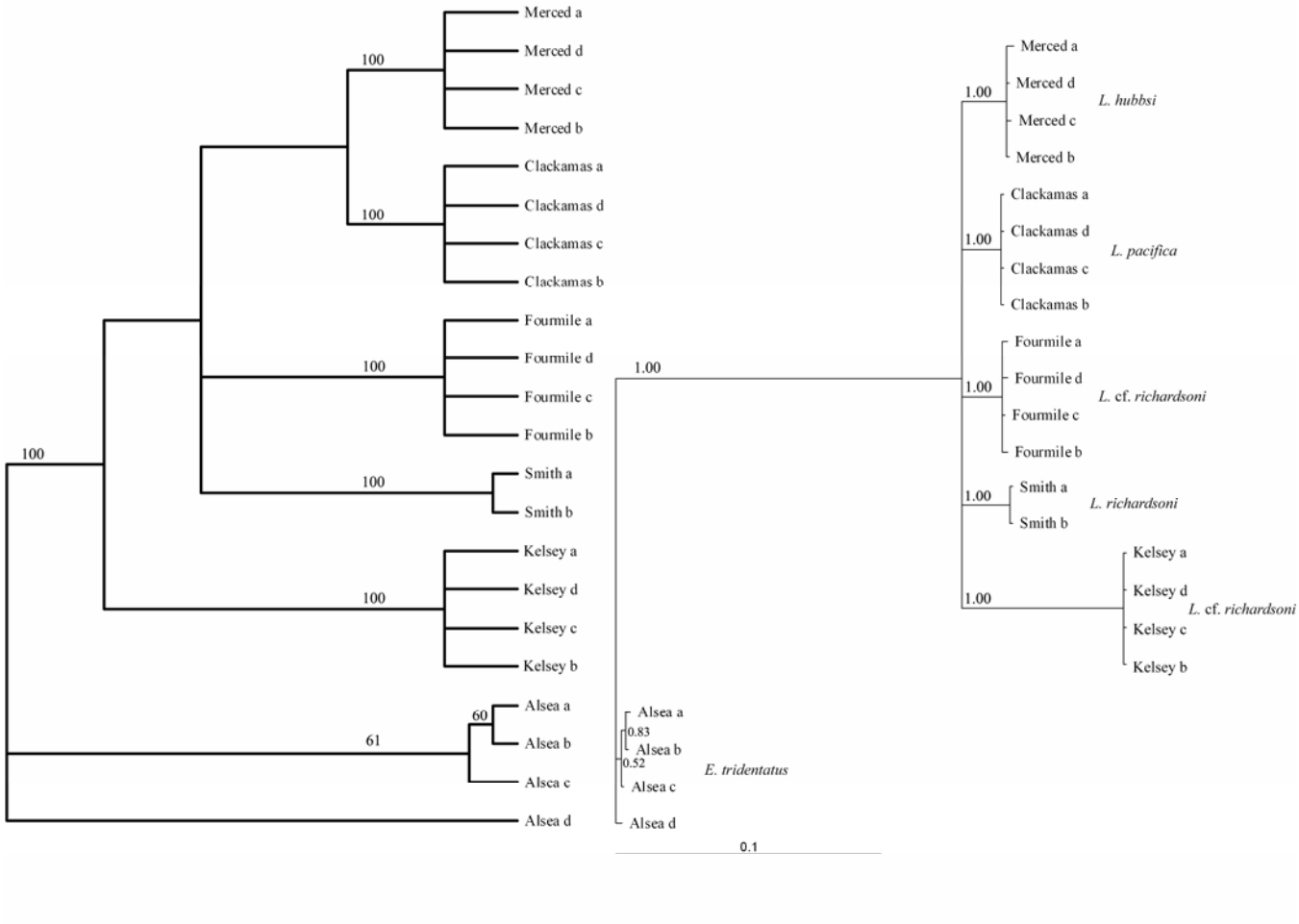

Merced a
Merced d
Merced c
Merced b
Clackamas a
Clackamas d
Clackamas c
Clackamas b
Fourmile a
Fourmile d
Fourmile c
Fourmile b
Smith a
Smith b
Kelsey a
Kelsey d
Kelsey c
Kelsey b
Alsea a
Alsea b
Alsea c
Alsea d
100
100
100
100
100
100
60
61
1.00
0.83
0.52
E. tridentatus
0.1
1.00
L. hubbsi
L. pacifica
L. cf. richardsoni
L. richardsoni
L. cf. richardsoni

Fig. 2-4

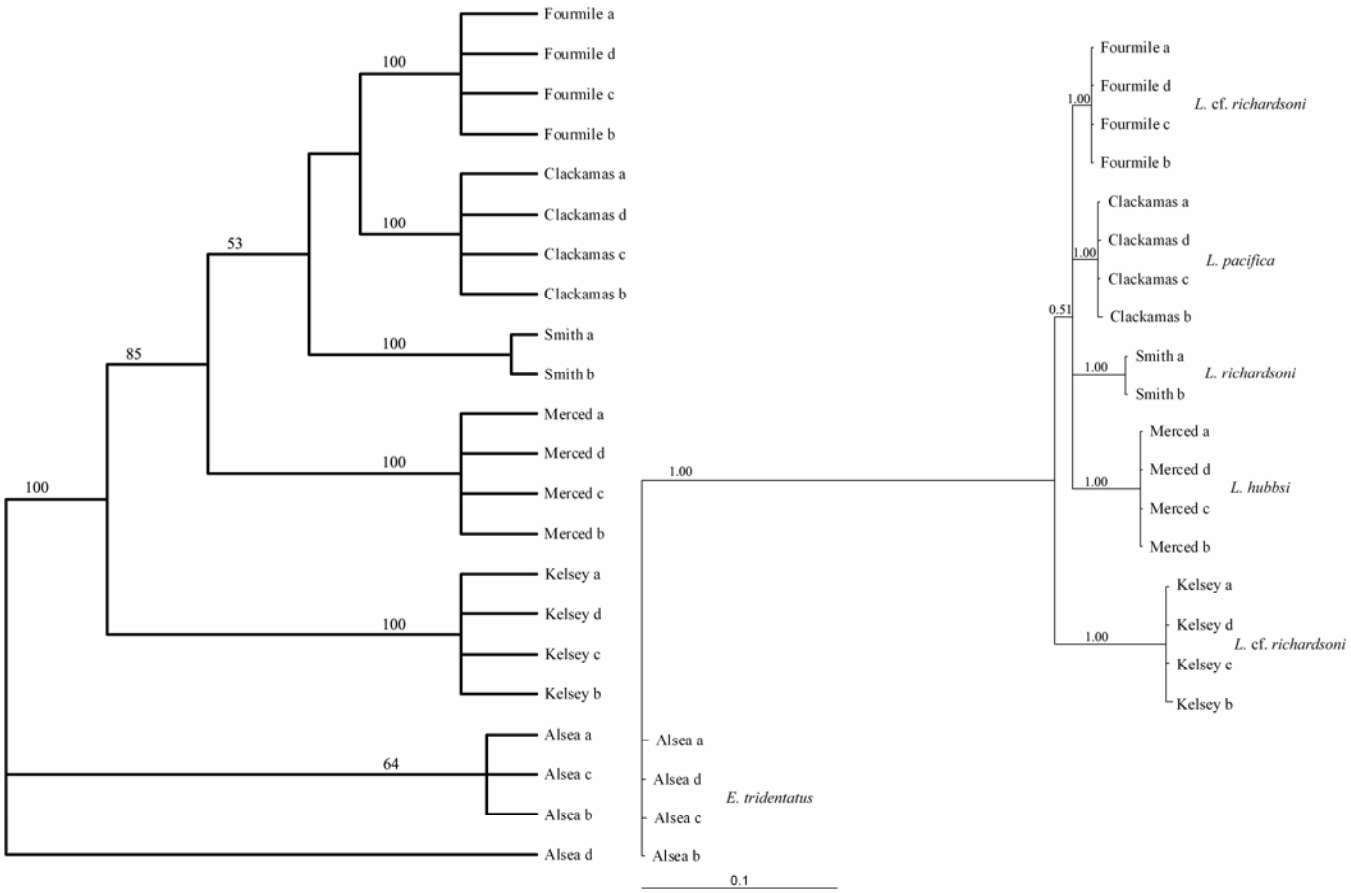

Fourmile a
Fourmile d
Fourmile c
Fourmile b
Clackamas a
Clackamas d
Clackamas c
Clackamas b
Smith a
Smith b
Merced a
Merced d
Merced c
Merced b
Kelsey a
Kelsey d
Kelsey c
Kelsey b
Alsea a
Alsea c
Alsea b
Alsea d
100
100
53
100
85
100
100
100
64
Fourmile a
Fourmile d
Fourmile c
Fourmile b
L. cf. richardsoni
Clackamas a
Clackamas d
Clackamas c
Clackamas b
L. pacifica
Smith a
Smith b
L. richardsoni
Merced a
Merced d
Merced c
Merced b
L. hubbsi
Kelsey a
Kelsey d
Kelsey c
Kelsey b
L. cf. richardsoni
Alsea a
Alsea d
Alsea c
Alsea b
E. tridentatus
1.00
1.00
1.00
0.51
1.00
1.00
1.00
0.1

Fig. 2-5

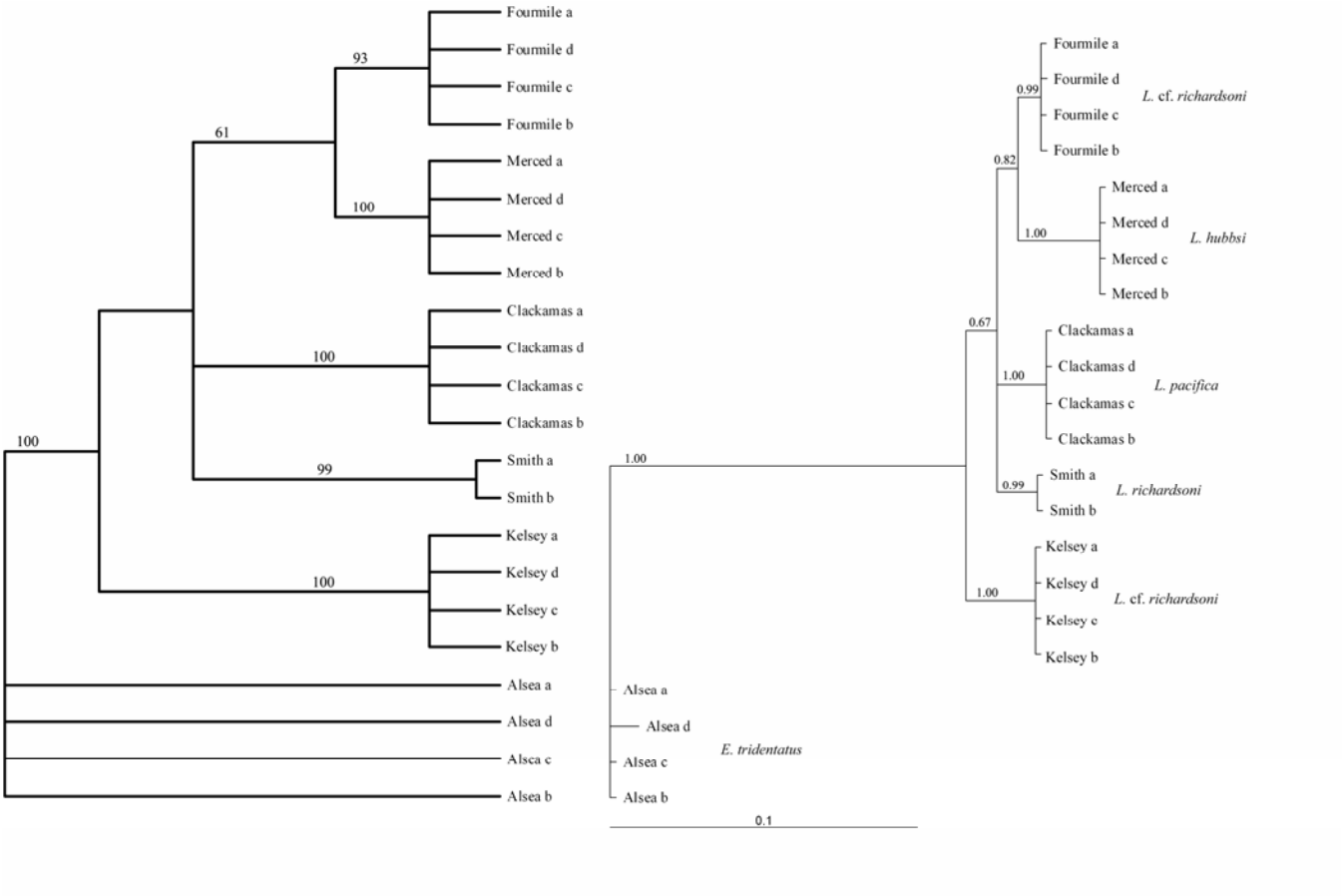

Fourmile a
Fourmile d
Fourmile c
Fourmile b
Merced a
Merced d
Merced c
Merced b
Clackamas a
Clackamas d
Clackamas c
Clackamas b
Smith a
Smith b
Kelsey a
Kelsey d
Kelsey c
Kelsey b
Alsea a
Alsea d
Alsca c
Alsea b
93
61
100
100
100
99
100
100
0.99
0.82
1.00
0.67
1.00
0.99
1.00
L. cf. richardsoni
L. hubbsi
L. pacifica
L. richardsoni
L. cf. richardsoni
E. tridentatus
0.1

Fig. 2-6

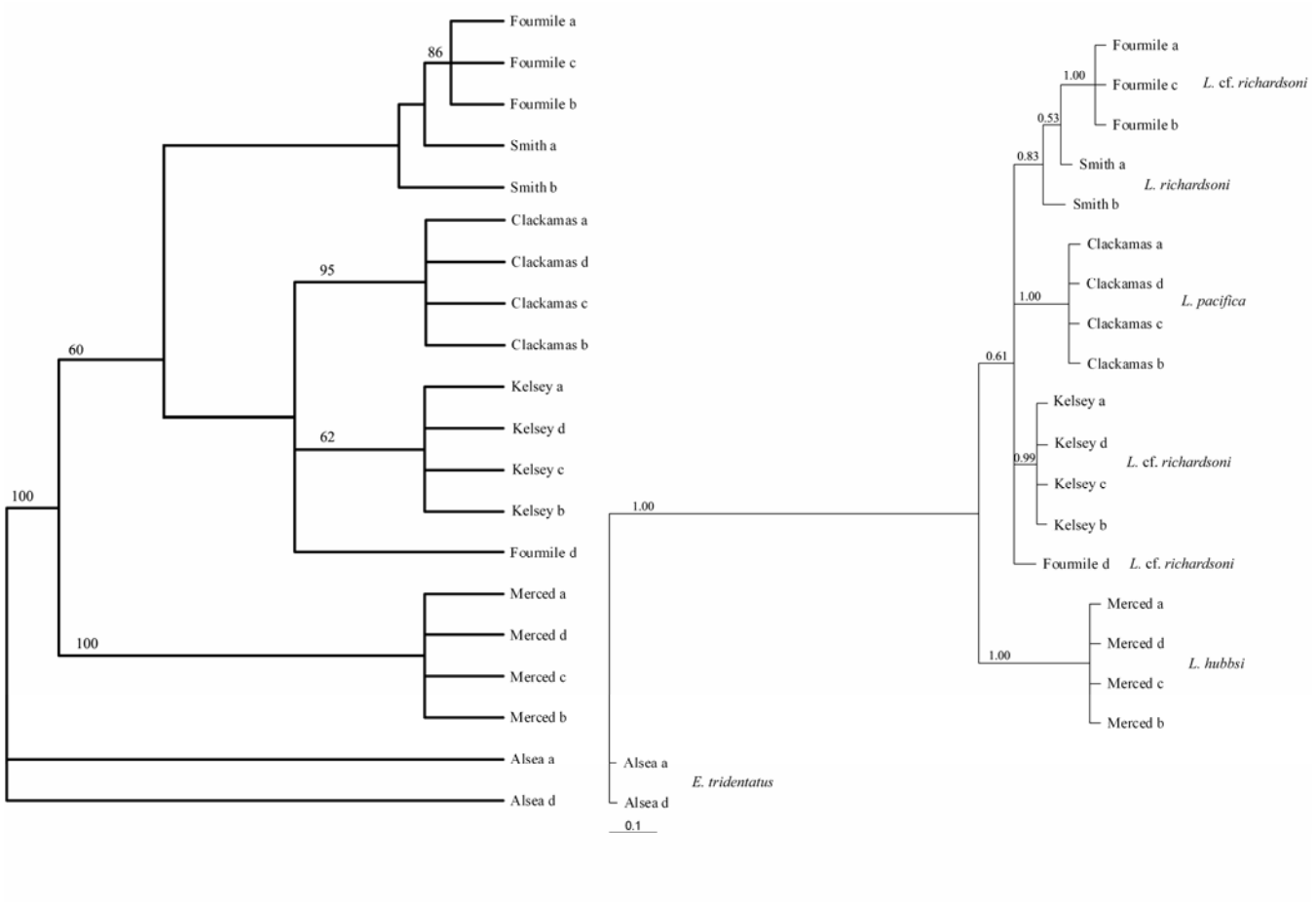

Fourmile a
Fourmile c
Fourmile b
Smith a
Smith b
Clackamas a
Clackamas d
Clackamas c
Clackamas b
Kelsey a
Kelsey d
Kelsey c
Kelsey b
Fourmile d
Merced a
Merced d
Merced c
Merced b
Alsea a
Alsea d
86
95
60
62
100
100
1.00
0.53
0.83
0.61
0.99
1.00
1.00
L. cf. richardsoni
L. richardsoni
L. pacifica
L. cf. richardsoni
L. cf. richardsoni
L. hubbsi
E. tridentatus
0.1

Fig. 2-7

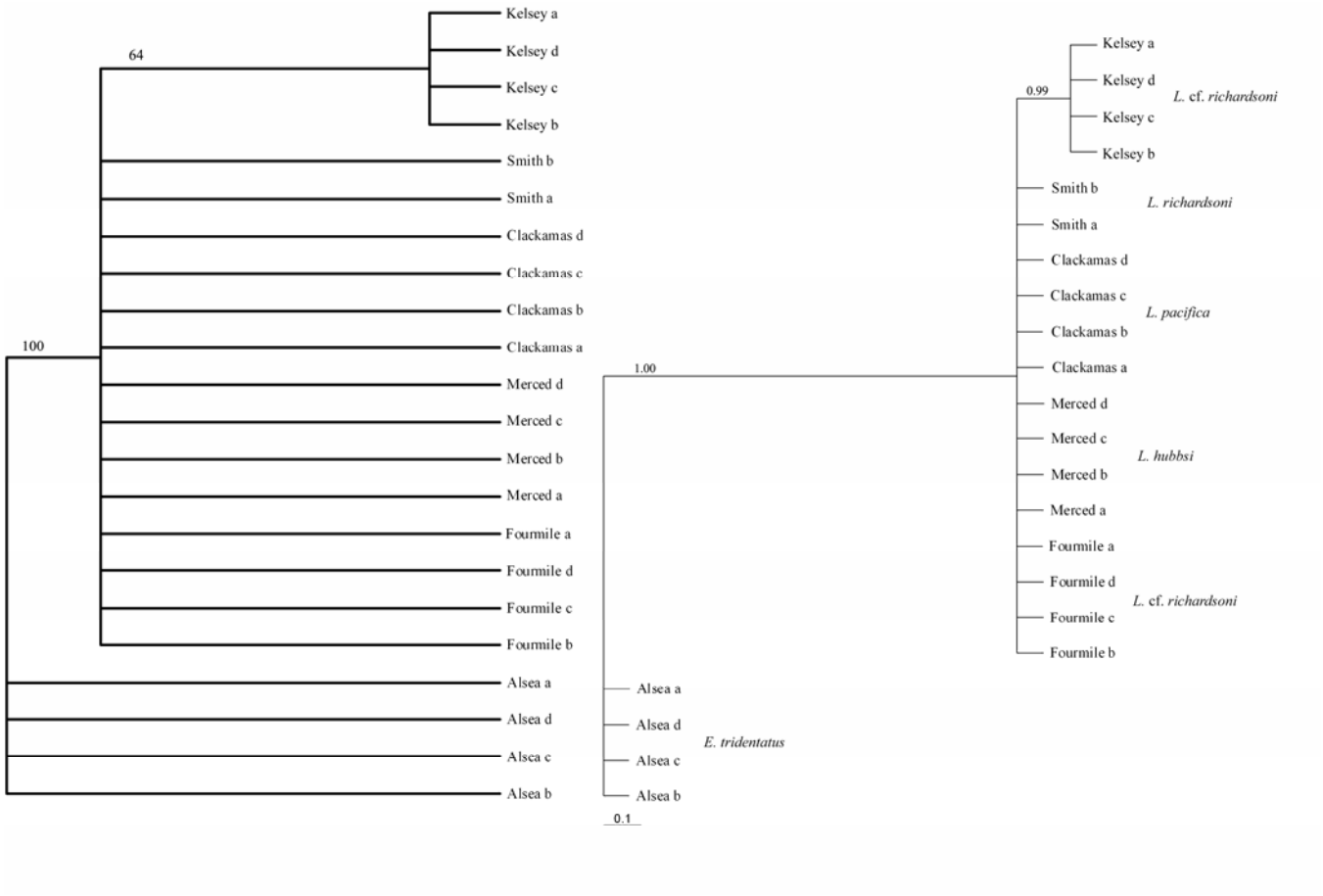

Kelsey a
Kelsey d
Kelsey c
Kelsey b
Smith b
Smith a
Clackamas d
Clackamas c
Clackamas b
Clackamas a
Merced d
Merced c
Merced b
Merced a
Fourmile a
Fourmile d
Fourmile c
Fourmile b
Alsea a
Alsea d
Alsea c
Alsea b
64
100
1.00
0.99
0.1
E. tridentatus
L. cf. richardsoni
L. richardsoni
L. pacifica
L. hubbsi
L. cf. richardsoni

Fig. 2-8

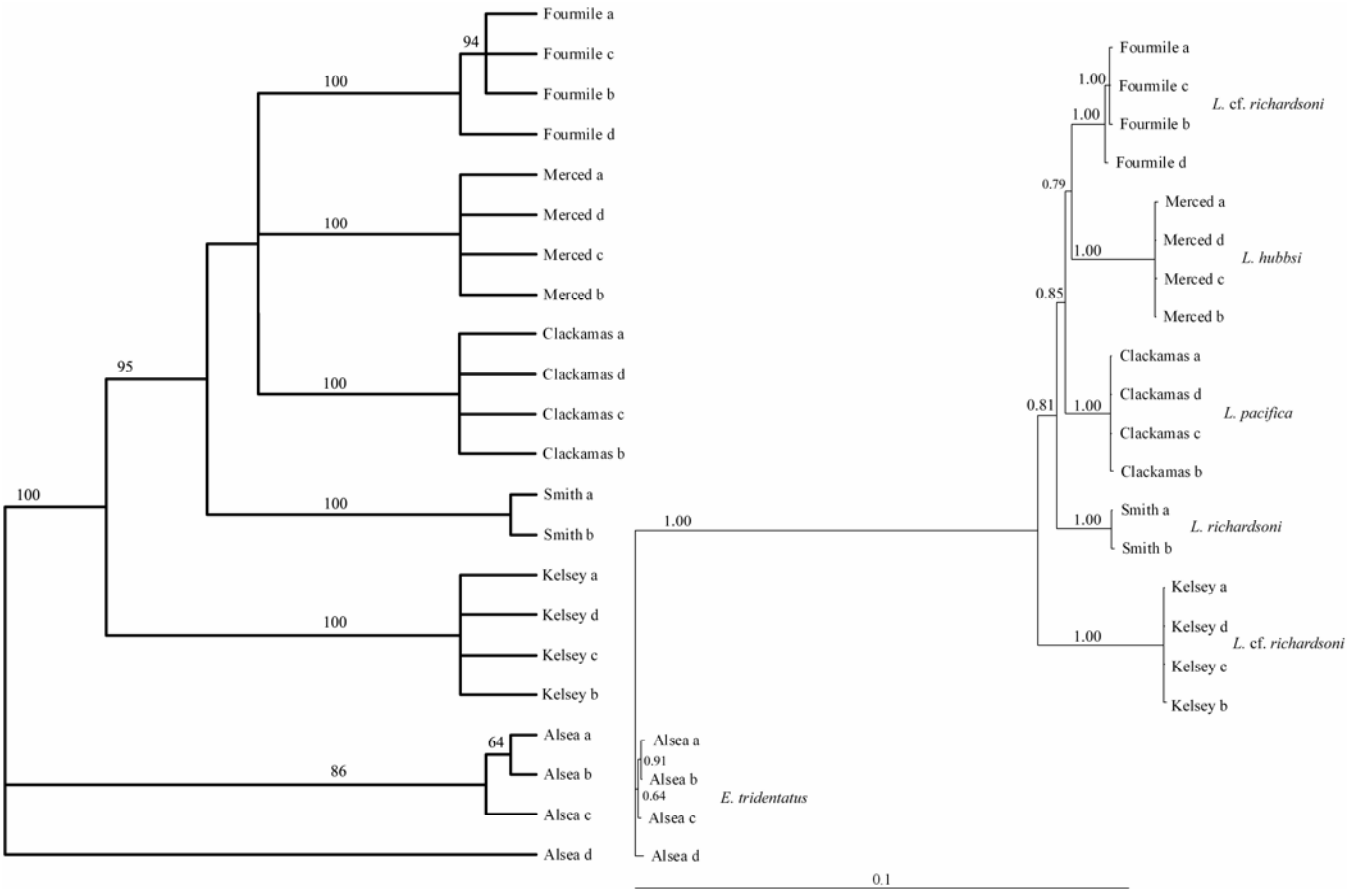

Fourmile a
Fourmile c
Fourmile b
Fourmile d
Merced a
Merced d
Merced c
Merced b
Clackamas a
Clackamas d
Clackamas c
Clackamas b
Smith a
Smith b
Kelsey a
Kelsey d
Kelsey c
Kelsey b
Alsea a
Alsea b
Alsea c
Alsea d
94
100
100
100
95
100
100
100
64
86
100
Fourmile a
Fourmile c
Fourmile b
Fourmile d
L. cf. richardsoni
Merced a
Merced d
Merced c
Merced b
L. hubbsi
Clackamas a
Clackamas d
Clackamas c
Clackamas b
L. pacifica
Smith a
Smith b
L. richardsoni
Kelsey a
Kelsey d
Kelsey c
Kelsey b
L. cf. richardsoni
Alsea a
Alsea b
Alsea c
Alsea d
E. tridentatus
1.00
1.00
1.00
0.79
1.00
0.85
0.81
1.00
1.00
1.00
1.00
0.91
0.64
0.1

Fig. 2-9

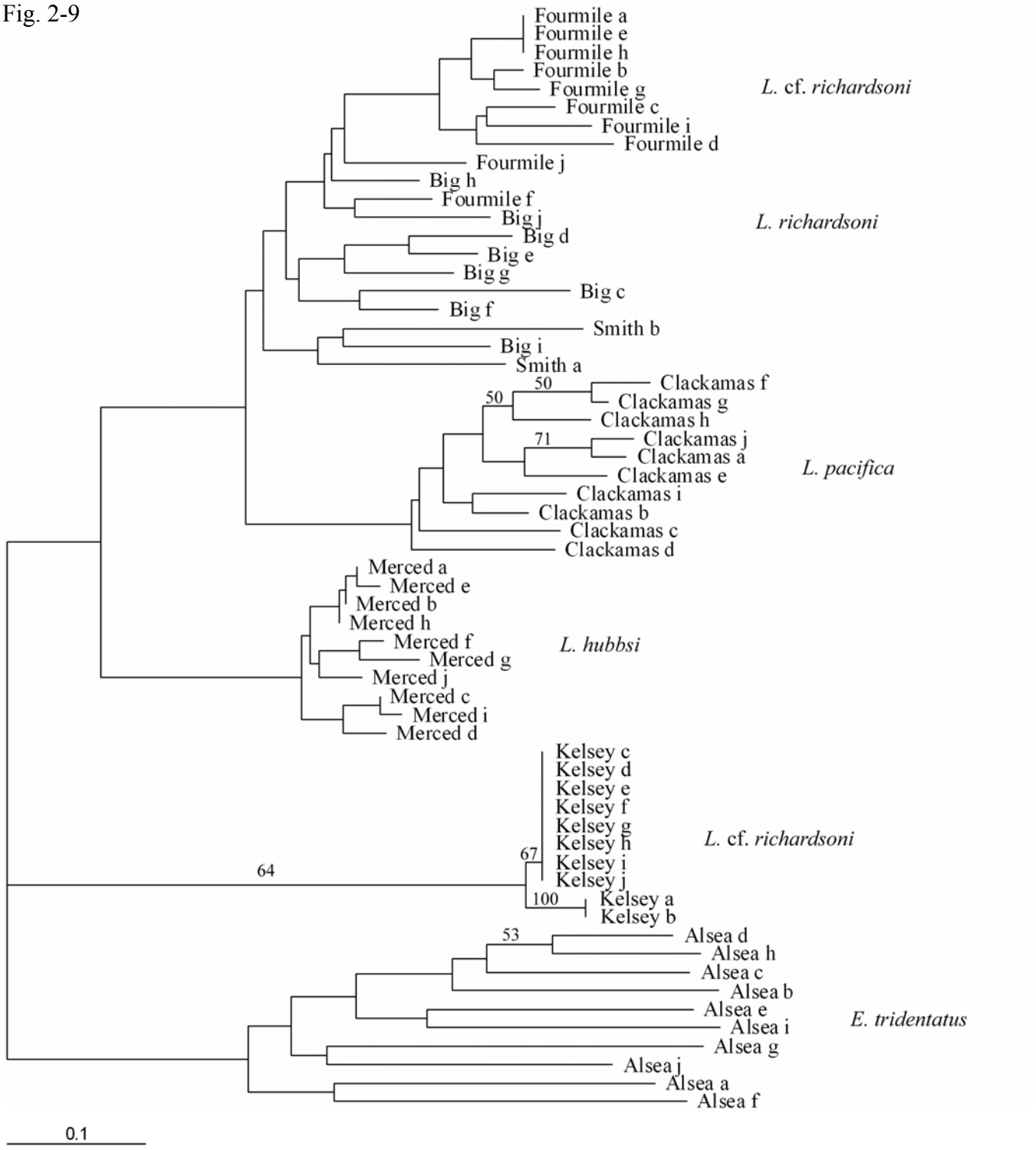
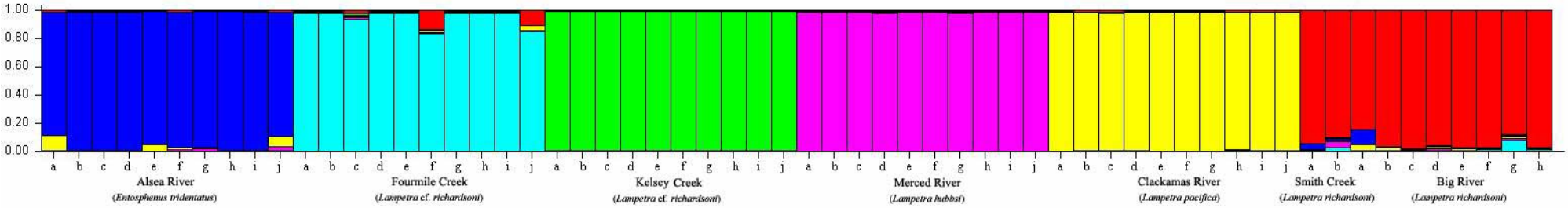

Fourmile a
Fourmile e
Fourmile h
Fourmile b
Fourmile g
Fourmile c
Fourmile i
Fourmile d
Fourmile j
Big h
Fourmile f
Big j
Big d
Big e
Big g
Big c
Big f
Smith b
Big i
Smith a
50
Clackamas f
50
Clackamas g
Clackamas h
71
Clackamas j
Clackamas a
Clackamas e
Clackamas i
Clackamas b
Clackamas c
Clackamas d
Merced a
Merced e
Merced b
Merced h
Merced f
Merced g
Merced j
Merced c
Merced i
Merced d
Kelsey c
Kelsey d
Kelsey e
Kelsey f
Kelsey g
Kelsey h
67
Kelsey i
Kelsey j
64
100
Kelsey a
Kelsey b
53
Alsea d
Alsea h
Alsea c
Alsea b
Alsea e
Alsea i
Alsea g
Alsea j
Alsea a
Alsea f
L. cf. richardsoni
L. richardsoni
L. pacifica
L. hubbsi
L. cf. richardsoni
E. tridentatus
0.1

Fig. 2-10


1.00
0.80
0.60
0.40
0.20
0.00
a b c d e f g h i j a b c d e f g h i j a b c d e f g h i j a b c d e f g h i j a b c d e f g h i j a b a b c d e f g h
Alsea River
(Entosphenus tridentatus)
Fourmile Creek
(Lampetra cf. richardsoni)
Kelsey Creek
(Lampetra cf. richardsoni)
Merced River
(Lampetra hubbsi)
Clackamas River
(Lampetra pacifica)
Smith Creek
(Lampetra richardsoni)
Big River
(Lampetra richardsoni)

General Discussion

Highlights and conclusions

The phylogeny of brook lampreys on the Pacific coast of North America is far more complex than previously thought. Nearly genetically indistinguishable *Lampetra richardsoni* cytochrome *b* haplotypes were discovered across their broad distribution (from Farragut River, AK to Navarro, CA – over 2700 km), but highly divergent haplotypes among presumed conspecifics were also discovered. One of the clearest and most notable findings was that *L. richardsoni* sensu lato is polyphyletic – presumtive *L. richardsoni* were found in at least three major clades, interspersed with *L. ayresii*, *L. pacifica*, and *L. hubbsi*. There was a total of 49 west coast *Lampetra* populations (excluding *L. fluviatilis*) analyzed, but nine populations fell outside the *richardsoni/ayresii* clade (only one of which, Merced River, is currently recognized as a distinct species; Nelson et al., 2004; while *L. pacifica* from the Clackamas River is considered to be synonymous with *L. richardsoni*; Robins et al., 1991) and were more than 2.9% divergent (and up to 6.4% divergent) from *L. richardsoni* from its type locale. These genetically distinct populations of *L. richardsoni* sensu lato display levels of genetic diversity that exceed that of congeneric species (approximately 2.6%; see Chapter 1). As such, some or all of these populations generally described as *L. richardsoni* (or tentatively described as *L.* cf. *richardsoni* in Chapter 2) potentially represent new cryptic species that have gone unrecognized due to their conserved body form and general lack of study. Lampreys from Kelsey Creek, California, were the most divergent and they were identified as *L. richardsoni* (Moyle, 2002). However, virtually all specimens collected – which were identified as *L. richardsoni* – came from populations where adult characteristics

were not provided, thus making identification questionable. The monophyly of *Lampetra* cf. *richardsoni* from Kelsey Creek is strongly supported by mtDNA and nuclear markers. The cyt *b* sequence des not correspond with any other known lamprey sequence (Tagliavini et al., 1994; Docker et al., 1999; Lorion et al., 2000; Docker, 2006; Espanhol et al., 2007; Blank et al., 2008). Additionally, it has the greatest pairwise genetic divergence between any congeneric species within this study (with one exception, i.e., at the TAP intron 9 locus) using both K2P% distance for sequence data and F_{st} values for microcrosatellite data. The TAP intron 9 sequence data recovered the greatest pairwise genetic divergence between *L. hubbsi* and *L. pacifica* (1.08%; Table 4-2), although *L. richardsoni* and *L.* cf. *richardsoni* from Kelsey Creek were still clearly distinct (0.21%). Moreover, *L.* cf. *richardsoni* from Kelsey was monophyletic based on all genetic data available, thus satifying phylogenetic species criteria.

Although species delineations cannot be based soley on genetic distance values (see Chapter 1), the major clades identified in this study appear to meet the species criteria according to several species concepts. For example, *L. pacifica* can be morphologically discriminated from *L. richardsoni* (Vladykov, 1973a), and is a strongly supported monophyletic group based on five of the six molecular markers used for evolutionary reconstruction (the sixth, ITS1, rarely displayed any genetic variation among congeneric species). This is consistent with at least two species concepts – the MSC and PSC. The fact that *L. pacifica* is as genetically distinct from *L. richardsoni* sensu stricto as the latter is from *L. hubbsi* (which has been deemed more closely related to *Entosphenus* species based on morphology, but shown to be more closely related to other *Lampetra* species using all genetic data; see General Introduction and above) suggests that they might also be

reproductively isolated. However, the BSC has not been directly tested. Nevertheless, the recognition of *L. pacifica* as a valid species should be reconsidered. To what extent morphology can be used to distinguish the most divergent *L.* cf. *richardsoni* from congenerics is not known since adult specimens were not available. Mature specimens from Kelsey Creek were captured this spring (2009) for morphological analysis by Dr. Stewart Reid (Western Fishes, Ashland, OR), but the analsis has not yet been completed.

Phylogeography and regions of endemism

Phylogeography departs from traditional phylogenetics by its focus on population history and demography. The phylogeography of brook lampreys, as well as *L. ayresii*, reveal both broadly distributed and endemic species. The proposed distribution of *L. richardsoni* has been subjected to amendments as authors have not agreed on a common distribution (e.g., Vladykov and Follett, 1965; Page and Burr, 1991; Moyle, 2002). Isolation-by-distance models do not appear to explain the degree of genetic variation between different populations of *Lampetra* species. *Lampetra richardsoni* populations distributed over 2700 km can be nearly genetically identical. This is of particular interest since these brook lamprey populations are isolated in fresh water and represent disjunct populations. One possible explanation for this lack of genetic differentiation over a broad geographical range could include a closely-related haplotype that occurred in a glacial refugium to the south and then served as the source of the parasitic migratory populations that recolonized northwards (and subsequently giving rise to non-parasitic populations) following glacial retreat. The geographical distribution of genetic diversity in many species of animals in general often implies that their current distribution was strongly affected by postglacial recolonization

(Hewitt, 1999). The greatest genetic distances within species are often between regions believed to have functioned as glacial refugia. Away from these regions, geographical ranges often yield genetically more uniform and less differentiated populations, explained by the dramatic loss of variation that occurred during postglacial range expansion (Ibrahim et al., 1996) from these refugia. Population bottlenecks may have subsequently occured as a result of colonization and isolation, and these populations would be more susceptible to founder effects due to a small effective population size. Accordingly, this stochasticity could lead to populations in close proximity to one another showing various degrees of genetic divergence. This might be apparent in the increasingly divergent *L. richardsoni* (Fig. 1-4; clade RA) that have become genetically distinct from their common haplotype (Fig. 1-4; clade T).

The phylogeographic analyses also revealed substantial genetic variation among various congeneric species in the southern coastal region of the United States. The findings are, in part only, consistent with Vladykov's (1973a) proposed distribution of *L. richardsoni* and *L. pacifica*. Populations that were not sampled by Vladykov (1973a) (e.g., Fourmile Creek, Siuslaw River, and Mark West Creek) may also represent *L. pacifica* (see Chapter 1 and Fig. 1-4a) and would therefore expand its distributional range. The Columbia River, separating the state of Washington to the north from Oregon to the south, acts as the northern limit of the most genetically divergent lampreys, but Vladykov (1973a) believed that *L. richardsoni* occurred only to the north of this divide and *L. pacifica* to the south. In contrast, Moyle (2002) suggests that *L. richardsoni* can be found in regions south of the Columbia River encompassing central California (Navarro River). Contrary to Moyle (2002), not all *Lampetra* brook lampreys in California are *L. richardsoni*.

Regarding the distribution of *L. hubbsi*, the single specimen collected from Paynes Creek may suggest a range extension of this species into the upper Sacramento River. In addition, after completion of the phylogeographic analyses performed for Chapter 1, I received tissue samples from specimens collected from Bear Creek (a second tributary approximately 30 km east of Clear Lake, California). I sequenced the cyt *b* gene in four individuals and found that they were genetically similar (1.27 K2P%) to *L. hubbsi* and using a maximum parsimony heuristic analysis, they form a monophyletic group with known *L. hubbsi* and *L.* cf. *richardsoni* from Paynes Creek. What is more, this adult specimen is morphologically consistent with *L. hubbsi* (Stewart Reid, Western Fishes, Ashland, OR, pers. comm.). The Clear Lake system therefore appears to support *L. hubbsi*, a potential new species of *L.* cf. *richardsoni* (Clear Lake, CA), and possibly *L. richardsoni* sensu stricto (Moyle, 2002). Broader geographical sampling is required (for both morphological and molecular analysis) to get better knowledge of the distribution of these (or related but as of yet undescribed) species.

The restricted distributions of highly genetically different clades (i.e., to areas not recently glaciated) suggest that the evolution of several populations of lampreys have been occurring in isolation for a long time. Several of these regions support high biological diversity, and this apparently includes lamprey taxa. Clear Lake, California, is renowned as one of the oldest lakes in North America (Sims, 1988; Sim et al., 1988) and may also represent an ancestral relict lake dating back to the early Pleistocene, making it 1.8 to 3.0 million years old (Casteel and Rymer, 1981; Hearn et al., 1988). It is host to several fish species considered important for ecosystem-level coordinated management strategies (Moyle et al., 1995). Subspecies classification has been given to several fish taxa endemic to Clear

Lake and its surrounding area including, but not limited to, the Clear Lake hitch (*Lavinia exilicauda chi*). Based on the molecular data presented here, lampreys collected from Kelsey Creek, a tributary to Clear Lake, may also warrant recognition. Despite Kelsey Creek lampreys being distributed in such close proximity to lampreys collected from Mark West Creek, a tributary to the Russian River, they are genetically distinct from one another, and likely represent a new cryptic species. This large discrepancy of genetic variation is peculiar since Clear Lake is hypothesized to have drained westward through the Russian River more than 10 000 years ago, at which point volcanic eruptions and subsequent landslides altered the landscape draining the lake eastward into the Central Valley (Davis, 1933; Suchanek et al., 2003). Therefore the events leading to the diversification between these two lamprey populations – separated by less then 500 km – remain unclear, especially when *L. richardsoni* distributed 2700 km along the Pacific coast of North America showed virtually no genetically differentiation.

Perhaps, isolated brook lamprey populations found further inland have had more time to sustain sequence divergence relative to conspecifics distributed in close proximity to the coast, and those cases of inland lampreys that are nearly genetically indistinguishable from their coastal-proximate-pair (e.g., Navarro River) are attributed to a recent undisrupted migratory corridor. It is possible that postglacial retreat (see above) differentially affected inland versus coastal populations of lampreys, but the extent is not known. A series of theoretical genetic barriers overlaying a geographical map of the Pacific coast of North America has been constructed (Appendix C). This provides an alternative means to interpreting phylogenetic trees, and also highlights particular geographical regions of endemism possibly corresponding to glacial refugia. It should however be interpreted

cautiously as a genetic barrier to one species may not influence that of another. The software program Barrier v2.2 supports eight regions of endemism corresponding to high levels of genetic variation. The species identification as well as the corresponding region of endemism are as follows: *L. richardsoni* (Fork Creek), *L. pacifica* (Columbia River drainage), *L.* cf. *richardsoni* (each endemic to their respective locale of Siuslaw River, Fourmile Creek, Paynes Creek, Kelsey Creek, and Mark West Creek), and *L. hubbsi* (Merced River).

The theory of ecological speciation may have contributed to the genetic structure suggested in brook lamprey phylogeny. Ecological speciation occurs as a response to different environmental stimuli, natural selection, as well as through reproductive isolation (reviewed by Schluter, 1996). As inferred from the historical geological records of central coastal California (Suchanek et al., 2003), both stochastic and large geological events may have had more of an influence on lamprey species found south of the Columbia River by isolating populations into new environments at an earlier time; this would lead to higher levels of genetic differentiation among them than among those populations north of the Columbia River that may have had more recent recolonization events. It is uncertain to what extent, if any, an ancestral lamprey from California contributed to the evolution of speciation in brook lampreys. Since Clear Lake, California (catchment to Kelsey Creek; discussed above), is believed to be the oldest lake in North America (Sims, 1988; Sims et al., 1988), it is possible that *Lampetra* brook lampreys throughout their range originated from an ancestral population in central California.

Paraphyly of Lampetra ayresii and the evolution of non-parasitism

Paraphyly is observed in many recognized species (Funk and Omland, 2003 and references therein). *Lampetra ayresii* from six locations distributed intermittently along the coast from northern British Columbia to California were not monophyletic (Fig. 1-4; clade T). Based on the most parsimonious explanation, the data suggest that parasitism has evolved multiple times, but conventional wisdom suggests that non-parasitism in derived (see "Paired and satellite species" in the General Introduction). It is not clear to what extent extant parasitic lampreys or closely-related ancestors have contributed to the evolution of non-parasitic brook lampreys, nor is it clear to the extent of their distribution range. Here, as in other molecular studies (e.g., Schreiber and Engelhorn 1998; Espanhol et al., 2007; Blank et al., 2008), data support that that non-parasitism has evolved multiple times within a genus. Particular to this study, further investigation is required to substantiate this hypothesis and might include a larger genetic survey of *L. ayresii* across its distributional range, as I report on only five populations some with a single representative specimen.

It is possible that parasitic lampreys are nested within other closely-related clades as the result of confusion of *L. ayresii* and/or *L. richardsoni* larvae in this study; there is a paucity of morphological characteristics identifying ammocoetes to adult species (e.g., Neave et al., 2007) and this study relied heavily on larval collections and existing distribution records. However, most *L. ayresii* were collected as adults (Table 1-1) and confirmed adults of *L. ayresii* and *L. richardsoni* were found within clade T (Fig. 1-4). Therefore, even if phylogenetic analyses were restricted to adult specimens, *L. ayresii* and *L.* richardsoni were not reciprocally monophyletic. This was consistent with previous reports by Docker et al. (1999); based on small mtDNA fragments, they found that *L. ayresii* was genetically

indistinguishable from *L. richardsoni* in British Columbia. This same pattern is observed in other paired lamprey species, where the parasitic and non-parasitic species are not reciprocally monophyletic (see Docker, in press). This example of morphotypes that are genetically indistinguishable does not appear to be unique to lampreys (e.g., the Mimic Shiner [*Notropis volucellus*] and the Ghost Shiner [*N. buchanani*] in Hubert et al., 2008). In contrast, two additional *L. ayresii* clades distributed further south in Oregon (Mill Creek) and California (Sacramento Delta and Feather Creek) are genetically distinct from type locale *L. richardsoni* (more than 1.5%). This demostrates that the search for diagnostic molecular differences between closely-related species (DNA 'barcodes') must consider intraspecific variation when comparing widely-distributed species.

Based on the hypothesis that non-parasitic lampreys have evolved from parasitic ancestors, it is uncertain to what extent the three genetically-distinct lineages of *L. ayresii* have contributed to the speciation events in non-parasitic brook lampreys. It is conceivable that an ancestral parasitic species distributing itself along the coast acted as a conduit through which brook lampreys evolved and diversified. As these anadromous ancestral parasitic river lampreys invaded new coastal streams with the aid of ocean currents and active transport on prey species, they were able to colonize new coastal habitats. Furthermore, the possibility of multiple ancestral parasitic lampreys cannot be ruled out, amplifying the complexity of this dynamic evolutionary system by independently giving rise to multiple non-parasitic lampreys. This is not unlikely, given that *L. ayresii* undergoes limited migration and does not appear to be distributed over a continuous range (i.e., local *L. ayresii* give rise to local *L. richardsoni*), but a greater similarity between local *L. ayresii* and *L. richardsoni* would be expected. Based on the relatively conserved body form of brook lampreys across their

distribution and the fact that life-history type (parasitic *versus* non-parasitic) is currently used to discriminate between species pairs (see "Paired and satellite species" in the General Introduction), the independent evolution of brook lampreys and moreover, independent speciation events, appear to be occurring in parallel. Similar studies have been supported by Schluter et al. (2001) who have studied parallel evolution in threespine sticklebacks. The successional events leading to the repeated emergence of almost identical brook lampreys may seem improbable, but if these events are dependent on similar selection constraints, closely-related paired and satellite species may represent the same evolutionary model.

The notion that non-parasitic lampreys have evolved from parasitic ones based on the repetitive trend towards the elimination of adult feeding (Hubbs, 1925) continues to persist in later discussions of 'paired' and 'satellite species' (Zanandrea, 1959; Vladykov and Kott, 1979; Docker, in press). The process through which this evolutionary transformation is attained may involve heterochrony (reviewed by Hardisty, 2006) or some intermediate life-history form (see below). For example, had a parasitic lamprey evolved the ability to feed facultatively (i.e., could mature without feeding post-metamorphosis), this would provide a unique method of colonizing new environments as a means to exploit new resources. Indeed, the example above is in part substantiated by anecdotal evidence of facultative feeding in a *L. richardsoni* population from Morrison Creek, BC (the population location is included in this study; Fig. 1-2; Beamish, 1985; Beamish and Withler, 1986). This population of lamprey incorporates a feeding variety intermediate to *L. richardsoni* and *L. ayresii* in its biology and morphology. The result of this discovery was the classification of a new morphologically distinct variant of *L. richardsoni*, the Morrison Creek lamprey (*L. richardsoni* var. *marifuga;* Beamish, 1985). I have found that the common *L. richardsoni* haplotype (Fig. 1-4; clade T)

can be found in Morrison Creek; however, the variants cannot be distinguished based on larval morphological characteristics (Beamish and Withler, 1986). These three life history forms have not yet been genetically tested, but I hypothesize that intermediate feeding varieties between species pairs are genetically indistinguishable at neutral loci over a sympatric distribution.

Direction for future study

This study supports *L. richardsoni* sensu lato as being polyphyletic with large genetic divergence from specimens collected from its type locale and therefore suggests that it is not a valid species based on the PSC. Genetic data also support *L. pacifica* as a valid species based on its original species description (Vladykov, 1973a) and the levels of genetic difference are on par with species-level differences. Additionally, populations tentatively identified as *L.* cf. *richardsoni* could be *L. pacifica* (e.g., from Mark West Creek) or they could represent cryptic species. Similarly, the specimen from Paynes Creek tentatively identified as *L.* cf. *richardsoni* could be *L. hubbsi* or could represent a new cryptic species. The population from Kelsey Creek is almost certainly a new species (tentatively described as *Lampetra* sp.). Adult specimens are required to be examined before formal taxonomic changes are recommended for these species, since there is a paucity of morphological data in larvae used for species-level identification. Future study should also include information such as the life-history strategy as well as a more detailed investigation into the distribution of each species.

Additional pertinent questions stem from this study that has provided information regarding the genetic diversification and phylogeny of brook lampreys and the closely-

related *L. ayresii*. For example, what is the extent and role of hybridization in paired and satellite species of lampreys? The widely distributed *L. richardsoni* haplotypes scattered within the molecular phylogeny may be heavily influenced by natural hybridization. There have been suggestions that gene flow may occur between the two life-history types, despite differences in adult body size (Docker, in press). A hybrid anadromous *L. ayresii/L. richardsoni* cross may act as a conduit by which *L. richardsoni* genes are propagated. This hybrid may be represented as the polytomy in the phylogeny (Fig. 1-4; clade T), and widely distributed from Alaska to California. If the maternal lineage of *L. richardsoni* is crossed with the paternal lineage of *L. ayresii*, and the result is an anadromous hybrid capable of invading new coastal freshwater habitats, then broad phylogeographic structure constructed using matrilineal genes would appear genetically similar. Other paired or satellite species that may exist within the phylogeny must also be explored.

Finally, what proportion of lamprey diversification along the Pacific coast results from allopatric versus sympatric divergences? Although most species-level diversification in *L. richardsoni* can be explained by vicariance events, some divergences may more adequately be explained by adaptive divergence. The support for genetically indistinguishable life-history types, the lack of knowledge regarding the mechanisms that influence a life-history shift, and the possibility of intermediate life-history types or facultative feeding, suggest that vicariance hypotheses may not be able to justify all the biological diversity within this model system, and that adaptive radiation may explain at least in part some of the variation.

Summarizing, the combined use of phylogeographic and phylogenetic analyses using multiple genetic datasets presents convincing evidence that brook lampreys along the Pacific

coast of North America are far more genetically diverse than previously thought. This study presents evidence that non-parasitism has evolved multiple times – likely at different times and in different places – within recognized taxa but has likely gone unrecognized due to convergent morphology. Studies into the mechanisms and selective pressures guiding this parallel evolution will be of interest to evolutionary biology.

Appendix A.

Partial list of lamprey species currently or previously recognized as belonging to the genus *Lampetra*; some Eurasian species not mentioned in the thesis are omitted. Scientific names used in the thesis, common names, scientific names following the recent American Fisheries Society (AFS) designations (Nelson et al., 2004), as well as comments regarding the classification of these species are given. Scientific names used in this thesis follow the generic designations of Vladykov and Kott (1979) and Renaud (1997). Nelson et al. (2004) use *Lampetra* collectively to include the *Lampetra, Entosphenus, Lethenteron*, and *Tetrapleurodon* subgenera (European *Eudontomyzon* would also be included as a subgenus of *Lampetra* (Bailey 1980) but is not dealt with explicitly by AFS) but AFS is currently reconsidering this decision and will recognize each as distinct genera in its next list of names (see Renaud et al., in press). Comments regarding classification are intended to facilitate the overall understanding of the thesis, and are not a comprehensive taxonomical review of each species.

Scientific name used in this thesis	Common name	Scientific name following AFS designation (Nelson et al., 2004)
Entosphenus		
En. folletti Vladykov and Kott, 1976b[1]	Northern California brook lamprey	*Lampetra* (*Entosphenus*) *lethophaga*
En. hubbsi Vladykov and Kott, 1976a[2]	Kern brook lamprey	*Lampetra* (*Entosphenus*) *hubbsi*
En. lethophagus (Hubbs, 1971)	Pit-Klamath brook lamprey	*Lampetra* (*Entosphenus*) *lethophaga*
En. macrostomus (Beamish, 1982)	Vancouver lamprey	*Lampetra* (*Entosphenus*) *macrostoma*
En. tridentatus (Gairdner in Richardson, 1836)	Pacific lamprey	*Lampetra* (*Entosphenus*) *tridentata*
Eudontomyzon		
Eu. danfordi Regan, 1911	Carpathian lamprey	*Lampetra* (*Eudontomyzon*) *danfordi*[9]
Eu. stankokaramani Karaman, 1974[3]	Drin brook lamprey	*Lampetra* (*Eudontomyzon*) *stankokaramani*[9]
Lampetra		
La. aepyptera (Abbot, 1860)	Least brook lamprey	*Lampetra* (*Lampetra*) *aepyptera*
La. ayresii (Günther, 1870)	Western river lamprey	*Lampetra* (*Lampetra*) *ayresii*
La. fluviatilis (Linnaeus, 1758)	European river lamprey	*Lampetra* (*Lampetra*) *fluviatilis*[9]
La. pacifica Vladykov, 1973a[4]	Pacific brook lamprey	*Lampetra* (*Lampetra*) *pacifica*
La. planeri (Bloch, 1748)	European brook lamprey	*Lampetra* (*Lampetra*) *planeri*[9]
La. richardsoni Vladykov and Follett, 1965	Western brook lamprey	*Lampetra* (*Lampetra*) *richardsoni*
La. richardsoni var. *marifuga* Beamish, 1985	Morrison Creek lamprey	----[10]
Lethenteron		
Le. appendix (DeKay, 1842)	American brook lamprey	*Lampetra* (*Lethenteron*) *appendix*
Le. camtschaticum (Tilesius, 1811)[5]	Arctic lamprey	*Lampetra* (*Lethenteron*) *camtschaticum*
Le. kessleri (Anikin, 1905)	Siberian brook lamprey	*Lampetra* (*Lethenteron*) *kessleri*[9]
Le. reissneri (Dybowski, 1869)[6]	Far Eastern brook lamprey	*Lampetra* (*Lethenteron*) *reissneri*[9]
Le. meridionale Vladykov, Kott, and Pharand-Coad, 1975[7]	Gulf lamprey	*Lampetra* (*Lethenteron*) *meridionale*
Le. zanandreai (Vladykov, 1955)[8]	Po brook lamprey	*Lampetra* (*Lethenteron*) *zanandreai*[9]

Appendix A continued

Tetrapleurodon		
T. geminis Alvarez del Villar, 1964	Mexican brook lamprey	*Lampetra* (*Tetrapleurodon*) *geminis*
T. spadiceus (Bean, 1887)	Mexican lamprey	*Lampetra* (*Tetrapleurodon*) *spadicea*

1 Synonymized with *En. lethophagus* (Robins et al., 1980)

2 Retained (by Nelson et al., 2004) in the subgenus *Entosphenus* based on its original morphological description, despite molecular similarity to *Lampetra* (Docker et al., 1999)

3 Had been synonymized with *Eudontomyzon danfordi* Regan, 1911, but has since been redescribed as a new species (Holčík and Šorić, 2004)

4 Synonymized with *La. richardsoni* (Robins et al., 1991) based on a statement in Bond and Kan (1986) that the morphological distinction between the two appears to be slight.

5 Also known as *Le. japonicum* (which is a junior synonym of *Le. camtschaticum*; Nelson et al., 2004)

6 Appears to represent at least three distinct species: *Le. reissneri* from its type locale, *Le.* sp. N from northern Japan (Yamazaki and Goto, 1998), and *Le.* sp. S from southern Japan and Korea (Yamazaki et al., 2006)

7 Synonymized with *Lampetra aepyptera* (Etnier and Starnes, 1993)

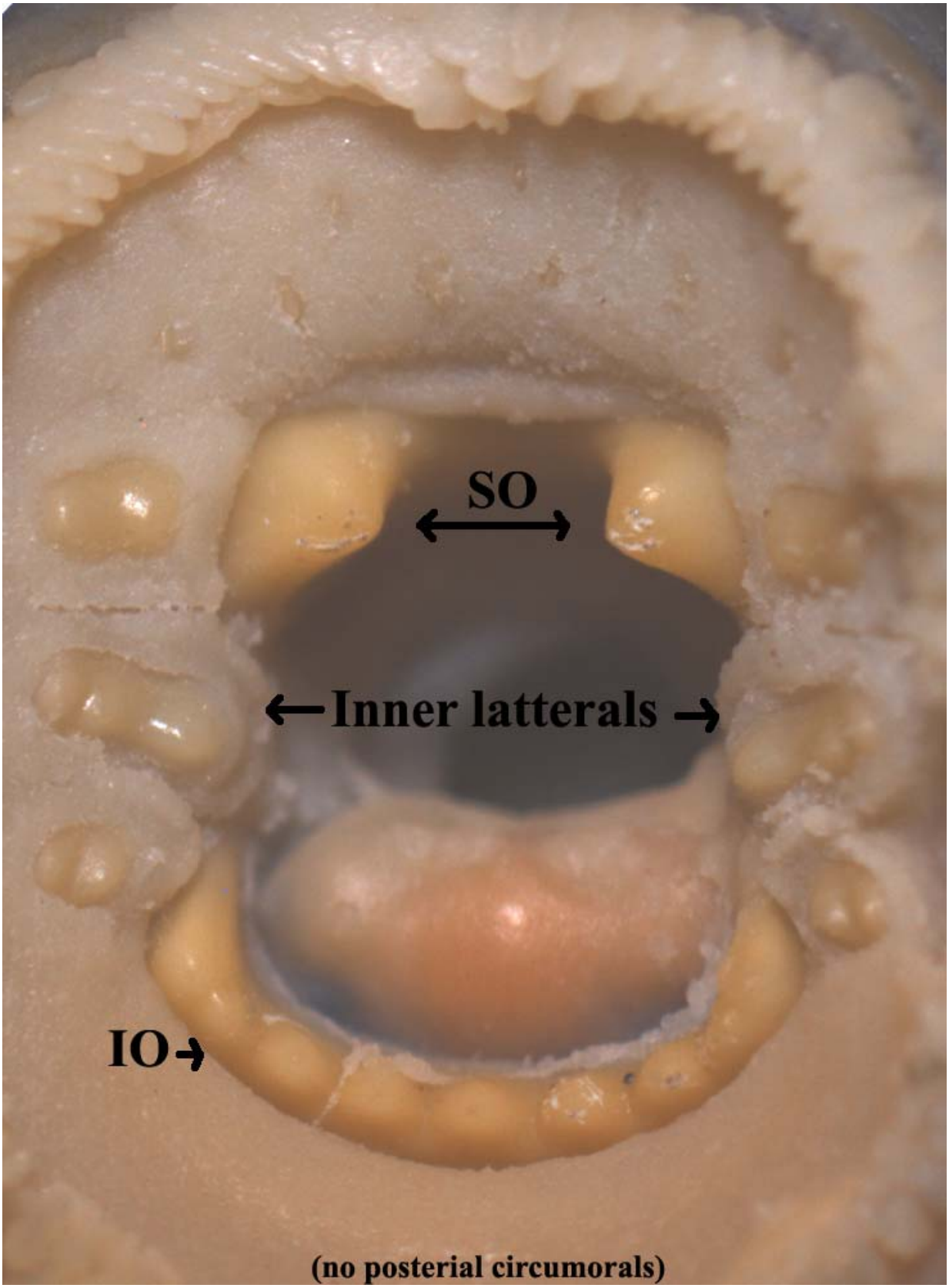
8 Originally described as *Lampetra.* Morphological characters (unicuspid posterials and 3 inner laterals with 2-2-2 cusp pattern; Potter, 1980) suggest *Lethenteron.* There are genetic data to support it is more closely related to *Lampetra* (Docker et al., 1999)

9 AFS does not report on species outside of North America, but Nelson et al. (2004) recognized the various nominal genera as subgenera.

10 Since a "variety" published after 1960 is an excluded taxonomic rank in zoological nomenclature (Article 15.2, International Commission on Zoological Nomenclature 1999), the Morrison Creek lamprey has not been given formal taxonomic status.

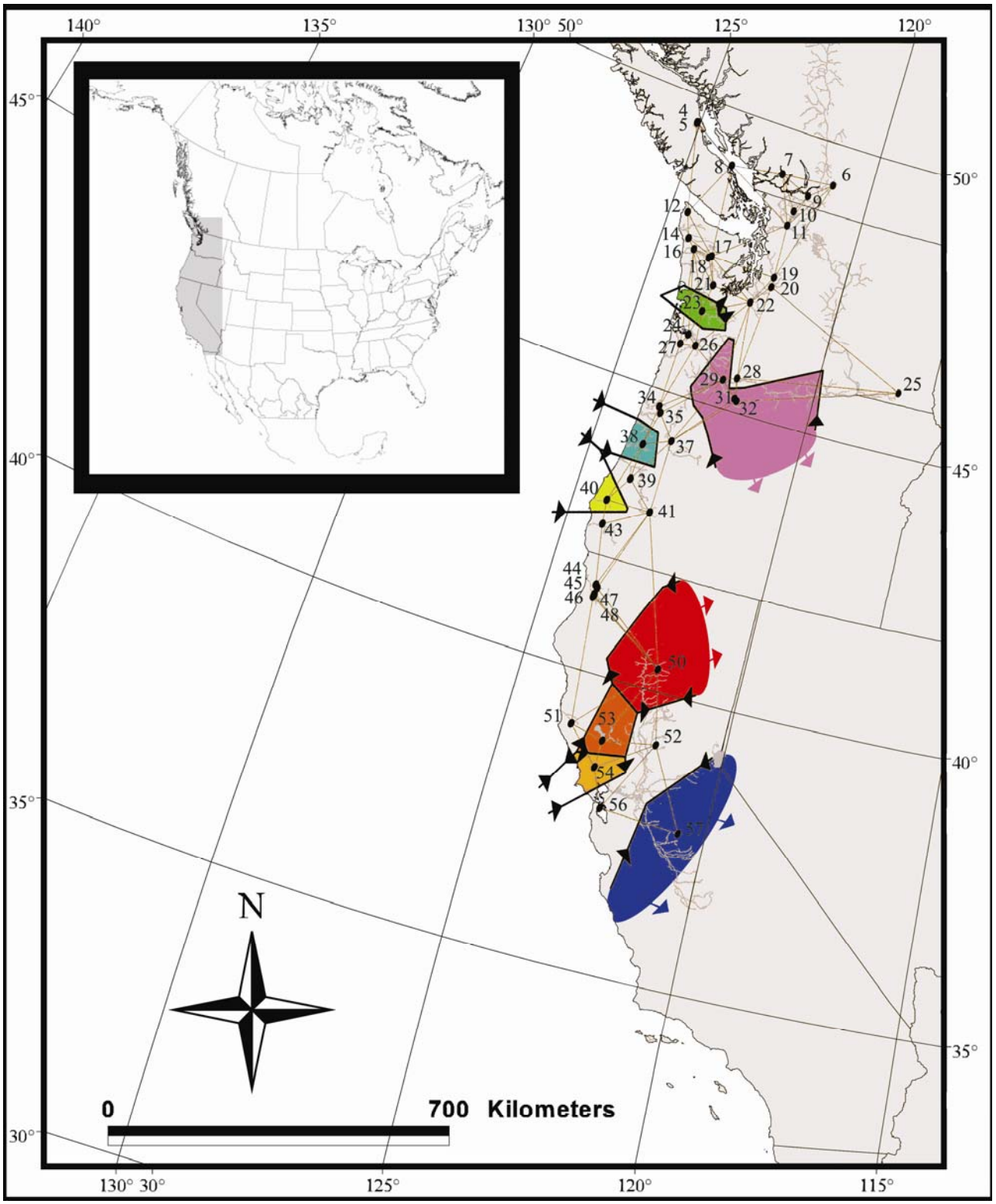
Appendix B.

Lampetra richardsoni from Smith Creek (see Table 1-1) identified as an adult by the following relevant morphological characteristics: a supraoral (SO) lamina with 2 cusps; 3 inner laterals with a 2-3-2 cusp arrangement; an infraoral (IO) lamina of 8 cusps; and the absence of posterial circumorals.


SO
Inner latterals
IO
(no posterial circumorals)

Appendix C.

Genetic boundaries of all *Lampetra* lampreys have been delineated using Barrier v2.2 (Manni and Guérard, 2004). A genetic distance matrix was compiled using the average number of character changes between each population. Ten barriers to gene flow were specified for output. Eight regions of endemism have been identified using the Monmonier algorithm (Monmonier, 1973) in conjunction with the Delaunay triangulation approach (Brassel and Reif, 1979). Map identification numbers correspond to those in Table 1-1. Each colour represents a potential isolated region of endemism. The black lines with arrows denote the genetic boundary, while coloured arrows denote a possible extension of a particular region of endemism unconfined by the analysis.


140°
135°
130°
50°
125°
120°
45°
40°
35°
30°
130°
30°
125°
120°
115°
N
0
700 Kilometers

Compiled literature cited

Agapow, P.M., Bininda-Emonds, O.R.P., Crandall, K.A., Gittleman, J.L., Mace, G.M., Marshall, J.C., 2004. The impact of species concept on biodiversity studies. Q. Rev. Biol. 79, 161-179.

Alvarez, J., 1964. Ictiología michoacana, IV. Contribución al conocimiento biológico y sistemático de las lampreas de Jacona, Mich., México. Anales de la Escuela Nacional de Ciencias Biologicas México. 13, 1-4.

Anikin, V. P., 1905. Description of new fish species from Asia. Izvestiya Tomskogo Universiteta. 27,1-17. [In Russian]

Arnheim, N., Krystal, M., Schmickel, R., Wilson, G., Ryder, O., Zimmer, E., 1980. Molecular evidence for genetic exchanges among ribosomal genes on nonhomologous chromosomes in man and apes. Proc. Natl. Acad. Sci. USA 77, 7323-7327.

Attardi, G., 1985. Animal mitochondrial DNA: an extreme example of genetic economy. Int. Rev. Cytol. 93, 93-145.

Avise J. C., 2000. Phylogeography: the history and formation of species Harvard Univ. Press, Cambridge, Massachusetts.

Avise, J.C., 2000. Phylogeography. Harvard Univ. Press, Cambridge.

Avise, J.C., Arnold, J., Ball., R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., Saunders, N.C., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18, 489-522.

Avise, J.C., Ball. M., 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Sur. Evol. Biol. 7, 45-67.

Avise, J.C., Walker, D., 1999. Species realities and numbers in sexual vertebrates: perspectives from an asexually transmitted genome. Proc. Natl. Acad. Sci. USA 96, 992-995.

Ayres, W.O. 1855. Description of new fishes from California. (Minutes of Academy meetings were printed in "The Pacific" (a newspaper) shortly after each meeting. New species date to publication in The Pacific. Dates of publication are given in each species account). The Pacific v. 4 (nos. 7-22). [Also appeared in Proc. Calif. Acad. Nat. Sci. v. 1.]

Bailey, R.M., 1980. Comments on the classification and nomenclature of lampreys – an alternative view. Can. J. Fish. Aquat. Sci. 37, 1626-1629.

Ballard, J.W.O, Whitlock, M.C., 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13, 729.

Ballard, J.W.O., Kreitman, M., 1995. Is mitochondrial DNA strictly a neutral marker? TREE 10, 485-488.

Baum, D.A., Shaw, K.L., 1995. Genealogical perspectives on the species problem. In: Hoch, P.C., Stephenson, A.G. (Eds.), Experimental and Molecular Approaches to Plant Biosystematics. Missouri Botanical Garden, St. Louis, Missouri. pp. 289-303.

Baxter, E.W., 1956. Observations on the buccal glands of lampreys (Petromyzonidae). Proc. Zool. Soc. Lond. 127, 95-118.

Beacham, T.D., 1985. Variation in number of vertebrae and gill rakers of sockeye salmon, *Oncorhynchus nerka*, in North America. Environ. Biol. Fishes. 14, 97-105.

Beamish, R.J., 1980. Adult biology of the river lamprey (*Lampetra ayresi*) and the Pacific lamprey (*Lampetra tridentata*) from the Pacific coast of Canada. Can. J. Fish. Aquat. Sci. 37, 1906-1923.

Beamish, R.J., 1982. *Lampetra macrostoma*, a new species of freshwater parasitic lamprey from the west coast of Canada. Can. J. Fish. Aquat. Sci. 37, 736-747.

Beamish, R.J., 1985. Freshwater parasitic lamprey on Vancouver Island and a theory of the evolution of the freshwater parasitic and non-parasitic life history types. In: Foreman, R.E., Gorbman, A., Dodd, J.M., Olsson, R. (Eds.) Evolutionary biology of primitive fishes. Plenum Publishing Corporation, New York, New York. pp. 123-140.

Beamish, R.J., Neville, C.E.M., 1992. The importance of size as an isolating mechanism in lampreys. Copeia 1992, 191–196.

Beamish, R.J., Withler, R.E., 1986. A polymorphic population of lampreys that may produce parasitic and a nonparasitic varieties. In: Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes, Tokyo, 1985. Ichthyological Society of Japan, Tokyo, Japan. pp. 31-49.

Bean, T.H., 1887. Descriptions of five new species of fishes sent by Prof. A. Dugès from the Province of Guanajuato, Mexico. Proceedings of the United States National Museum 10, 370-375.

Bermingham, E., Avise, J.C., 1986. Molecular zoogeography of freshwater fishes in the southestern United States. Genetics 113, 939-965.

Blank, M., Jurss, K., Bastrop, R., 2008. A mitochondrial multigene approach contributing to the systematics of the brook and river lampreys and the phylogenetic position of *Eudontomyzon mariae*. Can. J. Fish. Aquat. Sci. 65, 2780-2790.

Bloch, M.E., 1784. Oeconomische Naturgeschichte der Fische Deutschlands. III. Berlin.

Bond, C.E., Kan, T.T., 1986. Systematics and evolution of the lampreys of Oregon. In: Indo-Pacific fish biology, Proceedings of the second international conference on indo-pacific fishes. Uyeno, T., Arai, R., Taniuchi, T., Matsuura, K., (Eds.). The Ichthyological Society of Japan, Tokyo.

Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J.R., Cavalli-Sforza, L.L., 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368, 455-457.

Brassel, K.E., Reif, D., 1979. A procedure to generate Thiessen polygons. Geogr. Anal. 325, 31-36.

Brown, W.M., Prager, E.M., Wang, A., Wilson, A.C., 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. J. Mol. Evol. 18, 225-239.

Casteel, R.W., Rymer, M.J., 1981. Pliocene and Pleistocene fishes from the Clear Lake area. US Geological Survey Professional Paper 1141, 231-235.

Castric, V., Bernatchez, L., Belkhir, K., Bonhomme, F., 2002. Heterozygote deficiencies in small lacustrine populations of brook charr, *Salvelinus fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. Heredity 89, 27-35.

Cavalli-Sforza, L.L., and Edwards, A.W.F., 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21, 550–570.

Chakravarti, L., Roy, J., 1967. Handbook of Methods of Applied Statistics, v. 1, John Wiley and Sons. pp. 392-394.

Darwin, C., 1859. On the origin of species by means of natural selection. John Murray, London.

Davis, W.M., 1933. The lakes of California. Calif. J. Mine. Geol. 29, 175-236.

Degnan, S.M., 1993. The perils of single-gene trees – mitochondrial versus single-copy nuclear-DNA variation in white-eyes (Aves, Zosteropidae). Mol. Ecol. 2, 219-225.

DeKay, J. E., 1842. Zoology of New York or the New-York fauna. Part IV, Fishes. W. and A. White and J. Visscher, Albany, New York.

Docker, M.F., In press. The evolution of nonparasitism in lampreys: a review and update of the paired species concept. In: Brown, L. R., (Ed). Biology, Management and Conservation of Lampreys in North America. American Fisheries Society, Bethesda, Maryland.

Docker, M.F., 2006. Bill Beamish's contributions to lamprey research and recent advances in the field. Guelph Ichthyology Rev. 7, 1-52.

Docker, M.F., Haas, G.R., Goodman, D.H., Reid., S.B., Heath, D.D., 2007. Five PCR-RFLP markers detect 29 mitochondrial haplotypes in Pacific lamprey (*Entosphenus tridentatus*). Mol. Ecol. Notes 7, 350-353.

Docker, M.F., Youson, J.H., Beamish, R.J., Devlin, R.H., 1999. Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome *b* and ND3 gene sequences. Can. J. Fish. Aquat. Sci. 56, 2340-2349.

Domanico, M.J., Philips, R.B., Oakely, T.H., 1997. Phylogenetic analysis of Pacific salmon (genus *Oncorhynchus*) using nuclear and mitochondrial DNA sequences. Can. J. Fish. Aquat. Sci. 54, 1865-1872.

Donoghue, M.J., 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. The Bryologist 88, 172-181.

Dybowski, B.I., 1869. Vorläufige Mittheilungen über die Fischfauna des Ononflusses und des Ingoda in Transbaikalien. Verhandlungen der K.-K. Zoologisch-Botanischen Gesellschaft in Wien. 19, 945-958.

Ebach, M.C., Holdredge, C., 2005. DNA barcoding is no substitute for taxonomy. Nature 434, 697.

Echelle, A.A., Dowling, T.E., 1992. Mitochondrial DNA variation and evolution of the Death Valley pupfishes (*Cyprinodon*, Cyprinodontidae). Evolution 46, 193-206.

Espanhol, R., Almeida, P.R., Alves, M.J., 2007. Evolutionary history of lamprey paired species *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) as inferred from mitochondrial DNA variation. Mol. Ecol. 16, 1909-1924.

Esposti, M.D., DeVries, S., Crimi, M., Ghelli, A., Patarnello, T., Meyer, A., 1993. Mitochondrial cytochrome b: evolution and structure of the protein. Biocimica et Biophysica Acta. 1143, 243-271.

Etnier, D.A., Starnes, W.C., 1993. The fishes of Tennessee. Univ. Tennessee Press, Knoxville, Tennessee, USA.

Evans, B.J., Kelley, D.B., Tinsley, R.C., Melnick, D.J., Cannatella, D.C., 2004. A mitochondrial DNA phylogeny of African clawed frogs: phylogeography and implications for polyploid evolution. Mol. Phylogenet. Evol. 33, 197-213.

Eyre-Walker, A., Smith, N.H., Maynard, S.J., 1999. How clonal are human mitochondria? Proc. R. Soc. Lond. B. Sci. 266, 477-483.

Farmer, G.J., 1980. Biology and physiology of feeding in adult lamprey. Can. J. Fish. Aquat. Sci. 37, 1751–61.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39, 783-791.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368-376.

FitzSimmons, N.N., Limpus, C.J., Norman, J.A., Goldizen, A.R., Miller, J.D., Moritz, C., 1997. Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proc. Natl. Acad. Sci. USA 94, 8912-8917.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294-299.

Fritz, G.N., Conn, J., Cockburn, A., Seawright, J., 1994. Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from populations of *Anopheles nuneztovari* (Diptera: Culicidae). Mol. Biol. Evol. 11, 406-416.

Funk, D.J., Omland, K.E., 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34, 397-423.

Gill, H.S., Renaud, C.B., Chapleau, F., Mayden, R.L., Potter, I.C., 2003. Phylogeny of living parasitic lampreys (Petromyzontiformes) based on morphological data. Copeia 2003, 687-703.

Gladden, J.G.B., Fergusson, M.M., Friesen, M.K., Clayton, J.W., 1999. Population structure of North American beluga whales (*Delphinapterus leucas*) based on nuclear DNA microsatellite variation and contrasted with the population structure revealed by mitochondrial DNA variation. Mol. Ecol. 8, 347-363.

Gonzales, I.L., Sylvester, J.E. Smith, T.F., Stambolian, D., Schmickel, R.D., 1990. Ribosomal RNA gene sequences and hominoid phylogeny. Mol. Biol. Evol. 7, 203-219.

Goodman, D., Kinziger, A.P., Reid, S.B., Docker, M.F., In press. Morphological diagnosis of *Entosphenus* and *Lampetra* ammocoetes (Petromyzontidae) in Washington, Oregon and California. In: Brown, L. R., (Ed). Biology, Management and Conservation of Lampreys in North America. American Fisheries Society, Bethesda, Maryland.

Goodman, D.H., Reid, S.B., Docker, M.F., Hass, G.R., Kinziger, A.P., 2008. Mitochondrial DNA evidence for high levels of gene flow among populations of widely distributed anadromous lamprey *Entosphenus tridentatus* (Petromyzontidae). J. Fish Biol. 72, 400-417.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Goudet, J., 1995. Fstat version 2.9.3.2: a computer program to calculate Fstatistics. J. of Hered. 86, 485-486.

Graur, D., Li, W.H., 2000. Fundamentals of molecular evolution. Sinauer Associates, Sutherland, MA.

Grossman, L.I., Wildman, D.E., Schmidt, T.R., Goodman, M., 2004. Accelerated evolution of the electron transport chain in anthropoid primates. Trends Genet. 20, 578.

Günther, A., 1870. Catalogue of the fishes in the British Museum. British Museum. Cat. Fishes. 8, 1-549.

Gyllesten, U.B., Wharton, D., Josefsson, A., Wilson, A.C., 1991. Paternal inheritance of mitochondrial DNA in mice. Nature 352, 255-257.

Hagelberg, E., Goldman, N., Lió, P., Whelan, S., Sciefenhövel, W., Clegg, J.B., Bowden, D.K., 1999. Evidence for mitochondrial recombination in a human population of island Melanesia. Proc. R. Soc. Lond. B. Sci. 266, 485-492.

Halanych, K.M., Demboski, J.R., van Vuuren, B.J., Klein, D.R., Cook, J.A., 1999. Cytochrome *b* phylogeny of North American hares and Jackrabbits (*Lepus,* Lagomorpha) and the effects of saturation in outgroup taxa. Mol. Phylogenet. Evol. 11, 213-221.

Hardisty, M.W., 1963. Fecundity and speciation in lampreys. Evolution 17, 17-22.

Hardisty, M.W., 2006. Lampreys: Life without jaws. Tresaith, Cardigan. Forrest Text.

Hardisty, M.W., Potter, I.C., 1971. The behaviour, ecology and growth of larval lampreys. In: Hardisty, M.W., Potter, I.C., (Eds.). The Biology of Lampreys, vol. 1. London. Academic Press. pp. 85-126.

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 21, 160-174.

Hayashi, J.I., Tagashira, Y., Yoshida, M.C., 1985. Absence of extensive recombination between inter.and intraspecies mitochondrial DNA in mammalian cells. Exp. Cell. Res. 160, 387-395.

Hearn, B.C.Jr, McLaughlin, R.J., Donnely-Nolan, J.M., 1988. Tectonic framework of the Clear Lake Basin, California. Pages 9-20 in JD Sims, ed. Late Quaternary Climate, Tectonism, and Sedimentation in Clear Lake. USGS Special Paper 214. pp. 225.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2002. Biological indentifications through DNA barcodes. Proc. R. Soc. Lond. B. Biol. Sci. 270, 313-321.

Hedrick, P.W., 2005. Genetics of Populations. Third Ed. Jones and Bartlett Publishing, Sudbury, Massachusetts.

Hewitt, G.M., 1999. Post-glacial re-colonization of European biota. Biol. J. Linn. Soc. 68, 87-112.

Higgins, D.G., Sharp, P.M., 1989. Fast and sensitive multiple sequence alignments on a microcomputer. CABIOS 5, 151-153.

Hilliard, R.W., Bird, D.J., Potter, I.C., 1983. Metamorphic changes in the intestine of three species of lampreys. J. Morphol. 176, 181-196.

Hillis, D.M., Dixon, M.T., 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. Quart. Rev. Biol. 66, 411-453.

Holčík, J., Šorić, V., 2004. Redescription of *Eudontomyzon stankokaramani* (Petromyzontes, Petromyzontidae) – a little known lamprey from the Drin River drainage, Adriatic Sea basin. Folia Zool. 53, 399-410.

Huang, W.F., Tsai, S.J., Lo, C.F., Soichi, Y., Wang, C.H., 2003. The novel organization and complete sequence of the ribosomal RNA gene of *Nosema bombycis*. Fungal. Genet. Biol. 41, 473-481.

Hubbs, C. L., 1925. The life cycle and growth of lampreys. Mich. Acad. Sci., Arts and Lett. 4, 587-603.

Hubbs, C.L., Trautman, M.B., 1937. A revision of the lamprey genus *Ichthyomyzon*. Miscellaneous Publications of the Museum of Zoology, University of Michigan 35, 7-109.

Hubbs, C.L., 1971. *Lampetra (Entosphenus) lethophaga*, a new species, the nonparasitic derivative of the Pacific lamprey. Trans. San Diego Soc. Nat. Hist. 16, 125-164.

Hubbs, C.L., Potter, I.C., 1971. Distribution, phylogeny and taxonomy. In: Hardisty, M.W., Potter, I.C., (Eds.),The Biology of Lampreys, Volume 1. Academic Press. pp. 1-65.

Hubert, N., Hanner, R., Holm, E., Mandrak, N.E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., Bernatchez, L., 2008. Identifying Canadian freshwater fishes through DNA barcodes. PLoS ONE. 3, e2490.

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

Hurst, G.D.D., Jiggins, F.M., 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc. Biol. Sci. 272, 1525.

Ibrahim, K.M., Nichols, R.A., Hewitt, G.M., 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77, 282-291.

International Commission on Zoological Nomenclature, 1999. International code of zoological nomenclature. Fourth edition. The International Trust for Zoological Nomenclature c/o The Natural History Museum, London.

Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the cytochrome *b* gene in mammals. J. Mol. Evol. 32, 128-144.

Jin, L. Macaubas, C., Hallmayer, J., Kimura, A., Mignot, E., 1996. Mutation rate varies among alleles at microsatellite locus: phylogenetic evidence. Proc. Natl. Acad. Sci. USA 93, 15285-15288.

Johns, G.C., Avise, J.C., 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. Mol. Biol. Evol. 15, 1481-1490.

Karaman, M.S., 1974. *Eudontomyzon vladykov stankokaramani* n.ssp. a new subspecies of lamprey from tributaries of the Ohrid-Drim-Skaadar system in West Balkan peninsula. Folia Balcanica. 3, 1-14.

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

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in hominoidea. J. Mol. Evol. 29, 170-179.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196-6200.

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

Linnaeus, C., 1758. Systema naturae, Tenth edition, volume 1. Stockholm.

Loman, J.C.C., 1912. Über die Naturgeschichte des Bachneunauges *Lampetra planeri*. Zoologisches Jahrbuch [Suppl] 15, 243–270.

Lorion, C.M., Markle, D.F., Reid, S.B., Docker, M.F., 2000. Redescription of the presumed-extinct Miller Lake lamprey, *Lampetra minima*. Copeia 2000, 1019-1028.

Luzier, C.W., Docker, M.F., Whitesel, T.A., In press. Characterization of ten microsatellite loci for western brook lamprey *Lampetra richardsoni*. Conserv. Genet. Resour.

Lynch, M., Jarrell, P.E., 1993. A method for calibrating molecular clocks and its application to animal mitochondrial DNA. Genetics 135, 1197-1208.

Lyrholm , T., Leimar, O., Johanneson, B., Gyllensten, U., 1999. Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. Proc. R. Soc. Lond. Ser. B. 266, 347-354.

Mallatt, J., Sullivan, J., 1998. 28S and 18S ribosomal DNA sequences support the monophyly of lampreys and hagfishes. Mol. Biol. Evol. 15, 1706-1718.

Manni, F., Guérard, E., 2004. Barrier vs. 2.2 Manual of the user. Population genetics team, Museum of Mankind (Musée de l'Homme), Paris [Publication distributed by the authors].

Martin, H., White, M., 2008. Intraspecific phylogeography of the Least brook lamprey (*Lampetra aepyptera*). Copeia 2008, 579-585.

Mattern, M.Y., 2003. Molecular phylogeny of the Gasterosteidae: the importance of using multiple genes. Mol. Phylogenet. Evol. 30, 366-377.

Mayden, R. L., 1997. A hierarchy of species concepts: The denouement in the saga of the species problem. In: Claridge, M.F., Dawah, H.A., Wilson, M.R., (Eds.), Species: the units of biodiversity. Chapman & Hall, London. pp. 381-424.

Mayer, M.S., Soltis, P.S., 1999. Intraspecific phylogeny analysis using ITS sequences: insights from studies of the *Streptanthus glandulosus* complex (Cruciferae). Syst. Bot. 24, 47-61.

Mayr, E., 1942. Systematics and the Origin of Species. Chlumbia Liniv. Press, New York.

Mayr, E., 1963. Animal Species and Evolution. Harvard Univ. Press, Cambridge, Massachusetts.

Mayr, E., Provine, W., 1980. The Evolutionary Synthesis. Harvard Univ. Press, Cambridge, Massachusetts.

McPhail, J.D., Lindsey, C.C., 1970. Freshwater fishes of north-western Canada and Alaska. Bull. Fish. Res. Board. Can. No. 173.

Mejía, O., Polaco, O.J., Zúñiga, G., 2004. Genetic diversity of Mexican brook lamprey Lampetra (*Tetrapleurodon*) geminis (Alvarez del Villar, 1966). Genetica 122, 325–333.

Meyer, A., Zardoya, R., 2003. Recent advances in the (molecular) phylogeny of vertebrates. Annu. Rev. Ecol. Syst. 34, 311-338.

Miller, M.P. 1997. Tools for Population Genetic Analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population data. Computer software distributed by author.

Monmonier, M., 1973. Maximum-difference barriers: an alternative numberical regionalization method. Geogr. Anal. 3, 245-261.

Moyle, P.B., 2002. Inland fishes of California, revised and expanded. Univ. California Press, Berkeley.

Moyle, P.B., Yoshiyama, R.M., Williams, J.E., Wikramanayake, E.D., 1995. Fish Species of Special Concern in California. Second Edition. Prepared for California Department of Fish and Game, Rancho Cordova. Contract No. 2128IF.

Muir, G., Flemming, C.C., Schlötterer, C., 2001. Three divergent rDNA clusters predate the species divergence in *Quercus petraea* (Matt.) Liebl. and *Quercus robur*. L. Mol. Biol. Evol. 18, 112-119.

Mundahl, N.D., Ericson, C., Johnston, M.R., Sayeed, G.A., Taubel, S., 2005. Diet, feeding rate, and assimilation efficiency of American brook lamprey larvae. Environ. Biol. Fishes. 72, 67-72

Neave, F.B., Mandrak, N.E., Docker, M.F., Noakes, D.L., 2007. An attempt to differentiate sympatric *Ichthyomyzon* ammocoetes using meristic, morphological, pigmentation, and gonad analyses. Can. J. Zool. 85, 549-560.

Nei M., 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583-590.

Nelson, J.S., Crossman, E.J., Espinosa-Pérez, H., Findley, L.T., Gilbert, C.R., Lea, R.N., Williams, J.D., 2004. Common and scientific names of fishes from the United States, Canada, and Mexico. Sixth Edition. American Fisheries Society, Spec. Publ. 29, Bethesda, Maryland.

Nicholas, K.B, Nicholas, H.B., 1997. GeneDoc: a tool for editing ad annotating multiple sequence alignments. Distributed by the author at http://www.nrbsc.org/gfx/genedoc/index.html

Nichols, R., 2001. Gene trees and species trees are not the same. TREE 16, 358-364.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407-414.

Page, L.M., Burr, B.M., 1991. A field guide to freshwater fishes of North America north of Mexico. Houghton Mifflin Company, Boston.

Page, R.D.M., 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12, 357–358.

Palumbi, S.R., Baker, C.S., 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. Mol. Biol. Evol. 11, 426-435.

Pamilo P., M. Nei, 1988. Relationships between gene trees and species trees. Mol. Biol. Evol. 5, 568-583.

Phillips, R.B., Gudex, L.I., Westrich, K.M., DeCicco, A.L., 1999. Combined phylogenetic analysis of ribosomal ITS1 sequences and new chromosome data supports three subgroups of Doll Varden char (*Salvelinus malma*). Can. J. Fish. Aquat. Sci. 56, 1504-1511.

Pleyte, K.A., Duncan, S.D., Phillips, R., 1992. Evolutionary relationships of the salmonid fish genus *Salvelinus* inferred from DNA sequences of the first internal transcribed spacer (ITS 1) of ribosomal DNA. Mol. Phylogenet. Evol. 1, 223-230.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817-818.

Potter, I.C., 1980a. The Petromyzoniformes with particular reference to paired species. Can. J. Fish. Aquat. Sci. 37, 1595-1615.

Potter, I.C., 1980b. Ecology of larval and metamorphosing lampreys. Can. J. Fish. Aquat. Sci. 37, 1641-1657.

Potter, I.C., Gill, H.S., 2003. Adaptive radiation of lampreys. J. Great Lakes Res. 29 (Supplement 1), 95-112.

Presa, P., Pardo, B.G., Martinez, P., Bernatchez, L., 2002. Phylogeographic congruence between mtDNA and rDNA ITS markers in Brown Trout. Mol. Biol. Evol. 19, 2161-2175.

Purvis, A., 2004. The impact of speices concept on biodiversity studies. Quart. Rev. Biol. 79, 161-179.

Raney, E.C., 1952. A new lamprey, *Ichthyomyzon hubbsi*, from the Upper Tennessee River System. Copeia 1952, 93-99.

Raymond, M., and Rousset, F., 1995. An exact test for population differentiation. Evolution 49, 1280-1283.

Regan, C.T., 1911. A synopsis of the marsipobranchs of the order Hyperoartii. Ann. Mag. Nat. Hist. 7, 193-204.

Renaud, C.B., 1997. Conservation status of Northern hemisphere lampreys (Petromyzontidae). J. Appl. Ichthyol. 13, 143-148.

Renaud, C.B., Docker, M.F., Mandrak, N.E., In press. Taxonomy, distribution and conservation of lampreys in Canada. In: Brown, L. R., (Ed). Biology, Management and Conservation of Lampreys in North America. American Fisheries Society, Bethesda, Maryland.

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

Richards, J.E., Beamish, R.J., Beamish, F.W.H., 1982. Descriptions and keys for ammocoetes of lampreys from British Columbia, Canada. Can. J. Fish. Aquat. Sci. 39, 1484-1495.

Richardson, J., 1836. Fauna Boreali-Americana; or the zoology of the northern parts of British America: containing descriptions of the objects of natural history collected on the late northern land expeditions under command of Captain Sir John Franklin, R.N, part 3: the fish. J. Murray, London.

Rieppel, O., 1980. Homology, a deductive concept? J. Zool. Systemat. Evol. Res. 18, 315-319.

Robins, C.R., Bailey, R.M., Bond, C.E., Brooker, J.R., Lachner, E.A., Lea, R.N., Scott, W.B., 1991. A list of common and scientific names of fishes from the United States and Canada, American Fisheries Society. Bethesda, Maryland.

Robins, C.R., Bailey, R.M., Bond, C.E., Brooker, J.R., Lachner, E.A., Lea, R.N., Scott, W.B., 1980. A list of common and scientific names of fishes from the United States and Canada. American Fisheries Society. Bethesda, Maryland.

Rocha-Olivares, A., Fleeger, J.W., Foltz, D.W., 2001. Decoupling of molecular and morphological evolution in deep lineages of a meiobenthic harpacticoid copepod. Mol. Biol. Evol. 18, 1088-1102.

Rodrigues, A.S., Gaston, K.J., 2002. Maximising phylogenetic diversity in the selection of networks of conservation areas. Biol. Conserv. 105, 103-111.

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

Ronquist, F., Huelsenbeck, J. P., 2005. Bayesian analysis of molecular evolution using MrBayes. In: Nielsen, R., (Ed.), Statistical methods in molecular evolution. Springer, New York. pp. 183-232.

Ronquist, F., Huelsenbeck, J.P., van der Mark, P., 2005. MrBayes 3.1 Manual.

Roze, D., Rousset, F., Michalakis, Y., 2005. Germline Bottlenecks, Biparental Inheritance and Selection on Mitochondrial Variants. Genetics 170, 1385.

Saccone, C., Pesole, G., Sbisa, E., 1991. The main regulatory region of mammalian mitochondrial DNA: structure-function model and evolutionary pattern. J. Mol. Evol. 33, 83-91.

Sansom, I.J., Smith, M.M., Smith, M.P., 1996. Scales of thelodont and shark-like fishes from the Ordovician of Colorado. Nature 379, 628–629.

Schluter, D., 1996. Ecological speciation in postglacial fishes. Phil. Trans. R. Soc. Lond. B. 351, 807-814.

Schluter, D., Boughman, J.W., Rundle, H.D., 2001. Parallel speciation with allopatry. TREE 16, 283-284.

Schreiber, A., Engelhorn, R., 1998. Population genetics of a cyclostome species pair, river lamprey (*Lampetra fluviatilis* L.) and brook lamprey (*Lampetra planeri* Bloch). J. Zool. Syst. Evol. Res. 36, 85-99.

Schultz, L. P., 1930. The life history of Lampetra planeri Bloch, with a statistical analysis of the rate of growth of the larvae from Western Washington. Occ. Pap. Mus. Zool. Univ. Mich. No. 221, 1-35.

Schwartz, F.J., 1972. World literature to fish hybrids, with an analysis by family, species, and hybrid. Publ. Gulf Coast Res. Lab. Mus. 3, 1-328.

Selkoe, K.A., Toonen, R.J., 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol. Lett. 9, 615-629.

Shaw, K.L., 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. USA 99, 16122-16127.

Sims, J.D., 1988. Late Quaternary climate, tectonism and sedimentation rate in Clear lake, northern California coast ranges. Geological Society of America, Special Paper 214, pp. 225.

Sims, J.D., Rymer, M.J., Perkins, J.A., 1988. Late Quaternary deposits beneath Clear Lake, California; stratigraphy, age, and paleogeographic implications. In: Sims, J.D., (Ed.), Late Quaternary Climate, Tectonism, and Sedimentation in Clear Lake. USGS Special Paper 214, pp. 21-44.

Smith, G.R., 1992. Introgression in fishes: significance for paleontology, cladistics, and evolutionary rates. Syst. Biol. 41, 41-57.

Sturmbauer, C., Meyer, A., 1992. Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. Nature 358, 578-581.

Suchanek, T.H., Richerson, P.J., Nelson, D.C., Eagles-Smith, C.A., Anderson, D.W., Cech, J.J., Zierenber, J.R., Schladow, G., Mount, J.F., McHatton, S.C., Slotton, D.G., Webber, L.B., Swisher, B.J., Bern, A.L., Sexton, M., 2003. Evaluating and Managing a Multiply Stressed Ecosystem at Clear Lake, California: A Holistic Ecosystem Approach. In: Rapport et al., (Eds), Managing for Healthy Ecosystems. CRC Press. pp. 1239-1272.

Sutton, T.M., Bowen, S.H., 1994. Significance of organic detritus in the diet of larval lampreys in the Great Lakes basin. Can. J. Fish. Aquat. Sci. 51, 2380-2387.

Swafford, D.L., 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Massachusetts: Sinauer Associates.

Tagliavini, J., Tizzi, R., Contero, F., Mariottini, P., Gandolfi, G., 1994. Mitochondrial DNA sequences in three genera if Italian lampreys. Boll. Zool. 61, 331-333.

Takahata N., 1989. Gene genealogy in three related populations: consistency probability between gene and population trees. Genetics 122, 957-966.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial-DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512-526.

Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. In: Miura, R.M., (Ed.), Some mathematical questions in biology – DNA sequence analyis. Amer. Math. Soc., Providence, RI. pp. 57-86.

Tilesius von Tilenau, W.G., 1811. Piscium camtschaticorum descriptions et icones. Mémoires de l'Académie Impériale des Sciences de St. Pétersbourg. 3,225-285.

Uinuk-ool, T.S., Mayer, W.E., Sato, A., Takezaki, N., Benyon, L., Cooper, M.D., Klein, J., 2003. Identification and characterization of a TAP-family gene in the lamprey. Immunogenetics. 55, 38-48.

Vawter, L., Brown, W. M., 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. Science 234, 194-196.

Vladykov, V.D., 1955. *Lampetra zanandreai*, a new species of lamprey from northern Italy. Copeia 1955, 215-223.

Vladykov, V.D., 1973a. *Lampetra pacifica*, a new nonparasitic species of lamprey (Petromyzonidae) from Oregon and California. J. Fish. Res. Bd. Can. 30, 205-213.

Vladykov, V.D., 1973b. North American nonparasitic lampreys of the family Petromyzonidae must be protected. Can. Field Nat. 87, 235–239.

Vladykov, V.D., and Kott, E., 1980. Description and key to metamorphosed specimens and ammocoetes of Petromyzontidae found in the Great Lakes region. Can. J. Fish. Aquat. Sci. 37, 1616-1625.

Vladykov, V.D., Follett, W.I., 1958. Redescription of *Lampetra ayresii* (Günther) of Western North America, a species of lamprey (Petromyzontidae) distinct from *Lampetra fluviatilis* (Linnaeus) of Europe. J. Fish. Res. Bd. Can. 15, 47-77.

Vladykov, V.D., Follett, W.I., 1965. *Lampetra richardsoni*, a new nonparasitic species of lamprey (Petromyzontidae) from western North America. J. Fish. Res. Bd. Can. 22, 139-158.

Vladykov, V.D., Kott, E., 1976a. A new nonparasitic species of lamprey of the genus *Entosphenus* Gill, 1862 (Petromyzonidae) from south central California. Bull. South. Calif. Acad. Sci. 75, 60-67.

Vladykov, V.D., Kott, E., 1976b. A second nonparasitic species of *Entosphenus* Gill, 1862 (Petromyzonidae) from Klamath River system, California. Can. J. Zool. 54, 974-989.

Vladykov, V.D., Kott, E., 1979. Satellite species among the holarctic lampreys (Petromyzonidae). Can. J. Zool. 57, 860-867.

Vladykov, V.D., Kott, E., Pharand-Coad, S., 1975. A new nonparasitic species of lamprey, genus *Lethenteron* (Petromyzonidae), from eastern tributaries of the Gulf of Mexico, U.S.A. Publ. Zool., Natl. Mus. Nat. Sci., Natl. Mus. Can. 12, l-36.

von Martens, E., 1868. Ueber einige ostasiatische Süsswassertiere. Arch Naturgesch 34, 1-64.

Waples, R.S., Teel., D.J., 1990. Conservation genetics of Pacific salmon I. Temporal changes in allele frequency. Conserv. Biol. 4, 144-156.

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

Weir, B.S. and Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358-1370.

Wheeler, Q. D., 1996. The phylogenetic species. Cornell Univ. Press, Ithaca.

Will, K.W., Rubnoff, D., 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics 20, 47-55.

Willett, C.S., Burton, R.S., 2004. Evolution of Interacting Proteins in the Mitochondrial Electron Transport System in a Marine Copepod. Mol. Biol. Evol. 21, 443.

William, J., Ballard, O., Kreitman, M., 1995. Is mitochondrial DNA strictly a neutral marker? TREE 10, 485-488.

Yamazaki, Y., Goto, A., 2000. Breeding season and nesting assemblages in two forms of *Lethenteron reissneri*, with reference to reproductive isolating mechanisms. Ichthyol. Res. 47, 271-276.

Yamazaki, Y., Goto, A., Nishida, M., 2003. Mitochondrial DNA sequence divergence between two cryptic species of *Lethenteron*, with reference to an improved identification technique. J. Fish. Biol. 62, 591-609.

Yamazaki, Y., Yokoyama, R., Nishida, M., Goto, A., 2006. Taxonomy and molecular phylogeny of *Lethenteron* lampreys in eastern Eurasia. J. Fish. Biol. 68, 251-269.

Young, N.D., Healy, J., 2003. GapCoder automates the use of indel characters in phylogenetic analysis. BMC Bioinformatics 4, 6.

Youson, J.H., Beamish, R.J., 1991. Comparison of the internal morphology of adults of a population of lampreys that contains a nonparasitic life-history type, *Lampetra richardsoni*, and a potentially parasitic form, *L. richardsoni* var. *marifuga*. Can. J. Zool. 69, 628-637.

Yu, S., Zhang, W., Li, L., Huang, H., Ma, F., Li, Q., 2008. Phylogenetic analysis of 48 gene families revealing relationships between Hagfishes, Lampreys, and Gnathostomata. J. Genet. Genomics. 35, 285-290.

Zanandrea, G., 1959. Speciation among lampreys. Nature 187, 380.

Zardova, R., Meyer, A., 2001. Vertebrate phylogeny: limits of inference of mitochondrial genome and nuclear rDNA sequence data due to an adverse phylogenetic signal/noise ratio. In: Ahlberg, P.E., (Ed.), Major events in early vertebrate evolution. Palaeontology, phylogeny, genetics and development. Systematic Association special volume series, 61. London: Taylor and Francis. pp. 135-155.

Zouros, E., Freeman, K.R., Ball, A.O., Pogson, G.H., 1992. Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*. Nature 359, 412-414.